22nd ESACT Meeting

Vienna, Austria

May 15.-18., 2011

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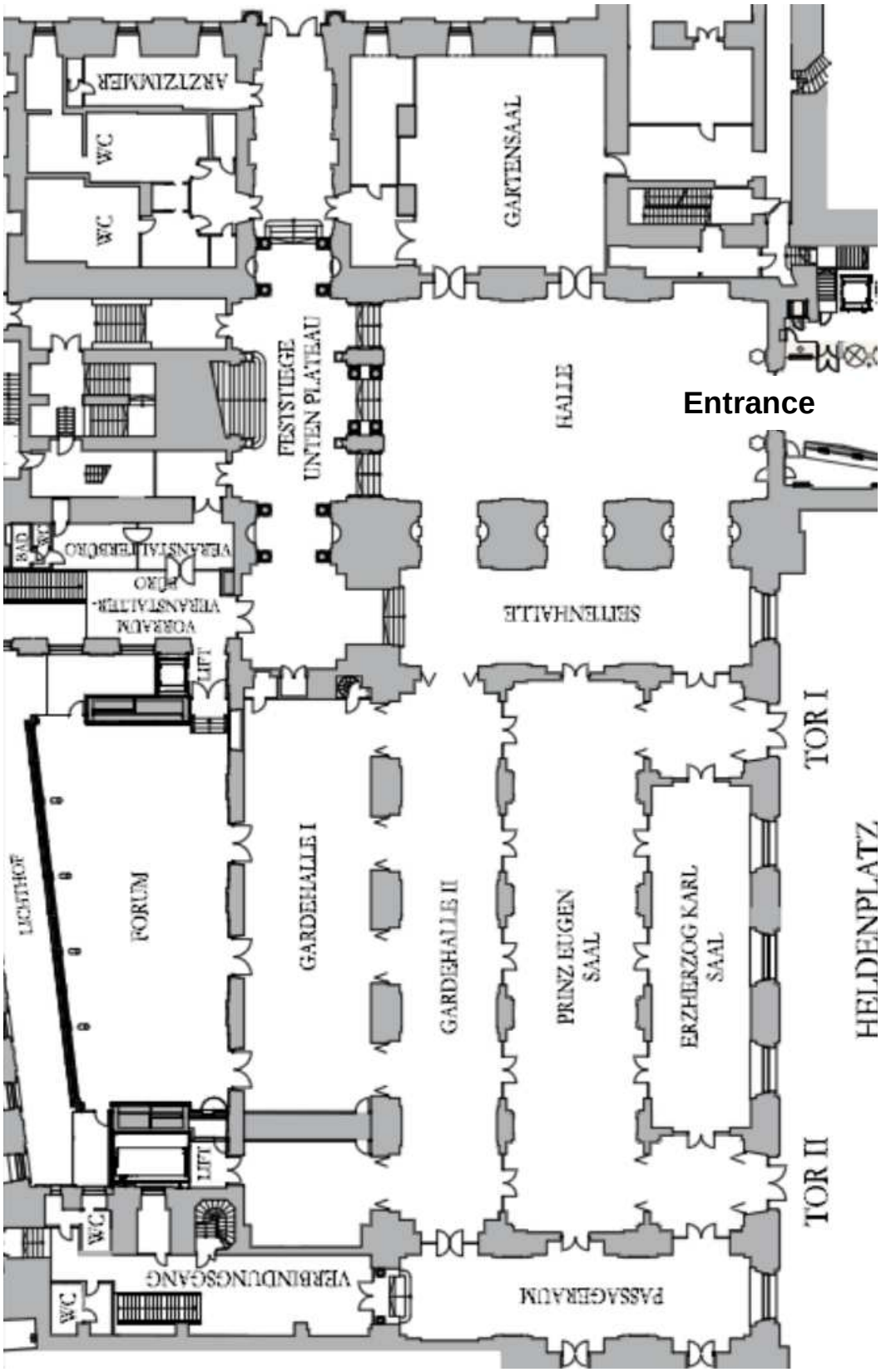
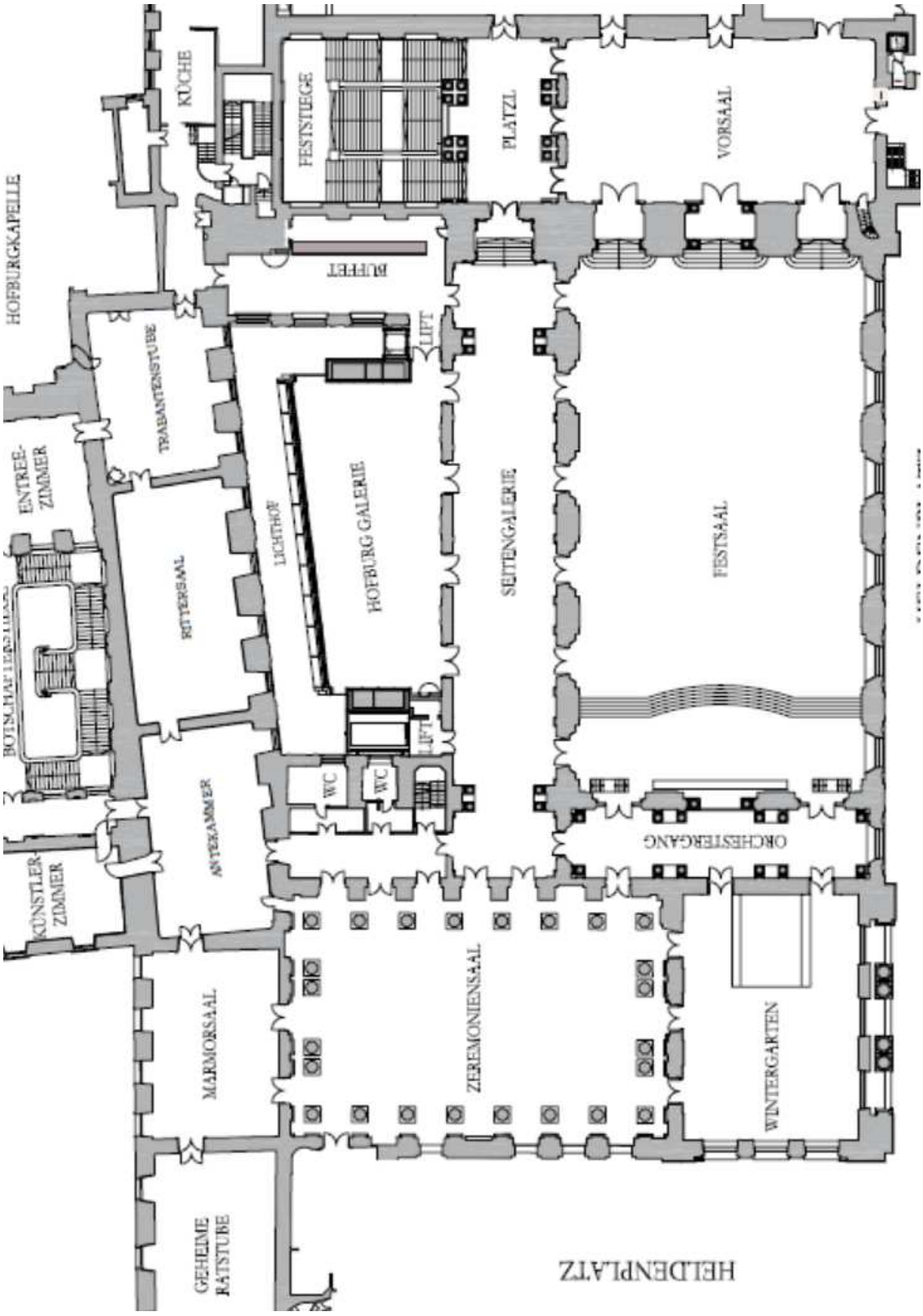
Map of Hofburg

Ground Floor



Map of Hofburg

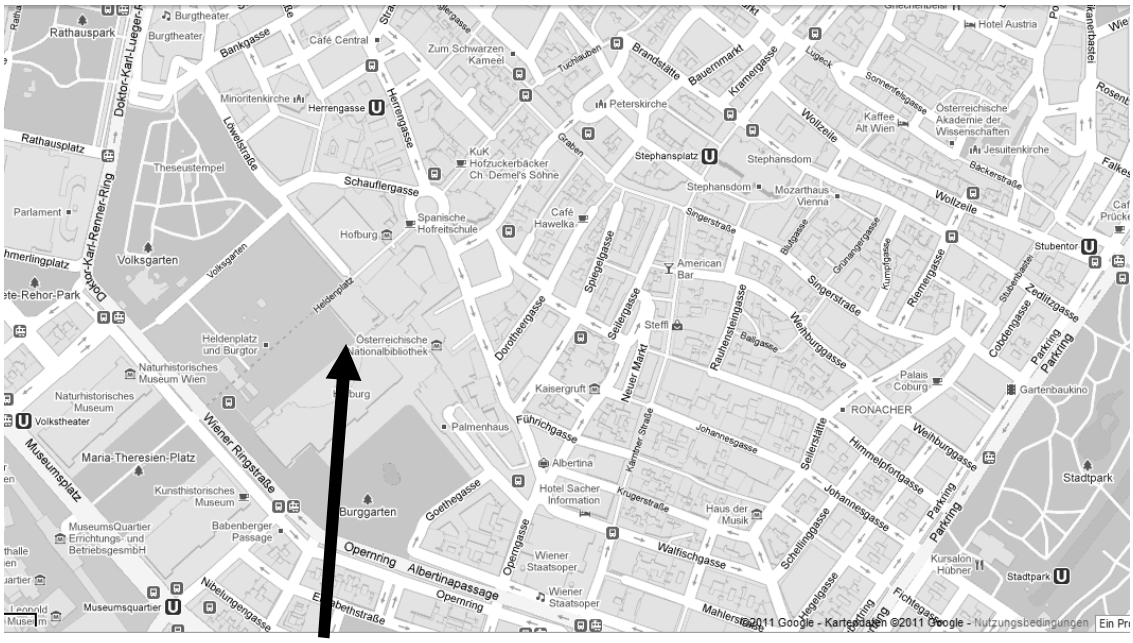
First Floor



Exhibitors

Company	Room	B.Nr.
Aber Instruments	Wintergarten	223
Applikon Biotechnology	Zeremoniensaal	220
ATMI BVBA	Marmorsaal	236
Bartelt	Wintergarten	229
Bayer Technology	Marmorsaal	235
Becton Dickinson	Seitengalerie	210
Bioengineering	Seitengalerie	204
Bioprocess International	Entrance Hall	101
Bioreliance	Seitengalerie	217
BioUetikon	Entrance Hall	102
BIS Industrietechnik	Wintergarten	224
BlueSens	Gardehalle I	115
Broadley Tech.	Seitengalerie	208
Cellon	Zeremoniensaal	233
Celonic	Hofburg Galerie	214
Cevec Pharma	Seitengalerie	218
Charles River	Gardehalle II	113
Chemometec	Entrance Hall	102
Covance CAPS	Entrance Hall	100
Cyntellect	Platzl	201
DASGIP	Hofburg Galerie	216
Eppendorf	Antekammer	243
Excellgene	Zeremoniensaal	219
Finesse	Seitengalerie	207
Fogale	Zeremoniensaal	234
Friesland Campina	Wintergarten	225
GE Healthcare	Seitengalerie	203
Genetic Eng. & Biotechn.	Gardehalle II	112
Genetix LTD	Entrance Hall	107
Greiner Bio-One	Zeremoniensaal	232
Hexascreen	Gardehalle II	111
Infors AG	Wintergarten	228
In Vitria	Wintergarten	227
Life Technologies	Seitengalerie	209
Irvine Scientific	Zeremoniensaal	230
JM Separations BV	Entrance Hall	100
Kühner	Zeremoniensaal	219
Lonza Inc.	Entrance Hall	105
LGC	Platzl	201
Miltenyi Biotec	Zeremoniensaal	221
Merck Millipore	Antekammer	238
M2p Labs	Zeremoniensaal	222
Nano F-M	Rittersaal	244
Nova Biomedical	Gardehalle I	116
Novozymes	Gardehalle I	117
PAA Laboratories	Antekammer	237
Pall Life Sciences	Entrance Hall	104
PBS Biotech	Wintergarten	226
Pneumatic Scale	Rittersaal	240
Polyplus	Gardehalle II	114
PreSens Precision Sensing	Antekammer	239
Protagen	Entrance Hall	106
Refine Technology	Seitengalerie	212
Rentschler	Seitengalerie	206
Roche	Rittersaal	242
SAFC	Hofburg Galerie	215
Saint Gobain	Seitengalerie	205
Sartorius	Hofburg Galerie	213
Sheffield Bio-Science	Zeremoniensaal	231
Sigma-Aldrich Chemie	Platzl	200
Solentim Ltd.	Gardehalle II	110
Stemcell	Rittersaal	241
TCS Biosciences	Entrance Hall	103
Thermo Scientific	Seitengalerie	211
The Automation Partnersh.	Seitengalerie	202
Veltek	Gardehalle I	119
Virussure	Gardehalle I	118

Map of City Centre



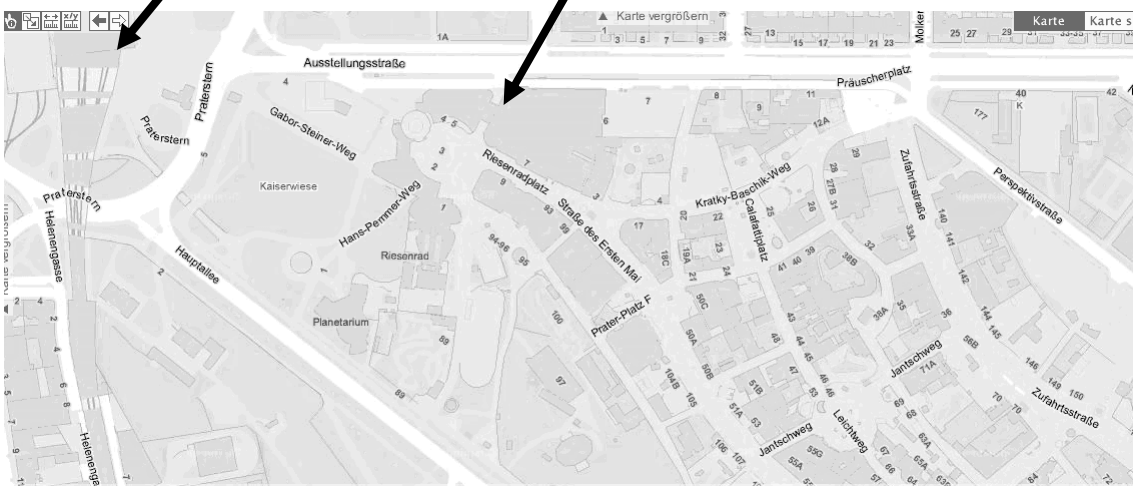
Entrance to Conference Centre

How to get to Pratergaleries for the Gala Dinner

Please use the Underground U1 or U2 and go to Praterstern. There will be signs and students to help you find the way. If you would like to stroll through the Prater entertainment before the Dinner, please refer to the map below. The Galeries are easy to find, as they are very close to the Riesenrad.

Underground station

Galeries – Galadinner



General Information

Registration

The registration desk, located in the lobby of the Hofburg Conference centre, will be open at

Sunday, May 15th	8:00–19:00
Monday, May 16th	8:30–19:00
Tuesday, May 17th	8:30–15:00
Wednesday, May 18th	8:30–18:00

The registration desk staff will be happy to help you with any activities, including reservation of city tours, ticket sercives and airport transport (please note that airport transfer in a normal taxi is approximately twice as expensive as if you book a reservation through limosine services. The later will cost between 30–38 €).

Access

The conference badges need to be worn during all lectures and social activities. Access will be strictly controlled.

Colour code:

Blue bar	Chair
Green bar	Executive, Organising and Scientific Committees
Red bar	Exibitor
Light Blue bar	Organiser´s Assistant
Pink bar	Accompanying person

Accompanying persons

Accompanying persons will be able to attend the Trader´s Reception on Sunday evening, the outing to Schloss Hof on Tuesday afternoon and the Gala Dinner on Wednesday evening.

Workshops

Workshops on Sunday morning will take place in three lecture halls:

- The main lecture hall on the first floor (Festsaal)
- The Gartensaal on the groundfloor, opposite to the registration desk
- The Geheime Ratsstube on the first floor, in the far back corner

Please refer to pages 12–24 for details on when and where each workshop will take place. Access is open to all registered participants.

Scientific Sessions

All Scientific Sessions will take place in the main lecture hall (Festsaal) on the first floor.

Meals

All coffee breaks and meals will be served at buffet stations spread throughout the exhibition and poster areas. Please note that these are evenly spread on both floors. Thus if there are long queues on the first floor or in the vicinity of the lecture hall, please move to the ground floor for faster access. Also, during lunch breaks, there will be tables and more extensive buffets provided in the following rooms (please refer to the maps on pages 2 and 3 for location:

Ground floor:

- Forum
- Prinz Eugensaal
- Gartensaal

First Floor:

- Geheime Ratstube

Public transport ticket

At registration you will be provided with a public transport ticket that is valid throughout the conference, from Sunday through Wednesday, will all public transport in Vienna, including underground, tram and buses. Please also use this ticket to reach the Prater Galeries for the Gala Dinner.

Speaker Preview

Computers will be set up in a room behind the Künstlerzimmer on the first floor. Please follow the signs. The computers will be staffed at all times to help you preview your presentation and also to receive the files, so that they are ready during the session you will be presenting in. Please contact the technical staff there or in the lecture hall well ahead of your scheduled presentation.

Poster Presentations

Please put up your poster as soon as you arrive. Please consult the Poster and Exhibitor Map you will receive at registration to locate your board. All posters will remain on display throughout the conference. Please be with your poster during the postersessions on Monday, May 16th:

Poster Session A: 12:10–15:00 Posters P1.1 to P4.54
Poster Session B: 17:20–20:00 Posters P4.55 to P5.53

On Tuesday before the lunch break, those poster presenters that were selected to give a short presentation for the poster prize will be announced. If you were amongst the preliminary selected presenters, please make sure to attend the conference at that time or watch out for a plaquette marking your poster as one of those selected. If you are selected, please make sure that your presentation follows the rules: 4 to maximum 5 slides, 5 minutes presentation followed by a short discussion. Please bear in mind that you will be relentlessly cut short if you exceed the time limit, as we need to make sure that everybody has the chance to speak.

Computers and WLAN

The computers in the speaker preview room are connected to the internet and can also be used by participants. In addition, throughout the building there is a free WLAN system in operation. Please connect to Hofburg Secure and use ESACT011 as password.

Smoking Policy

Smoking is not allowed inside the Hofburg or any other buildings. You will have to step outside to Heldenplatz to smoke. In Schloss Hof also please go outside, there will be a Smoker´s Corner with ashtrays provided. During the Gala Dinner there will be a Smoking area on one of the terraces, again outside.

Lost and Found

Anything found should be taken to the registration desk, likewise if you are looking for something lost, please ask at the registration desk.

Insurance

The conference organising committee and it agents will not be responsible for any medical expenses, loss or accidents incurred during the conference, nor for any damage done to the historical surroundings. In case of any emergencies please contact the registration desk immediately for help.

ESACT General Assembly

The General Assembly will take place on Tuesday, May 17th, at 12:20 in the main lecture hall (Festsaal)

Program at a glance

Time	Sunday	Monday	Tuesday	Friday
08:00	Workshops			
09:00		S2: Cells as Therapies and Stem Cells	S4: Biopharmaceutics and Vaccine Production	S5: Complex Interactions and Cell engineering
10:00				
11:00				
12:00		General Assembly		
13:00		Poster Session A	Poster Session	
14:00				Poster Prize Session
15:00		S3: Protein Synthesis, Processing and Secretion	Outing to Schloss Hof	
16:00	S1: Cell Stability and Differentiation			
17:00				K2: R. Lerner
18:00	K1: G. Winter	Poster Session B		
19:00	Trader´s Reception			Gala Dinner
20:00				
21:00				

Sunday May 15th 2011

Workshops	
08:30	WS A: **Recent Advancements in Viral Vector Manufacturing** (Gartensaal)
08:30	WS 1: **Massively Parallel Sequencing (MP-Seq)** (Geheime Ratsstube)
08:30	WS 2: **Using a rational approach to develop cell culture manufacturing processes** (Main Hall)
10:15	WS B: **CHO Genome Workshop** (Main Lecture Hall)
10:15	WS 3: **BD Mosaic™ MSC SF: A new, high performance serum free culture medium for the expansion of Mesenchymal Stem Cells** (Geheime Ratsstube)
10:15	WS 4: **Evolution of Risk Mitigation Requirements for Platform Expression Systems** (Gartensaal)
12:00	WS 6: **From molecules to market: PD solutions from Life Technologies** (Gartensaal)
12:30	WS 5: **Towards a fully single use protein production facility** (Geheime Ratsstube)
14:15	WS C: **FC Fusion Proteins: A growing Class of Therapeutics** (Gartensaal)
14:15	WS 7: **Rethinking Media Supplementation: Identifying Bioactive Molecules that drive protein production (Main** Lecture Hall)
14:15	WS 8: **How QbD is changing upstream bioprocessing design** (Geheime Ratsstube)

Conference Opening

16:00	Welcome	H. Katinger, BOKU Vienna N. Borth, BOKU Vienna
Session I: Cell stability and differentiation		
16:10	Chair´s Introduction	T. von Zglinicki, U Newcastle, UK
16:15	Molecular mechanisms of cellular senescence	F. d´Adda di Fagagno, IFOM, Italy
16:45	MicroRNA-31: an inhibitor of osteogenic differentiation of mesenchymal stem cells	J. Grillari, BOKU Vienna, Austria
17:15	Immortalized Endothelial Cell Lines	T. May, HZI Braunschweig, Germany
17:35	Senescence-associated genes and their implication in the induction of cellular senescence	M. Udono, Kyushu University
Keynote Lecture I:		
17:50	The antibody revolution	G. Winter, MRC, UK
18:30	Trader´s Reception	

Monday May 16th 2011

Session II: Cells as Therapies and Stem Cells		
09:00	Chairs Introduction	J. Friedl, Medical University Vienna H. Hauser, HZI, Germany
09:05	Critical role of IFNg for the production of highly immunogenic dendritic cell-derived exosomes	N. Chaput, Institute Gustave Roussy, France
09:35	ATMPs – The Regulatory Perspective	I. Reischl, AGES Pharmed, Austria
10:05	Cell-based medicinal products and the development of GMP-compliant processes and manufacturing	L. Romagnoli, Areta International, Italy
10:25	Coffee Break	
11:00	Ultra High Yield Manufacture of Red Blood Cells in Fully Defined Animal Component Free Medium	N. Timmins, U Queensland, Australia
11:20	Road to the clinics and in vitro disease modelling using iPSCs: Wilson's disease	M. Esteban, Chinese Academy of Science
11:50	Manufacturing Process Optimization Strategies for Autologous Active Cellular Immunotherapies (ACIs)	C. Ramsborg, Dendreon Corporation, USA
12:20	Poster Session A and Lunch	
12:20	ESACT General Assembly	Main Lecture Hall

Session III: Cellular Mechanisms of protein synthesis, processing & secretion		
15:00	Chair´s Introduction	R. Jefferis, U Birmingham M. Butler, U Manitoba
15:05	Quality control of immunoglobulin biosynthesis: life and death decisions	L. Hendershot, St. Jude´s Hospital, USA
15:35	Golgi Biogenesis in a protozoan parasite	G. Warren, Max Perutz Laboratories, Austria
16:05	Coffee Break	
16:20	Impact of Glycosylation and Sialylation on Ion Channels and the Nervous System	D. Baycin Hizal, Johns Hopkins U, USA
16:40	Antibody Disulfide Bond Reduction in CHO Cell Culture Processes	M. Laird, Genentech, USA
16:50	Evaluation of Impact of Cell Culture Process Conditions on Consistency of Trisulfide vs Native Disulfides Linkages in an IgG1 Molecule	R. Kshirsagar, Biogen Idec, USA
17:00	Using Intact Protein Accurate Mass Data and Differential Gel Electrophoresis (DIGE) to Assess Clonal Difference in High-Mannose Oligosaccharide Content in Recombinant Humanized IgG Producing Chinese Hamster Ovary Cells	N. Lin, SAFC, USA
17:20	Postersession B and drinks	

Tuesday May 17th 2011

Session IV: Biopharmaceutics and Vaccine Production		
9:00	Chair´s Introduction	O. Kistner, Baxter Austria H. Katinger, BOKU, Austria
9:05	Protein Pharmaceuticals: Future State	M. Sliwkowski, Genentech, USA
9:35	Recombinant antibody mixtures: optimization of cell line generation and single-batch manufacturing processes	S. Rasmussen, Symphogen, Denmark
9:55	High yield antibody production using a perfusion process in a disposable WAVE bioreactor	C. Kaisermayer, GE Healthcare
10:15	Highly efficient, chemically defined and fully scalable production of host-restricted pox viruses	I. Jordan, ProBioGen, Germany
10:35	Coffee Break	
11:20	Vaccine Production	J. Aunins, Merck USA
11:50	Fluorescence-based tools to support biopharmaceutical process development	A. Teixeira, IBET, Portugal
12:10	Detection and Remediation of a Vesivirus 2117 Related Contamination in Large-Scale CHO Cell Cultures	W. Zhou, Genzyme Corporation, USA
12:30	Posters and Lunch, ESACT General Assembly	
15:00	Outing to Schloss Hof	

IMPORTANT

Busses for the outing will leave from the Conference Centre entrance starting at 15:00. The last bus will leave at 15:45, so please do not miss that one! The ticket for the outing that you received has a time for your departure marked on it, in the interest of a smooth departure, please adhere to the time you were assigned. Please wear comfortable shoes and bring an extra sweater or jacket, as it may get cool in the evening!

The buses will take you to Schloß Hof, a baroque hunting palace buildt in the 17th century by Prince Eugene of Savoy, an extremely successful military commander of the Habsburgs.

Upon arrival you will be welcomed by a tour guide who will lead you through the palace and onto the terrace, where you can refresh yourself with drinks and snacks. The gardens surrounding the palace are beautifully laid out and include a husbandry, stables and animals. Feel free to stroll around at any time.

At approx. 19:00 trumpets will call you to enjoy a baroque concert. After that we will convene in the riding hall for dinner, followed by entertainment time either in the gardens or on the terrace for a chat, or in the stables for disco dancing or on another guided tour through the underground passages and the hidden destillery.

From 22:00 onwards busses will be waiting at the entrance to drive you back to Vienna. Busses will leave approx. every half hour when they are full. We´ll try to make sure no one is left behind ;-)

Wednesday May 18th 2011

Session V: Understanding Complex Interactions and Cell Engineering		
9:00	Chair´s Introduction	Martin Fussenegger, ETH Zurich
9:05	Integrated Single-Cell Analysis	Ch. Love, MIT, USA
9:35	Biosynthetic Pathway Deflection – a new engineering approach and efficient strategy for ADCC enhancement	V. Sandig, ProbioGen, Germany
9:55	Improved cell line selection through in situ metabolic profiling and offline product analysis	A. Behjousiar, Imperial College London, UK
10:10	Coffee Break	
11:00	Influenza Viruses and host cell signaling – from molecular mechanisms to novel antiviral approaches	S. Ludwig, U Münster, Germany
11:30	Towards rational engineering of cells: Recombinant Gene Expression in Defined Chromosomal Loci	D. Wirth, HZI Braunschweig Germany
11:50	Establishment of a large scale functional screening method for microRNAs in Chinese hamster ovary cells	M. Hackl, BOKU Vienna, Austria
12:05	Announcement of Posters selected for presentation during the afternoon session	
12:05	Posters and Lunch	

Short Poster Presentations	
Chairs:	Poster Committee
14:00	Chair´s Introduction and Explanation of Voting Procedure
14:10	Short Poster presentations
15:20	Coffee Break
15:50	Short Poster presentations
17:00	Collection of votes

Keynote Lecture II:

17:10	The Chemistry of Large Numbers	Richard Lerner, The Scripps Research Institute, USA

19:00 Gala Dinner at the Prater Galeries

Please refer to page 5 for a map and instructions how to get there

Workshop A: Recent Advancements in Viral Vector Manufacturing

8:30-10:00 Gartensaal, Ground Floor

Chairs: *Amine Kamen, BRI-NRC, Bioprocess Centre, CA*
Otto Merten, Genethon, France

Viral vectors are extensively used as delivery systems for gene and cell therapies, oncotherapies and vectors for display or expression of antigens in different vaccination strategies. Also, viral vectors are important tools for acceleration of drug discovery.
Over many years, developments in cell culture technologies have been critical to enable mass production of viral vectors and have greatly contributed in facilitating pre-clinical and clinical trials for therapeutic applications. However, progress reports are confined to specialized conferences in these fields and results are published in journals often not accessible by Animal Cell Culture Technologists. The purpose of this workshop is to revue the main technological advancements in the field of cell culture-based manufacturing of viral vectors including:

- Adenovirus and Adeno-Associated viruses
- Lentiviruses and retroviruses
- Baculoviruses and other enveloped vectors
- Other vectors for vaccination

This workshop will address novel approaches in upstream and downstream processing as well as critical developments in quantification of viral particles, and process intensification. Short presentations will be delivered by experts from academia and industry to inform the cell culture community about key advancements in the field of viral vector manufacturing. We also wish to engage the audience in discussing the remaining challenges that cell culture technology can address in further advancements in Gene and Cell Therapy and Novel Vaccine development.

Speakers :

Amine Kamen, BRI-NRC, CA	Large scale transfection for viral vector manufactoring
Otto Merten, Genethon, France	Progress in lentiviral vector processing
Andrew Bakker, AMT Biopharma The Netherlands	AAV manufacturing using baculovirus systems
Udo Reichl, Max Planck Inst. DE	Cell culture derived influenza virus processing
Majid Mehtali, VIVALIS, France	New cell line for viral vaccine manufacturing

Workshop B: CHO Genome Workshop

10:15–13:45 Festsaal / Main Lecture Hall

Chairs: *Mike Betenbaugh, Johns Hopkins University, USA*
Kelvin H. Lee, University of Delaware, USA
Nicole Borth, BOKU University, Austria

Speakers :

10:15	B. Palsson, UCSD	The genomic sequence of the CHO K1 cell line
10:35	B. Loo, a* star, Singapore	The Chinese Hamster Genome: A foundation for CHO Genome engineering
10:55	N. Borth, BOKU, Austria	A draft Chinese Hamster Genome using a combination of Roche 454 and Illumina sequencing
11:15	Discussion	
11:45	*Break and Lunch*	
12:15	T. Omasa, Tokushima Univ., JP	Chromosome identification and its application in CHO cells
12:30	I. Famili, GT Life Sciences, USA	A genome scale metabolic model for CHO
12:45	C. Clarke, DCU, Ireland	Predicting productivity in CHO production cells
13:00	R. Setterquist, Life Techn., USA	Comparative analysis of CHO transcriptional dynamics under different culture conditions using NGS
13:15	Discussion and next steps	

Members of www.CHOgenome.org will meet at 14:15 in Künstlerzimmer

THE GENOMIC SEQUENCE OF THE CHINESE HAMSTER OVARY (CHO) K1 CELL LINE

Palsson, B.O.[1], Xu, X.[2], Nagarajan, H.[1], Liu, X.[2], Lewis, N.E.[1], Pan, S.K.[2], Chen, W.[2], Wang, J.[2]
[1] *University of California, San Diego, La Jolla, USA* [2] *BGI, Shenzhen, China*

We have recently sequenced the CHO-K1 ancestral cell line using next-generation sequencing technology. Our assembly has 24,383 genes with 29,291 transcripts. In addition to a global comparative analysis of this genome with respect to human, mouse and rat genomes, we also present an analysis of genes involved in protein glycosylation. Most human and mouse genes involved in protein glycosylation are identified. However, genes for several potentially immunogenic glycosylation pathways are not expressed. We also investigate the genomic basis for CHO's resistance to viral infection. The availability of this genomic sequence will usher in an era of genome-scale science for CHO-produced biopharmaceuticals and allow for improved bioprocess optimization.

THE CHINESE HAMSTER GENOME: A FOUNDATION FOR CHO GENOME ENGINEERING

Loo, B.[1*], Jacob, N.M.[2*], Yusufi, F.N.K.[1*], Lee, T.S.[1*], Chin, J.X.[1*], Johnson, K.C.[2], Ramaraj, T.[4], Crow, J.A.[4], Mudge, J.[4], Woodward, J.E.[4], Bharti, A.K.[4], Farmer, A.D.[4], Retzel, E.F.[4], Gao, S.[5], Sung, W.-K.[5], Nagarajan, N.[5], Ruan, X.[5], Ruan, Y.[5], Karypis, G.[3], Lee, DY.[1], Yap, M.[1], Hu, W.S.[2]
[1] *Bioprocessing Technology Institute, A-STAR, Singapore*
[2] *Dep. Chemical Engineering and Materials Science, U Minnesota, Minneapolis, MN, USA*
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[4] *National Center for Genomic Resources, Santa Fe, NM, USA*
[5] *Genome Institute of Singapore, A-STAR, Singapore* * *Equal contributors*

The Chinese hamster is arguably the economically most important industrial organism. The availability of a well-annotated Chinese hamster genome will open up many new opportunities for cell engineering and metabolic intervention for process enhancement. We employed high throughput sequencing technologies and completed high coverage deep sequencing of an inbred laboratory female Chinese hamster. The genome sequence data was complemented by ~80,000 long EST sequences from traditional Sanger sequencing as well as transcript contigs obtained through deep coverage of RNA-seq

data. As current approaches for assembly of high throughput sequencing reads are still underdeveloped, to minimize assembly errors, three independent assemblies were completed along with an EST based extension into non-coding genome region before scaffolding. Integration of the assemblies from the four independent approaches resulted in a draft genome of ~2.5 Gb, with more than 1.7Gb in specific hamster chromosomes. The annotation was aided by a synergistic integration of a CHO EST collection with more than 13,000 genes annotated with high confidence. Those genes span a range of functional classes. Comparative analysis of the Chinese Hamster genome with the mouse genome permitted the examination of sequence orthology and the expansion of a draft hamster-mouse synteny map. This diploid hamster reference genome will greatly enhance our ability to examine the genetic variation underlying the development of desirable traits in CHO cells. The availability and application of these genomic resources will facilitate fundamental studies employing CHO cells as well as enable the engineering of CHO at a genomic scale.

CHROMOSOME IDENTIFICATION AND ITS APPLICATION IN CHINESE HAMSTER OVARY CELLS

Omasa,T. [1,2],, Cao, Y.[1], Kimura, S.[1], Park, J.-Y.[1], Yamatani, M.[1], Honda K.[1], Ohtake H.[1]
[1] *Graduate School of Engineering, Osaka University, 2-1 Yamadaoka, Suita, Osaka, Japan*
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Chinese hamster ovary (CHO) cells are the most dependable host cells for the industrial production of therapeutic proteins. At present, CHO cells are the most important "industrial mammalian cell line", similarly to *Escherichia coli* or *Saccharomyces cerevisiae* cells, among various mammalian cell lines (1). Recently, we constructed CHO genomic bacterial artificial chromosome (BAC) library from a mouse dihydrofolate reductase (DHFR) gene-amplified CHO DR1000L-4N cell line for genome-wide analysis of CHO cell lines (2). The CHO BAC library consisted of 122,281 clones and was expected to cover the entire CHO genome five times.

A CHO chromosomal map was constructed by fluorescence *in situ* hybridization (FISH) imaging using BAC clones as hybridization probes (BAC-FISH). More than 300 BAC clones were mapped on chromosome ideogram. Thirteen BAC-FISH marker clones were necessary to identify all 20 individual chromosomes in a DHFR-deficient CHO DG44 cell line because of the aneuploidy of the cell line. The chromosome homology map showed that a large scale chromosome rearrangement had been occurred in CHO-DG44 cell line. In order to determine the genomic structure of the exogenous *Dhfr* amplicon, a 165-kb DNA region containing exogenous *Dhfr* was cloned from the BAC library using high-density-replica (HDR) filters and Southern blot analysis. The nucleotide sequence analysis revealed a novel genomic structure in which the vector sequence containing *Dhfr* was sandwiched by long inverted sequences of the CHO genome. To investigate the effect of the palindrome structure derived from the BAC clone Cg0031N14 on *Dhfr* amplification in CHO cells, we constructed plasmids that contain part or the whole junction region of the palindrome structure. The transfected CHO DG44 cells containing part or the whole junction region of the palindrome structure could adapt quickly to high methotrexate (MTX) concentrations.

References

1. T.Omasa, M.Onitsuka, W.-D. Kim, Curr. Pharm. Biotechnol. 11 (2010) 233.
2. T.Omasa *et al.*, Biotechnology and Bioengineering , 104 (2009)986.

A GENOME-SCALE METABOLIC MODEL FOR CHO

Famili, I.[1], Usaite, R.[1,*], Feist, A.M.[2,*], Velasco, A.M.[1], Rosenbloom, J.D.[1]
[1] *GT Life Sciences, Inc., San Diego, USA*
[2] *Currently at University of California, San Diego, La Jolla, USA*
[*] *Authors contributed equally*

In a joint collaboration with BGI, we have recently completed sequencing and annotating the CHO genome. The availability of this information is now bringing genome-scale science to CHO-based production of biopharmaceuticals. Using our proprietary CHOmics platform and genome-scale model of CHO metabolism, we have successfully developed strategies for media optimization and experimentally shown significant increase in product titers and decrease in byproduct accumulation. This modeling approach has also been successfully used to identify metabolically advantageous selectable markers in CHO cells. Genetically engineered CHO cell lines expressing these novel metabolic markers showed higher integrated viable cell density and lower peak byproduct concentrations compared with the parental cell lines. These results demonstrate that metabolic modeling combined with genome-scale technologies can significantly improve and accelerate research, discovery, and development of therapeutic proteins in mammalian cell lines.

PREDICTING PRODUCTIVITY IN CHINESE HAMSTER OVARY PRODUCTION CELLS

Doolan, P.[1], **Clarke, C.[1]**, Barron, N.[1], Meleady, P.[1], O'Sullivan, F.[1], Gammell, P.[2], Melville, M.[3], Leonard, M.[3], Clynes, M.[1]
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[3] *Bioprocess R&D, Pfizer Inc., Andover, MA 01810, USA*

Improving the rate of recombinant protein production in Chinese hamster ovary (CHO) cells is an important consideration in controlling the cost of biopharmaceuticals. However, the molecular factors and complex pathways underpinning productivity in CHO are poorly understood. Here we present the first predictive model of productivity in CHO bioprocess culture based on gene expression profiles. We utilised a supervised regression algorithm, partial least squares (PLS), to produce a predictive model of cell-specific productivity (Qp) derived from transcriptomic data on stationary phase, temperature-shifted CHO production cell line samples. The final model was capable of accurately predicting Qp to within 4.44 pg/cell/day root mean squared error in cross model validation ($RMSE^{CMV}$) with a $Q^{2,CMV}$ of 0.72. To incorporate additional validation of the model, we utilised 10 unseen samples with a similar range of Qp values as an independent testing set, generating a $RMSE^{prediction}$ of 3.11 pg protein/cell/day. Several of the genes constituting the model are linked with biological processes relevant to protein metabolism and some have been identified to be differentially expressed in previous CHO and NS0 bioprocess profiling studies specifically examining productivity. Additionally, three of the selected genes have been demonstrated to functionally impact productivity in bioprocess culture. Additional preliminary siRNA functional screening results demonstrating that some of the selected genes exhibit a functional impact on productivity in CHO will be presented.

COMPARATIVE ANALYSIS OF CHO CELL TRANSCRIPTIONAL DYNAMICS UNDER DIFFERENT CELL CULTURE CONDITIONS USING NEXT GENERATION RNA-SEQUENCING TECHNOLOGY

Setterquist, R.[2], Gorfien, S.[1], Donahue-Hjelle, L.[1], Wang, S-Y.[1], Liu, M.[1], Schageman, J.[2]

[1] *Life Technologies, Grand Island, NY, USA*
[2] *Life Technologies Austin Texas, USA.*

Next Generation RNA-Sequencing (RNA-Seq) is a methodology for comprehensive measurements of cellular transcription at a scale, accuracy and precision never seen with previous technologies. We conducted a comparative RNA-Seq study of basic cell growth conditions to understand and improve high quality therapeutic protein production in CHO cells. Whole transcriptome analysis is a pre-requisite for full understanding of cellular processes that govern efficient bioproduction. In this study we examined global changes in gene expression in CHO cells under common bioproduction parameters. Eight CHO RNA samples from different cultures were sequenced on two full slides of a SOLiD™ System resulting in approximately 850 million sequence reads. These reads were mapped to multiple references including CHO EST, recently sequenced CHO genome content, as well as known annotated mouse reference transcripts. From this we report estimates of transcript expression levels and use known annotation to infer functional differences that can be associated with changing basic bioproduction growth conditions. These findings may uncover novel genetic mechanisms that could be optimized for improved bioproduction. This analysis of this data set represents the characterization of the CHO transcriptome in an unprecedented way.

Workshop C: FC Fusion Proteins: A growing Class of Therapeutics

14:00–15:45 Gartensaal, Ground Floor
Chair: *Steven Chamow, S & J Chamow, Inc., USA*

The potential therapeutic value of many proteins—including enzymes, cell-surface receptors, cytokines and peptides—can be realized by fusing these proteins to the Fc region of human immunoglobulin G. Of the 30 mAb products approved as human therapeutics in the USA to date, 4 are Fc fusion proteins, and many more are in clinical testing. Considerations in fusion protein design and production will be presented.

Speakers:
Pierre-Alain Girod, Selexis
Thomas Ryll, Biogen Idec
Michiel Ultee, Laureate Pharma
Kevin Bailey, Regeneron

Workshop 1: Massively Parallel Sequencing (MP-Seq)

8:30-10:00 Geheime Ratsstube

Organised by

MASSIVELY PARALLEL SEQUENCING (MP-SEQ): A NEW TOOL FOR ADVENTITIOUS AGENT DETECTION AND VIRUS DISCOVERY

David Onions, Bioreliance

Recent contaminations of manufacturing processes by porcine circovirus and vesiviruses have highlighted the need for broadly based and rapid methods to detect adventitious agents in cell banks, virus seeds and bulk product (drug substance). Massively parallel sequencing (MP-Seq) is a powerful new method for the identification of viruses and other adventitious agents, without prior knowledge of the nature of the agent. BioReliance have developed MP-Seq methods to detect free viruses in raw materials and fermenter samples. Our application of this technology has resulted in the discovery of a new parvovirus in bovine serum capable of infecting human cells and we have used this technology in the investigation of fermenter contaminations. In some cells, the genomes of latent or transforming viruses may be present in a cell but no virus particles are produced. However, latency associated or transforming gene mRNAs are expressed. We have developed a method to identify these latent viruses by sequencing the total transcriptome of the cell and applying an algorithm to identify the viral specific transcripts. Enormous amounts of data (~400Mb) are generated in this process and a robust algorithmic process is required to analyse the data. Using this method we have been able to identify a new retrovirus expressed in Vero cells and we have identified nodavirus and errantivrius contamination of insect cells. The definitive nature of the methodology provides considerable reassurance that cell banks are free of unexpected contaminating agents.

We are also developing MP-Seq to provide rapid end points in *in vitro* virus detection assays. We have shown that we can detect virus infection as early as day 4 post infection with MP-Seq while conventional methods may require 14 or 28 days to reveal infection.

Reference: Onions D Kolman JMassively parallel sequencing, a new method for detecting adventitious agents. Biologicals. 2010 377-80. 2010

APPLICATION OF MASSIVELY PARALLEL SEQUENCING (MP-SEQ) TO IDENTIFY HIGH PRODUCING CLONES AND VALIDATE THE GENETIC STABILITY OF EXPRESSION CONSTRUCTS

John Kolman, Bioreliance

Application of Massively Parallel Sequencing (MP-Seq) methods to the analysis of genetic stability of cells has resulted in a quantum change in the quality of data. The complete sequence of the construct genes are obtained with extraordinarily high coverage. In addition the flanking sequences of inserts and the concatamer junction points are resolved. Sequencing of the transcripts also provides data not available from conventional Sanger sequencing where only a consensus sequence results. In contrast, MP-Seq provides the sequence of rare variants that may be present within the cell.

We have coupled MP-Seq approaches with Automated Spectral Karyotyping (SKY) methods to provide additional data on the genetic stability of the cell. SKY is capable of detecting fine translocations that can be missed by traditional karyotyping methods and can give warning of genetic instability that lies outside of the target construct. Perhaps the most exciting application of MP-Seq is in the developing field of clone selection. Recently, nucleotide arrays have been used to identify genes differentially expressed in high and low producing CHO cells and the data has been used to modify media to optimise production (Schaub et al 2010). MP-Seq provides additional data above that available from arrays. BioReliance has data sets on the transcriptome of multiple CHO clones and is developing pathway analysis tools to identify signatures associated with high producing cells.

Reference : Schaub et al Biotechnology & Bioengineering. 2010 Feb 1;105(2):431-8.

Workshop 2: Using a rational approach to develop cell culture manufacturing processes

8:30–10:00 Festsaal / Main Lecture Hall

Organised by

Dr. Tom Fletcher, Director of R&D Cell Culture

Successful upstream manufacturing processes for new biopharmaceutical products are dependent on two key parameters; the development of optimal cell culture media formulas (both growth media and feed solutions), and on the development of manufacturing protocols which result in consistent yields and product quality. Optimization of cell culture media formulas can be accomplished using a variety of approaches, each featuring certain strengths and weaknesses. Various methods and tools will be discussed and compared, including media library screening, component heat mapping, metabolic profiling, DoE optimization of components individually and as groups, and metabolomics, using data from actual case studies as examples. The most effective approach often involves combining several methods and tools chosen in combinations to fit particular project needs. The role of a media partner in supporting innovation during cell culture manufacturing process development will also be discussed.

Workshop 3: BD Mosaic™ MSC SF: A new, high performance serum free culture medium for the expansion of Mesenchymal Stem Cells

10:15–11:45 Geheime Ratsstube

Organised by

Speakers

James W. Brooks, BD Advanced Bioprocessing

Cell based therapeutics using Mesenchymal Stem Cells (MSC) are emerging for the treatment of a wide range of acute and degenerative human diseases. BD Mosaic™ MSC SF is a unique, high performance medium, which, through several key features, enhances MSC expansion with reduced culture time, labor, and medium requirements.

Workshop 4: Evolution of Risk Mitigation Requirements for Platform Expression Systems

10:15-11:45 Gartensaal, Ground Floor

Organised by

In order to continue to provide the industry with solutions for the future, SAFC as a member of Sigma Aldrich has ventured into new technology and is developing new media and feed formulations to support industrially relevant platform expression systems. Historically, a platform expression system that meets industrial standards must be animal component free, use chemically defined media and have a supply chain that is transparent and robust to ensure continuity of supply and minimal TSE/BSE transmission risk.

In addition to the known regulatory demands to minimize TSE/BSE risk, now there is a new requirement to decrease the risk of of drug therapeutics to both raw material adulteration (e.g. melamine etc.) and manufacturing shutdowns. Manufacturing interruptions due to virus contamination events (e.g. Genzyme, Genentech/Roche etc.) have gained increased awareness within the industry and regulatory agencies on how a viral contamination event can have a huge impact on patient access to critical therapeutics. As a result the industry has increased their focus on the transparency of the supply chain and put processes in place to build "layers of protection" that will systematically reduce the risk of all potential sources for viral contamination. This viral risk management strategy will start with cell line generation (e.g. ACF transfection systems) and continues through with treatment strategies for all raw materials that go into the upstream production processes where the risk of viral replication in a host cell line is the greatest.

SAFC has developed a strategic partnership with Sartorius Stedim Biotech and in coordination SAFC-SSB have evaluated and will present the 4 different strategies to reduce the viral contamination risk from the raw materials used in the fermentation processes. The strategies for viral risk reduction will address sourcing and analytical equivalence of raw materials. the application of viral filtration, ultra-violet radiation and pasteurization (HTST) as preventive measures for the treatment of raw materials in the fermentation process used in industrial biopharmaceutical production systems. As part of the talk, SAFC will introduce their strategies for RM sourcing and HTST treatment and Sartorius Stedim Biotech will give an update on Viral Filtration and UVC treatment for viral decontamination of raw materials.

Finally a case study will be presented by Amgen, a biopharmaceutical industry leader, on their experiences in implementing a virus risk mitigation strategy on their current platform expression system. A question and answer session will follow with a panel of industry experts.

Speakers

Kevin Kayser, SAFC
David Kolwyck, SAFC
Tjebbe van der Meer, Sartorius Stedim Biotech

Workshop 5: Towards a fully single use protein production facility

12:30-14:00 Geheime Ratsstube

Organised by

Christel Fenge, Sartorius Stedim Biotech

During the last decade the advent of larger scale single use process solutions have changed our approach to making proteins from cell cultures dramatically. Driven by a growing pipeline of biopharmaceutical drugs in development and the cost pressure that the Pharma industry is experiencing, more and more companies are adopting single use bioreactors up to and beyond the 1000L scale. A similar trend can be observed in downstream processing although probably less matured yet. Membrane adsorption lends itself to single use applications with the potential of combining cell removal, clarification and capture into one unit operation.
A number of companies have already taken the step and implemented hybrid or even single use production facilities for monoclonal antibodies or vaccine production. Saleability of the process and consistency of results from bench-top bioreactors used in process development and as scale-down models in process validation to commercial scale production bioreactors is a key prerequisite for successful drug development. Therefore, ideally the design of the single use bioreactors should be as close as possible to the full scale systems to reduce the number of variables that may influence product quality and process performance.
Finally, with all this different disposable solutions used in GMP production, not only the vendor – user relationship has changed but also the approach to validation of single use equipment such as bioreactor bags and containers for process fluids.

During the workshop we will discuss current status of single-use process solutions, challenges and future outlook. In order to stimulate a lively discussion the following discussion primers will be prepared.

Speakers

Thorsten Peuker, Sartorius Stedim Biotech	Current status of single use process solutions
Gerhard Greller, Sartorius Stedim Biotech	Characterisation and scale-up considerations of single-use bioreactors
Aziz Cayli, Cell Ca	High titer mAb production in single-use Bioreactors
Sybille Ebert, Rentschler Biotechnologie	DOE supported optimisation of membrane adsorption as a sapture step
Heike Frankl, Sartorius Stedim Biotech	Extractable and Leachable – a concern

Workshop 6: From molecules to market: PD solutions from Life Technologies

12:00-14:00 Gartensaal, Ground Floor

Organised by

Freedom™ CHO-S™ Platform: Gene to clone in 3-4 months achieving mAb titers of 1-3 g/L with no milestone or royalty payments

Peggy Lio and Volker Sandig

Stable cell line development is a critical phase of biotherapeutic development. While several CHO based platforms are widely marketed they are costly to access even for research use only evaluation. To enable everyone to perform stable cell line development we have developed a CHO-S™ based kitted platform that allows the end user to go from transfection to stable clone in 3-4 months. Only one scientist is needed for the optimized workflows that are included in the kit. All components necessary to complete the workflow are included. Key to the optimized workflow is how transfection, selection and cloning have been co-optimized. mAb titers achieved with these platforms are ~1 g/L for an un-optimized simple glucose fed batch process and up to 3 g/L when more complex nutrient feeds are employed. Research use rights are granted upon kit purchase. Commercial licensing is unprecedented and requires only a onetime fee.

Media and Feed Platforms: Control of cell function by nutrient and process integration

Steve Gorfien

Sustained growth of the biopharmaceutical market has created a need for high-titer processes to meet increasing demand and to reduce manufacturing costs. Advances in recombinant cell line engineering have resulted in high producing clones with high nutritional demands. Depletion of critical nutrients in a production process can limit the potential of these clones, causing reduced titers and resulting in inefficient, costly processes to compensate for media or feed deficiencies. Through integration of base and feed media development, we have addressed nutrient limitation issues in rCHO cultures and obtained substantial improvements in titer by sustaining specific productivity for extended periods of time. Such approaches are clearly effective, but are time consuming and are limited by the large number of possible component combinations and the need to integrate process parameters like pH, temperature, dissolved oxygen, agitation rate and time of feed addition with a balanced nutrient composition. We have applied high throughput (HT) tools that enable simultaneous evaluation of multiple nutrient and process variables in up to 420 simultaneous conditions. Case studies will be presented demonstrating the power of HT tools to evaluate broad design spaces making possible rapid creation of chemically defined processes with multi-fold increases in titers while maintaining final product quality.

Highly sensitive PCR based assays for In-Process testing: Rapid detection of cell culture contamination

Michael T. Brewer

The possibility of viral or Mycoplasma contamination of mammalian cell culture during large scale manufacturing of biological therapeutics has always been a concern in the industry. Because the traditional adventitious agent or Mycoplasma tests take considerable time, contaminant testing is generally only conducted during cell banking and following final harvest. We have recently developed Real-Time PCR based assays that allow for rapid, highly sensitive detection of Mycoplasma, Mouse Minute Virus (MMV) and Vesivirus 2117. These rapid assays allow for testing for the presence of these agents at multiple points during the cell culture manufacturing process, allowing for the earliest possible detection of a contamination event. The importance of rapid detection using molecular based testing has been further highlighted recently in public reports of viral contamination events that affected both product supply and product quality. We will review the assay designs, sensitivity, sample preparation and proprietary discriminatory positive/extraction controls developed for these assays during our presentation.

Workshop 7: Rethinking Media Supplementation: Identifying Bioactive Molecules that drive protein production

14:15–15:45 Festsaal / Main Lecture Hall

Organised by

Moderator

Elisabeth Dodson, BD Advanced Bioprocessing

Serum. Hydrolysates. Chemically-defined. Rethinking media supplementation requires the identification of molecules that drive growth and protein production. Biochemical deconstruction of hydrolysates coupled with high resolution analysis was used to identify these molecules. Using DoE, a chemically defined media supplementation has been developed to substitute for yeast extract peptone.

Workshop 8: How QbD is changing upstream bioprocessing design

14:15-15:45 Geheime Ratsstube

Organised by

This workshop session will focus on the evolving demands of quickly and accurately improving the quantitative output from bioprocessing expression systems, while also maintaining (or in some cases increasing) the qualitative bioactivity of the expressed molecule. Examples of regulatory directives and process control technologies interfacing with bench-level cell culture optimization will be shown.

How QbD is Changing Upstream Bioprocess Design

William G. Whitford, Sr. Manager, BioProcessing Market, Thermo Scientific Cell Culture & BioProcessing

In light of QbD, PAT, and process platform imperatives, bioproduction process design now requires capabilities and capacity beyond essential facilities and personnel. The goals of producing high levels of quantity product in a robust and flexible production process require capabilities beyond even subject matter experts with access to a repertoire of reference formulations. In-house technologies now demanded include a full complement of cell and culture media analytics and HTS capabilities. In most cases, some level of product quality and attribute assays beyond simple product level quantitation is highly recommended. Process optimization toward highly regulated manufacturing can also draw upon such capabilities as regulatory certifications, quality management systems, and qualified raw materials sourcing. Modern demands for increased process understanding, CPP determination, and robust design space development virtually require access to qualified scale-up and technology transfer equipment and methods rather early on in process development. Increased demands in process implementation efficiency are best supported by early consideration and testing of appropriate product containment and transfer technologies. The case-studies presented here illustrating such approaches are the fastest way to communicate the art and science of modern process design.

Fast-track process development using commercial feed solutions

Karlheinz Landauer, Director, R&D, Celonic AG, Basel

New biological entities, NBEs, as well as biosimilar development have high demands in rapid process development leading in high yielding, and robust processes. To achieve these necessities industry needs platform technologies for the development of high quality processes producing high quality recombinant products with stable features. The prerequisite of such platform technologies is the use of an excellent host cell line, which is safe in a regulatory point of view, proven to produce high amount of recombinant protein over many generations (stability) and works within a proven design space. Based on such cell lines media and feed-media development can also be based on a platform technology. Here the prerequisites are animal component free media and solutions; large scale availability, low costs and high lot to lot consistency. New industrial standard for production of recombinant proteins is the usage of chemically defined media. Albeit hydrolysates and peptones are still widely used, the issue of lot to lot variability and the dependency on the production process of a specific peptone and its features leads to a certain uncontrolled status within a production strategy. Therefore it is inevitable to control all substances used to produce the product, also “goodies” of conventional peptones, such as vitamins, fatty acids and trace elements. Media requirements for animal cells strongly depend on the recombinant product, the mode of fermentation and last but not least on the cell line itself. The slightest changes in the media composition may lead to changes in post-translational modifications. Such differences could change the behavior of a product in-vivo and may compromise timelines and whole development schedules. The use of chemically defined media in the processes are favorable and the future for biologics. In addition it helps to keep time lines and opens the possibility of a fast-track process development.

Session I: Cell Stability and Differentiation

Sunday May 15th, 2011

Chair: Thomas von Zglinicki, University of Newcastle, UK

16:15	F. d´Adda di Fagagno, IFOM, Italy	Molecular mechanisms of cellular Senescence
16:45	J. Grillari, BOKU, Austria	MicroRNA 31: an inhibitor of osteogenic differentiation of mesenchymal stem cells
17:15	T. May, HZI Braunschweig, DE	Immortalised Endothelial Cell Lines
17:35	M. Udono, Kyushu University, JP	Senescence-associated genes and their Implication in the induction of cellular senescence

MOLECULAR MECHANISMS OF CELLULAR SENESCENCE

d'Adda di Fagagna, F.[1]
[1]*IFOM Foundation – FIRC Institute of Molecular Oncology Foundation, via Adamello 16, 20139 Milan, Italy*

Early tumorigenesis is associated with the engagement of the DNA-damage checkpoint response (DDR). Cell proliferation and transformation induced by oncogene activation are restrained by cellular senescence. We have previously shown that expression of an activated oncogene in cultured normal human cells results in a permanent cell-cycle arrest caused by the activation of a robust DDR. Experimental inactivation of DDR abrogates senescence and promotes cell transformation. Oncogene-induced senescence is also associated with a global heterochromatinization of nuclear DNA. Our most recent results on the interplay between DDR and heterochromatin formation, the differential repair of the human genome, the regulation of DDR in stem cells and our search for novel pathways regulating genome stability will be discussed.

MICRORNA-31 IS SECRETED BY SENESCENT ENDOTHELIAL CELLS AND INHIBITS OSTEOGENIC DIFFERENTIATION OF MESENCHYMAL STEM CELLS

Grillari, J.[1,6],
Schraml, E.[1], Wieser, M.[1,5], Fortschegger, K.[1], Messner, P.[2], Maier, A.[3], Westendorp, R:[3], Wolbank, S.[4], Redl, H.[4], Grillari-Voglauer, R.[1,6]
[1] *Dep. Biotechnology, University of Natural Resources and Life Sciences Vienna, Austria*
[2] *Dep. Nanobiotechnology, University of Natural Resources and Life Sciences Vienna, Austria*
[3] *Leiden University Medical Center, Leiden, The Netherlands*
[4] *LBI for Traumatology, Vienna, Austria*
[5] *Austrian Center of Industrial Biotechnology (ACIB), Vienna, Austria*
[6] *Evercyte GmbH, Austria*

One major driving force of aging is the struggle between the accumulation of damage in cells and tissues and the counteracting repair systems of organisms. Stem and progenitor cells range among such repair systems and recently their functionality has been found to depend on the age of the systemic environment, as factors contained in young blood increase the functionality of muscle stem cells, indicating a negative influence of the old systemic environment. In order to address which molecules might be the factors contained in the old blood serum and what might be the source of such factors, we tested the effects of the senescence associated secretory phenotype (SASP) of young versus senescent human umbilical vein endothelial cells on mesenchymal stem cells.
Recently, miRNAs have been found in human serum. In order to test, if miRNA levels in blood might be dependent on age, we tested if miR-31 is enriched in the cellular supernatants of senescent versus early passage cells, and is packaged into exosomes as visualized by electron-microscopy in-situ-hybridization. Furthermore, we found that miR-31 from these vesicles is taken up by mesenchymal stem cells and inhibits osteogenic differentiation via downregulation of its target FZD3. Finally, analysis of blood samples from young and elderly healthy individuals shows a strong variability of miR-31 levels in older age, indicating that it might be used as a biomarker of aging and age-associated disease, but might also represent a therapeutic target whenever osteogenesis is a limiting factor.

IMMORTALIZED ENDOTHELIAL CELL LINES

May T.
Schucht, R.[1,2], Schuller, F.[1], Zauers, J.[1,2], Wirth, D.[1]
[1] *Helmholtz Centre for Infection Research, Braunschweig, Germany*
[2] *InSCREENeX GmbH, Germany*

Endothelial cells are specialized cells that line blood vessels. They control blood pressure, form a barrier between vessel and surrounding tissue, and are involved in inflammation processes and blood vessel formation. Dysfunction of the endothelium is observed in diabetes, hypertension, cancer and coronary artery diseases. For the efficient development of novel drugs and treatments an endothelial cell system is highly desirable which closely reflects the *in vivo* properties of endothelial cells.
We have identified a set of immortalizing genes that allows the amplification of primary endothelial cells while the functions of the originating cells are fully retained.
Primary endothelial cells from the umbilical cord and from the skin were transduced with these immortalizing genes which led to the establishment of cell lines with a robust proliferation phenotype. The resulting cell lines were cultivated for more than nine months which corresponds to more than 120 cumulative population doublings. An in-depth characterization of these cell lines was performed side-by-side with primary cells and demonstrated that the established cell lines retained the expression of endothelial specific marker proteins as well as endothelial specific functions. Importantly, this phenotype was stable throughout the whole cultivation period.
We regard these novel endothelial cell lines as physiological relevant *in vitro* test systems which can greatly support the drug discovery process.

IDENTIFICATION OF SENESCENCE-ASSOCIATED GENES AND THEIR IMPLICATION IN THE INDUCTION OF CELLULAR SENSCENCE

Udono, M.[1],
Katakura, Y.[2]
[1] *Graduate School of Bioresource and Bioenvironmental Sciences, Kyushu University, Fukuoka, Japan*
[2] *Faculty of Agriculture, Kyushu University, Fukuoka, Japan*

Purpose

In the present study, we used human diploid fibroblast cells (TIG-1) as model cell lines. Replicative senescence was induced in TIG-1 cells by serial passagings. Membrane proteins from young and replicatively senesced TIG-1 cells (old TIG-1 cells) were subjected to 1D and 2D electrophoresis. Bands and spots for proteins whose expressions were changed between young and old TIG-1 cells were excised and subjected to MALDI TOF-MS analysis after in-gel digestion. We identified 16 candidates for senescence-associated gene. We investigated the expression profiles of these genes in replicative senescence by quantitative real-time PCR, and identified 14 genes as senescence-associated genes (SAGs). Here, we tried to evaluate contributions of these SAGs to cellular senescence program.

Materials and Methods

Knockdown and overexpression of SAGs in TIG-1 cells were performed by using retrovirus gene expression system. Senescence-associated □-galactosidase (SA-□-Gal) activity and intracellular level of reactive oxygen species (ROS) was measured by using imaging cytometer, IN Cell Analyzer 1000.

Results

Firstly, we established 6 cell lines that stably reduce the expression of SAGs by using shRNA. Some of cell lines showed vigorous proliferation as compared to control. Next we established 6 cell lines that stably express SAGs and analyzed their cell phenotypes by using IN Cell Analyzer 1000. Results showed that several cell lines expressing SAGs decreased proliferative potential, increased cellular/nuclear size, augmented SA-□-Gal activity and intracellular ROS level.

Discussion

We carried out knockdown and overexpression experiment on SAGs. Knockdown experiment indicated that two SAGs play an indispensable role in the induction of replicative senescence. One SAG was found to be important for the induction of SA-□-Gal activity, but not for the growth retardation, suggesting that induction of SA-□-Gal activity and growth retardation at the cellular senescence stage would be separable, and differently regulated.

Next we overexpressed SAGs in TIG-1 cells, and analyzed the profiles by using IN Cell Analyzer 1000. Results showed that almost all of SAGs except for p16 induced temporal growth retardation in TIG-1 cells, but these cells resume vigorous growing during the long-term culture. These results suggest that these SAGs have abilities to induce cellular senescence, but do not have abilities to maintain cellular senescence state. We are now trying to identify the molecular mechanisms and factors required for the permanent induction of cellular senescence state.

Keynote I:

The antibody revolution

Gregory Winter,
MRC Laboratory of Molecular Biology,
Cambridge, UK

THE ANTIBODY REVOLUTION

Winter, G.
MRC Laboratory of Molecular Biology, Cambridge, UK

In recent years there has been a revolution in the pharmaceutical industry; antibodies are displacing small molecule drugs. There are already six antibodies in the top 20 best-selling pharmaceutical drugs, and in 2014 it is predicted that there will be six antibodies in the top ten, with the first three slots occupied by antibodies. I will trace the technology that led to this revolution, and attempt to predict where the antibody revolution will go next, with the opportunities for science, medicine and commerce.

Session II: Cells as Therapies and Stem Cells

Monday May 16th, 2011

Chairs: Josef Friedl, University of Medicine Vienna, Austria
Hansjörg Hauser, HZI Braunschweig, Germany

09:05	N. Chaput, Inst. G. Roussy, France	Critical role of IFNg for the production of Highly immunogenic dendritic cell-derived Exosomes
09:35	I. Reischl, AGES Pharmed, Austria	ATMPs- The regulatory perspective
10:05	L. Romagnoli, Areta Int., Italy	Cell based medicinal product: development of GMP-compliant processes and manufacturing
10:25		*Coffee Break*
11:00	N. Timmins, U Queensland, AU	Ultra high yield manufacture of red blood cells in fully defined animal component free medium
11:20	M. Esteban, Chinese Academy of Sciences, China	Road to the clinics and in vitro disease modelling using iPSCs: Wilson's disease
11:50	C. Ramsborg, Dendreon, USA	Manufacturing process optimisation Strategies for autologous active cellular immunotherapies
12:20		*Poster Session A and Lunch*
12:20		***ESACT GENERAL ASSEMBLY***

CRITICAL ROLE OF IFN*G* FOR THE PRODUCTION OF HIGHLY IMMUNOGENIC DENDRITIC CELL-DERIVED EXOSOMES

Chaput, N.[2,3,4]
Viaud, S.[1,4], Théry, C.[2,5], Ploix, S.[2,4], Lapierre, V.[3,4], Lantz, O.[2,5], Zitvogel, L.[1,2,4]
[1] *INSERM U1015*
[2] *Centre of Clinical Investigation in Biotherapy, CIC BT 507*
[3] *Cellular Therapy Unit*
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Dendritic cell-derived exosomes (Dex) are nanovesicles promoting T cell-dependent antitumor effects in mice. Two phase I clinical trials aimed at vaccinating cancer patients with peptide pulsed-Dex demonstrated the feasibility and safety of inoculating clinical grade Dex but failed to show their immunizing capacity. These low immunogenic capacities have led us to develop strategies to boost Dex immunity. First strategy was to administrate Dex along with Toll Like Receptor (TLR) agonists, second strategy was to combine Dex with drugs that could decrease tumor-induced tolerance and third strategy was to produce Dex from mature Dendritic cells (DC). Preclinical studies demonstrated that these three strategies allowed enhancing immunity of Dex. These results prompted us to develop a second generation Dex with enhanced immuno-stimulatory properties for clinical implementation. Metronomic cyclophosphamide (CTX) was identified as capable of inhibiting regulatory T cells leading to restoration of T cell proliferation and NK cell cytotoxicity. Moreover, CTX was showed to enhance Dex immunity in preclinical models. Furthermore, we showed that IFNg is a key cytokine conditioning DC to induce the expression of CD40, CD80, CD86 and CD54 on gDex, endowing them with direct and potent peptide-dependent CD8+ T cell activation. Thus, recombinant IFNg represents a suitable MD-DC maturation agent for the production of human clinical grade Dex. We validated a new process in accordance with good manufacturing practices (GMP) for the vaccination of cancer patients. We will describe the Dex² clinical trial, currently ongoing at Gustave Roussy and Curie institutes, aiming at vaccinating advanced and inoperable non small cell lung cancer patients with gDex.

ATMPS – THE REGULATORY PERSPECTIVE

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The Regulation for Advanced Therapies (REG/2007/1394/EC) has come into force in December 2008 and lays down specific rules concerning the authorisation, supervision and pharmacovigilance of advanced therapy medicinal products (ATMPs). It is a *lex spezialis*, expanding on Directive 2001/83/EC with the intent to provide a framework, guidance and harmonization for the specialized emerging field of biomedicine. REG/2007/1394/EC further includes the legal definition of tissue engineered products, complementing existing definitions for somatic cell therapy and gene therapy which are found in Annex I of DIR/2001/83/EC.
To ensure the harmonization amongst the member states and to concentrate expertise, the Committee for Advanced Therapies (CAT) was established at the European Medicines Agency (EMA). The CAT is not only involved in licensing applications for ATMPs but also in the classification of ATMPs, their certification and scientific advice procedures. This committee is further the driving force in the provision of scientific guidance to facilitate and foster the regulatory development ATMPs
The underlying principles for the regulation of ATMPs and their practical translation from donation to administration to patients will be outlined in the presentation.

CELL-BASED MEDICINAL PRODUCTS REQUIRE A UNIQUE APPROACH FOR THE DEVELOPMENT OF GMP-COMPLIANT PROCESSES AND MANUFACTURING OF SAFE AND HIGH-QUALITY PRODUCTS.

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When performing the GMP process development and scale up of cellular therapies, a critical review of the manufacturing process and all the materials and reagents involved in the production steps is the mandatory starting point to avoid potential issues related to the quality and safety of the product.
The choice of the raw materials and all components such as plastics and other equipment that comes into direct contact with the product must be performed always keeping in mind that the cells as drug products cannot be terminally sterilized. The quality of the materials and reagents utilized is therefore directly related to the quality and degree of purity of the final product. Information about the available certification must be gathered for every component and, for critical materials, audits must be performed to the manufacturing sites to qualify the supplier.
The protocol used for the cell expansion and processing (if necessary) must be designed trying to reduce at a minimum the dependance on growth factors and medium suppments. Each additional component that is added to the culture medium must be justified and its absence from the final product must be validated. Residues that are not removed during the production process must be accurately measured and limits must be set after performing a risk evaluation analysis, to ensure that they have no adverse effects on the patient. Supplements such as FBS are still allowed for the manufacturing of cellular therapies, as long as the serum is sourced from a TSE-free area. Anyway, the choice of a medium with FBS must be done only in the absence of efficient alternatives. In this case, continuos research and development is strongly advised at the laboratory level in order to keep up to date with the latest developments in medium formulations, being ready to switch to an animal-free medium as soon as it is feasible. The reduction of growth factors and supplements is also important in order to control the manufacturing costs of a cell therapy. An evaluation of the economical aspects and market sustainability should be performed at an early stage if an industrial development of the cellular product is desired.
The manipulation steps performed during manufacturing stage should be kept to a minimum, in order to reduce the human intervention and the possibility of contaminations. Media fill simulations must be performed in purposely stressed conditions to ensure that the process and the facility are able to support the production of a sterile product.
When manufacturing patient-specific therapies, extensive efforts should be directed towards the reduction in the variability of the starting material, that is usually a tissue sampled from the patient during hospitalization. Working with well-defined starting material allows for the set-up of a more robust process with comparable characteristics between batches dedicated to different patients. The specifications of the final product for parameters such as cell number, purity and potency must be wide enough to tolerate the normal biological variability of living organisms, but sufficiently narrowed down to generate comparable batches of drug. This uniformity is mandatory for the set up of clinical trials aiming at gathering a reliable analysis of the safety, tolerability and efficacy data obtained from treated patients, in order to speed up the clinical development of innovative medicinal products such as cellular therapies.

ULTRA HIGH YIELD MANUFACTURE OF RED BLOOD CELLS IN FULLY DEFINED ANIMAL COMPONENT FREE MEDIUM

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Günther, M.[1], Nielsen, L.K.[1]
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Provision of a safe and secure supply of transfusible red blood cells (RBC) is a major global health challenge. It has been proposed that manufactured RBC could help to alleviate the constraints of the current donor system. While it has been demonstrated that, in principle, up to 5 units of RBC could be manufactured from a single umbilical cord blood (UCB) donation, this is insufficient for routine clinical use and the laboratory scale methods employed are incapable of producing the large numbers of cells required.
We have developed a simple feeder-free RBC manufacturing process capable of producing over 500 units of RBC per UCB donation. Culture generated cells undergo terminal differentiation and enucleation at frequencies of > 90%, with a mean cell hemoglobin content (30.8 pg/cell) in the range of normal donor blood (27 - 33 pg/cell). This process has been successfully adapted for scale-up in wave-type bioreactor systems. Using recombinant transferrin and albumin products produced and supplied by Novozymes Biopharma, we have eliminated animal and donor derived medium components. Cell yields in fully defined media were equivalent to those using our standard formulation.
In simultaneously addressing the limited cell yields obtained by existing methods, use of feeder-layers, the limited capacity of manual flask-based cell culture operations, and the use of animal derived medium components, our process represents a major step forward in the direction of industrialized RBC manufacture.

ROAD TO THE CLINICS AND IN VITRO DISEASE MODELLING USING iPSCS: WILSON'S DISEASE

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The generation of induced pluripotent stem cells (iPSCs) by nuclear reprogramming with exogenous factors of somatic cells is an outstanding discovery with implications at all levels. Many caveats need to be solved before we realize the potential of this technology but given the pace of research this seems likely. Among other things directed hepatocyte differentiation from human iPSCs provides a unique platform for modeling liver genetic diseases, drug toxicity screening, and potentially also autologous transplantation therapies. Wilson's disease is a relevant human genetic liver disease caused by mutations in the ATP7B gene, whose product is a liver enzyme responsible for copper export into bile and blood. Interestingly, the spectrum of ATP7B mutations is vast and can influence clinical presentation (a variable spectrum of hepatic and neural manifestations), though the mechanism is not well understood. Here we describe the successful generation of iPSCs from a Chinese patient with Wilson's disease that bears the R778L Chinese hotspot mutation in the ATP7B gene. These iPSCs were pluripotent and could be readily differentiated into hepatocyte-like cells that display abnormal cytoplasmic localization of mutated ATP7B and defective copper transport. Importantly, gene correction using a self-inactivating lentiviral vector that expresses codon optimized-ATP7B or treatment with the chaperone drug curcumin could reverse the functional defect in vitro. Our work thus describes an attractive model for studying the pathogenesis of Wilson's disease that could also be valuable for screening compounds or gene therapy approaches aimed to correct the abnormality. Once relevant safety concerns are solved, including the stability of a mature liver-like phenotype, hepatocyte-like cells from similarly genetically corrected iPSCs may be as well an option for autologous transplantation in Wilson's disease.

MANUFACTURING PROCESS OPTIMIZATION STRATEGIES FOR AUTOLOGOUS ACTIVE CELLULAR IMMUNOTHERAPIES (ACIS)

Ramsborg CG.[1]
Meagher; TC.[1], Chinn; J:[1], Wagener; F.[1], Khuu-Duong; K.[1], Emde; M.[1], Trager; JB.[1]
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Dendreon has developed a specialized manufacturing platform to develop active cellular immunotherapies (ACIs) that are designed to stimulate a patient's own immune system against cancer. On April 29, 2010, PROVENGE® (sipuleucel-T) was the first autologous cellular immunotherapy to receive FDA approval. PROVENGE is designed to be an ACI. PROVENGE is approved for the treatment of asymptomatic or minimally symptomatic metastatic castrate resistant (hormone refractory) prostate cancer. ACIs are designed to stimulate a T-cell response to cancer cells. An immune response is started by a specialized class of immune system cells called antigen-presenting cells (APCs). APCs take up antigen from their surroundings and process the antigen into fragments that are then displayed on the APC surface. Once displayed, these antigens can be recognized by specific classes of immune cells called T lymphocytes (T cells), which are activated as a result of their engagement with APCs and combat cancer by directly targeting antigen-bearing cells.

The development of an ACI manufacturing process has several unique challenges. First, the manufacturing process must be robust to the large patient-to-patient variability in both total cell number and cell composition. Second, component of variance analysis has shown that for some product quality attributes, significant process variability can be attributed to the patient-specific raw material. Third, ACI process characterization requires the quantitation of diverse assays, such as cell-based functional assays, multi-color flow cytometry and multi-plex enzyme-linked immunosorbent assays (ELISAs).

We have explored the utility of applying multifactor design-of-experiments (DOE) strategies using a small scale model paired with linear mixed models to optimize ACI ex vivo culture conditions. Small scale model qualification methodology as well as our observed scale-based bias will be discussed. In addition, studies using both cell culture engineering and immunological methods to delineate the effects of adjuvants, APC concentration, target antigen concentration and time on various response variables and product quality attributes such as APC activation, biological function and cytokine profile will be discussed.

As expectations for ACI process knowledge have grown, these process characterization and optimization methods have proven to be useful in the development of robust manufacturing processes on short clinical development timelines and represent a step toward implementing Quality-by-Design in ACI process development.

Session III: Cellular mechanisms of protein synthesis, processing and secretion

Monday May 16th, 2011

Chairs: Roy Jefferis, University of Birmingham, UK
Mike Butler, University of Manitoba, Canada

15:05	L. Hendershot, St.Jude´s Hospital, USA	Quality control of immunoglobulin biosynthesis: Life and death decisions
15:35	G. Warren, Max Perutz Labs, Austria	Golgi Biogenesis in a protozoan parasite
16:05		*Coffee Break*
16:20	D. Baycin Hizal, Johns Hopkins U, USA	Impact of glycosylation and sialylation on Ion channels and the nervous system
16:40	M. Laird, Genentech, USA	Antibody disulfide bond reduction in CHO Cell culture processes
16:50	R. Kshirsagar, Biogen Idec, USA	Impact of process conditions on consistency of trisulfide vs native disulfide linkages in an IgG1 molecule
17:00	N. Lin, SAFC, USA	Clonal Differences in high-mannose oligosaccharide content in recombinant CHO
17:20 - 20:00		*Postersession B and Drinks*

QUALITY CONTROL OF IMMUNOGLOBULIN BIOSYNTHESIS: LIFE AND DEATH DECISIONS

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The endoplasmic reticulum (ER) is the site of synthesis of antibodies that are either destined to be expressed on the cell surface or secreted. These proteins enter the ER through a protein channel as extended polypeptide chains and interact with a variety of molecular chaperones and folding enzymes that help them achieve their proper mature tertiary and quaternary structures, which in their simplest form requires the assembly of two heavy and two light chains. If successful, the antibody leaves the ER to travel to its appropriate destination outside the cell. If however, the nascent heavy or light chain fails to fold or assemble properly, it must be identified and targeted for retrotranslocation to the cytosol for degradation by the 26S proteasome, which involves some of the same molecular chaperones. There are a number of unresolved issues concerning how the ER quality control machinery can distinguish between proteins that have not yet folded and those that cannot fold, as it is expected that both types of proteins would have very similar features. In addition, the folding of many nascent proteins is dependent on the oxidizing environment of the ER, whereas the degradation of misfolded proteins often requires that those portions of the protein that have folded be reduced to allow them to pass through the retrotranslocon. Finally, any protein that is synthesized in the ER has the potential to misfold, arguing that the ubiquitin/proteasome system must be poised to recognize every type of secretory pathway produced in that cell. How such broad specificity is executed remains unclear. Progress from our lab on these issues and how this contributes to understanding how the ER orchestrates seemingly opposing functions will be presented.

GOLGI BIOGENESIS IN A PROTOZOAN PARASITE

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The Golgi processes the entire output of newly-assembled proteins from the endoplasmic reticulum, modifying the bound oligosaccharides then sorting them to their final destinations. As with all other cellular organelles, the Golgi undergoes duplication during the cell cycle and partitioning during mitosis, so as to ensure propagation through successive generations. The process of duplication - making another copy of the Golgi - has been difficult to study since many cells have several hundred Golgi, making it difficult to follow the appearance of new Golgi. We have solved this problem by focusing on protozoan parasites, many of which have only one Golgi that can be followed using XFP fluorescence technology. Through studying the Golgi in *Trypanosoma brucei* (the causative agent of sleeping sickness in man and Nagana in cattle), we have been able to tackle the mechanism that ensures duplication and partitioning of this organelle. Our studies to date highlight a particular role for a hooked bilobe structure that appears to regulate Golgi biogenesis as well as certain functions of the flagellar pocket.

IMPACT OF GLYCOSYLATION AND SIALYLATION ON ION CHANNELS AND THE NERVOUS SYSTEM

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Treatment of channelopathy diseases such as ataxia, paralysis, epilepsy, memory and learning loss, cardiac arrhythmia and cancer requires a broad understanding of ion channel functioning. Ion channels are membrane proteins that play critical roles in a number of cell functions including communication and neuromuscular activity. In this study, sialic acid was used as a model to understand the effect of biochemical environment on the electrophysiology of potassium channels which leads to changes in the neuronal system of organisms. Cell culture techniques, proteomics, electrophysiology and immunostaining methods were used as tools to prove the functional effects of glycosylation and sialylation on potassium channels in *in vitro* and *in vivo* studies. Both HEK 293 and *N*-acetylglycosaminyltransferase deficient HEK GNTI (-) cells were transfected with potassium channels and the sialylation effect on the action potentials were shown. After these achievements in *in vitro* systems, *Drosophila melanogaster* was used as a model to prove the sialylation effect on the neuronal system of an organism. The membrane proteins of wild type and sialic acid deficient flies were enriched by solid phase extraction of N-linked glycopeptides and sialylated-peptides (SPEG) methods. Mass spectrometry was performed to identify the glycosylated and sialylated glycoproteins of wild type and mutant flies. Furthermore, electrophysiology was performed to examine the role of glycosylation and sialylation on the neuromuscular system of *Drosophila*. In addition, the sialylation effect on the neuromuscular system of *Drosophila* was also observed physiologically.

ANTIBODY DISULFIDE BOND REDUCTION IN CHO CELL CULTURE PROCESSES

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In the biopharmaceutical industry, therapeutic monoclonal antibodies are primarily produced in mammalian cell culture systems. After initiating a production bioreactor, various process additions and parameter manipulations are performed to maximize growth and antibody production and yield suitable product quality. At the end of the production culture, the antibody product is typically separated from the cells using centrifugation and/or filtration. During the scale-up of a monoclonal antibody production process, we observed significant reduction of the antibody's disulfide bonds during the harvest operations. This antibody reduction event was catastrophic as the product failed to meet the drug substance specifications and the bulk product was lost. Subsequent laboratory studies demonstrated that cells subjected to mechanical shear during the harvest operations released cellular components that resulted in this antibody reduction phenomenon. Several methods to prevent this antibody reduction event were developed using a small-scale model. Some of these methods were implemented at manufacturing-scale and shown to successfully prevent the reduction of the antibody's disulfide bonds. The results of the small-scale reduction prevention studies and the translation into manufacturing processes will be discussed here as well as some of our understandings into the molecular mechanism of antibody disulfide bond reduction by endogenous intracellular enzymes.

EVALUATION OF IMPACT OF CELL CULTURE PROCESS CONDITIONS ON CONSISTENCY OF TRISULFIDE VS NATIVE DISULFIDES LINKAGES IN AN IgG_1 MOLECULE

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Molecular heterogeneity was detected in a recombinant IgG1 mAb and attributed to the presence of a protein trisulfide moiety. The predominant site of trisulfide modification was the bond between the heavy and light chains. Trisulfide bond formation has been recently observed in immunoglobulins (IgGs) produced in culture by mammalian cells. This molecular heterogeneity has no observable effect on antibody function based on in-house studies at Biogen Idec. Nevertheless, to minimize the heterogeneity of therapeutic monoclonal antibody (mAb) preparations, an understanding of impact of cell culture process conditions on trisulfide vs disulfide linkage formation is desirable.

Trisulfide levels in samples from 2000-L bioreactors using conditions developed at the 200 L scale showed consistency. To investigate variables that might impact trisulfide formation, a number of cell culture parameters such as cell density and feed strategies were varied in bench bioreactor studies. Trisulfide analysis of the samples from these runs revealed that the trisulfide content in the LC5-HC5 linkage varied considerably from <1% to 39%. Changes in culture conditions that seemed relatively minor and did not significantly affect growth and culture productivity resulted in large differences in trisulfide levels. In particular, culture duration and feeding strategy were important variables and product with reproducible trisulfide levels can be obtained by tightly controlling cell culture conditions. Therefore, trisulfide/disulfide heterogeneity may be eliminated from IgG1 molecules via control of cell culture process conditions.

USING INTACT PROTEIN ACCURATE MASS DATA AND DIFFERENTIAL GEL ELECTROPHORESIS (DIGE) TO ASSESS CLONAL DIFFERENCE IN HIGH-MANNOSE OLIGOSACCHARIDE CONTENT IN RECOMBINANT HUMANIZED IGG PRODUCING CHINESE HAMSTER OVARY (CHO) CELL LINES

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N-glycans structures of therapeutic glycoproteins are well known to affect bioactivity, immunogenicity and clearance rate. High-mannose oligosaccharide population (Man5 - Man9) indicates accumulation of high-mannose precursor and failure to form complex bi-antennary N-glycans in recombinant IgG production. The mechanism of Man5-9 formation remains poorly understood. The biopharmaceutical industry has set the goal to control and eliminate this population. To achieve this, we developed a high throughput analytical method using intact protein accurate mass data to perform glycoprofiling directly from culture supernatant. We selected 20 clonal CHO cell lines producing a humanized IgG to characterize the content of the Man5 population among total glycosylated IgG population. These lines were derived from a parental CHO cell line using Zinc-Finger Nuclease (ZFN) mediated gene knockout of Dihydrofolate Reductase (Dhfr). The cell lines demonstrated different % Man5 under a simple fed-batch culture condition in a proprietary chemically-defined culture media. Three of these cell lines (one with low Man5, two high Man5) demonstrated clone-dependent difference in Man5 that was reproducible under batch, simple-fed batch and low temperature (31℃) culture conditions. To further understand the genetic basis of such clonal difference, we carried out quantitative RT-PCR (qRT-PCR) studies to assess relative expression levels of seven genes that are critical in N-glycan chain extension and branching. Cells were also harvested for total protein extraction at mid- and late-exponential phases of culture, and DIGE studies were carried out to identify the differentially expressed genes between the three clones. These results are the first steps towards biomarker discovery for clonal differences in high-mannose oligosaccharide formation. The biomarkers will be used for early clonal screening and selection as well as cell engineering to eliminate high-mannose populations.

Session IV: Biopharmaceutics and Vaccine Production

Tuesday May 17th, 2011

Chairs: Otfried Kistner, Baxter Innovations, Austria
Hermann Katinger, BOKU University, Austria

09:05	M. Sliwkowski, Genetech, USA	Protein Pharmaceuticals: Future State
09:35	S. Rasmussen, Symphogen, Denmark	Recombinant antibody mixture: optimisation of cell line generation and single-batch manufacturing process
09:55	C. Kaisermayer, GE Healthcare	High yield antibody production using perfusion in a disposable WAVE bioreactor
10:15	I. Jordan, Probiogen, Germany	Highly efficient, chemically defined and fully scalable production of host-restricted pox viruses
10:35		*Coffee Break*
11:20	J. Aunins, Merck USA	Vaccine Production
11:50	A. Texeira, IBET, Portugal	Fluorescence-based tools to support biopharmaceutical process development
12:10	W. Zhou, Genzyme Corp. USA	Detection and remediation of a Vesivirus related contamination
12:30		*Posters and Lunch*
		General Assembly
15:00		Outing to Schloss Hof

RECOMBINANT ANTIBODY MIXTURES; OPTIMIZATION OF CELL LINE GENERATION AND SINGLE-BATCH MANUFACTURING PROCESSES

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At Symphogen A/S the proprietary Sympress™ expression platform is used for controlled production of mixtures of recombinant monoclonal antibodies for therapeutic use in cancer and infectious disease. A second generation of the Sympress platform has been developed that results in a 20-fold titer increase compared to the previous generation where constructs were integrated in the host cell by site-specific integration.
The second generation Sympress technology is based on expression in a CHO DG44 derivative as host cell line. Random integration of expression constructs together with a DHFR-based selection system is utilized for generation of stable cell lines. The cell line development processes have been automated and robotic handling of cell cultures has been implemented to increase throughput and process documentation.
The first step in cell line generation is the creation of the individual cell line transfected with a desired antibody expression construct. These cell lines, collectively termed the polyclonal antibody library cell stocks (PALS) are characterized with regard to production and growth properties. The resulting best clone candidates are selected for generation of polyclonal master cell banks.
The cell banking and manufacturing of antibody mixtures by Sympress technology is based on generation of a polyclonal master cell bank (pMCB) and a polyclonal working cell bank (pWCB) which, apart from the new concept of combining several individual cell lines during generation of the pMCB, follows a conventional two-tier cell banking approach. For manufacturing, one pWCB ampoule is thawed, expanded and applied as inocula in bioreactor processes.
Here we present the key features of the expression technology and demonstrate that mixtures of recombinant monoclonal antibodies can be produced in a cost-efficient single-batch process under predictable, reproducible, and stable conditions using the Sympress technology.

HIGH YIELD ANTIBODY PRODUCTION USING A PERFUSION PROCESS IN A DISPOSABLE WAVE BIOREACTOR

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[2] *GE China Research and Development Center Co. Ltd. Shanghai, China*

The production of a monoclonal antibody in S2 insect cells was compared in batch and perfusion cultures, both run in disposable WAVE bioreactors. A floating filter, integrated into the bioreactor, was used for cell retention during perfusion. Compared to a parallel batch process, a tenfold higher cell concentration; more than 10^8 cells/mL, was achieved in the perfusion process. In combination with a 2.5 fold higher cell specific productivity of monoclonal antibody, this resulted in a 24 fold improvement of the average volumetric productivity. The perfusion process was run at stable operating conditions for three weeks. During this time, a more than 30 fold higher total protein production was achieved compared to the two week batch process. Cell specific rates for glucose consumption and lactate production were compared in both cultivation modes. In the perfusion process cell metabolism changed only slightly after inducing recombinant protein production. Contrastingly, induction triggered a 50% decrease of glucose consumption and a substantial increase in lactate production in the batch culture. These results indicate that the stable metabolite concentrations in the perfusion process had a beneficial impact on cell viability and specific productivity. Combining high cell concentrations and extended cultivation time, perfusion systems are an attractive option for high yield processes.

HIGHLY EFFICIENT, CHEMICALLY DEFINED AND FULLY SCALABLE PRODUCTION OF HOST-RESTRICTED POX VIRUSES

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Vectorial vaccines are predicted to yield novel therapeutic and protective approaches. They consist of recombinant live carriers that express antigen from an unrelated pathogen in the recipient. Especially promising viral carriers are host-restricted pox viruses that trigger a strong immune response without ability to replicate in the human organism. The block in replication is an important safety feature that allows application even in immunocompromized recipients. However, with this type of attenuation very high numbers of infectious units have to be given per dose for full efficacy. Hence, if these viruses are to be used in global vaccine programs highly efficient production processes will be required. Furthermore, to combat diseases such as AIDS, hepatitis C, tuberculosis, or malaria with these vectors, millions of the concentrated vaccine units will have to be provided annually. Any production process therefore should also be scalable and transferrable to newly industrialized countries. Finally, production should be robust and independent of the demanding logistics and uncertainties associated with the current industrial substrate, primary chicken cells.

We believe that we have solved most of the upstream challenges and describe a chemically defined production process for three disparate members of the highly attenuated poxviruses: modified vaccinia Ankara (MVA), fowlpoxvirus (FPV) and canarypoxvirus ALVAC. The process is independent of primary material and based on the continuous duck suspension cell line AGE1.CR specifically created as a vaccine substrate. For MVA, titers in the crude lysate without any processing reliably exceed the critical threshold of 10^8 pfu/mL and often are in the range of 5×10^8 to 2×10^9 pfu/mL.

In contrast to production of influenza virus, development of a production process for poxviruses was surprisingly complicated and involves dedicated media formulations. Our process was readily adjusted to the different kinetics and requirements of the three examined viruses, and was studied in Wave and disposable bioreactors up to 50 L scale. Superior yields were also obtained for a vector not related to poxviruses suggesting that we may have developed a more general approach for production of viruses that initially may appear not to replicate in suspension cultures free of animal-derived components.

VACCINE PRODUCTION

Aunins J.
Merck Research Laboratories, USA

Vaccines from cell culture have been one of the most effective public health instruments in history, dramatically decreasing diseases in many instances, and even eliminating it from general circulation for smallpox and poliovirus. The challenges of the past five decades have been to mature the technology generally, build concepts of and ensure product safety, and to address 'first world' diseases. This has succeeded spectacularly, despite some bumps in the road for vaccines cell culture which continue to this day. The technical challenges of the next decades promise to be no less formidable. Advances in biology and immunology enable the application of cell culture to novel types of vaccines, and bring forward new concepts and new challenges, as well as refining and redefining older problems of cell culture. This talk will discuss the current state-of-the-art of cell culture in vaccines, issues that the industry has faced, and will provide a perspective on future needs that will encompass new viruses, diseases, and ways to vector and deliver vaccines; new markets, their needs and expectations; and new competitive forces in the industry.

FLUORESCENCE-BASED TOOLS TO SUPPORT BIOPHARMACEUTICAL PROCESS DEVELOPMENT

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[2] *Instituto de Biologia Experimental e Tecnológica (IBET), Oeiras, Portugal*

Clone screening and media design are time-consuming steps during the development of new biopharmaceutical production processes. Several hundreds of transfected clones are first screened to select a subset with better expression characteristics followed by several parallel small-scale experiments to identify the optimal culture medium composition and nutrient feeding strategies. To support and accelerate these development phases, we developed a fluorescence-based method for high throughput analysis of secreted product and cell density in 96-well plates. It consists of recording fluorescence excitation/emission maps from cell cultures over time to collect information on multiple fluorophores (amino acids, vitamins and cellular cofactors) and then using multivariate statistical analysis to establish correlations between the fluorescence changes and off-line measurements of the target bioprocess variables. The method was applied to cultures of CHO-K1 cell clones expressing different amounts of an IgG_4 monoclonal antibody. Both cell density and secreted antibody could be predicted with good accuracies in culture supernatant samples, demonstrating the potential to shorten the time spent in early phases of bioprocess development. In addition, the same approach was successfully implemented for real-time monitoring of viable cells and antibody titer in bioreactor cultures, allowing continuous evaluation of bioprocess performance and opening the possibility for future implementation of closed-loop control strategies based on these critical process variables.

Acknowledgments
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DETECTION AND REMEDIATION OF A VESIVIRUS 2117 RELATED CONTAMINATION IN LARGE-SCALE CHO CELL CULTURES

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A vesivirus contamination was identified as the cause for failure of three large-scale cell cultures at Genzyme in 2008 and 2009. These events were first indicated by rapid declines of on-line process indicators monitoring cell metabolism and density. A standard panel of *in vitro* assays did not reveal consistent evidence of virus contamination in bioreactor samples. However, in-house studies using a production CHO cell line demonstrated that the agent could affect naïve cultures. Subsequent analysis of bioreactor samples by mass spectrometry detected Vesivirus isolate 2117 related peptides. This finding was confirmed by RT-PCR indicating the presence of this infectious agent. In parallel with studies to identify the root cause, a viral contamination was assumed as the worse case for remediation activities. Related raw materials were discarded, affected facilities sanitized, potential sources of the virus entry considered, and corrective and preventive measures implemented.

Session V: Understanding complex interactions and Cell Engineering

Wednesday May 18th, 2011

Chairs: Martin Fussenegger, ETH Zürich, Switzerland

09:05	Ch. Love, MIT, USA	Integrated Single-Cell analysis
09:35	V. Sandig, Probiogen, Germany	Biosynthetic Pathway deflection – a new engineering approach and efficient strategy for ADCC enhancement
09:55	A. Behjousiar, Imperial College, UK	Improved cell line selection through metabolic profiling and offline product analysis
10:10		*Coffee Break*
11:00	S. Ludwig, U Münster, DE	Influenza virus and host cell signalling – from molecular mechanisms to novel antiviral approaches
11:30	D. Wirth, HZI Braunschweig, DE	Rational engineering of cells: recombinant gene expression in defined chromosomal loci
11:50	M. Hackl, BOKU Vienna, Austria	A large scale functional screening method for microRNAs in CHO cells
12:05		*Posters and Lunch*

INTEGRATED SINGLE-CELL ANALYSIS

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The majority of analytical technologies used to assess the identities and functional capacities of cells yield average measures of their phenotypes. These measures obscure unique individuals that contribute significantly to the collective behavior or that may be of particular interest in discovery-based research. This talk will describe the development of a modular collection of techniques that use microfabricated arrays of subnanoliter containers (10^5-10^6) to enable flexible, reconfigurable processes for integrated and dynamic single-cell analysis. Some examples of measurements include phenotypic analysis of surface-expressed proteins, profiles of cytokine secretion, cytolytic activity, and gene expression. Specific applications of these technologies for massively parallel single-cell analyses will be presented in the areas of bioprocess development, clonal selection, antibody discovery, and immune monitoring of chronic human diseases, such as HIV/AIDS. The approach described establishes a new paradigm for tailoring bioanalytical processes to a host of cell-based problems important in drug discovery, monitoring, and manufacturing.

BIOSYNTHETIC PATHWAY DEFLECTION – A NEW ENGINEERING APPROACH AND EFFICIENT STRATEGY FOR ADCC ENHANCEMENT

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With increasing information on genome, transcriptome and metabolome of commonly used production cell lines, engineering becomes an increasingly popular approach to achieve desired product attributes, growth behavior and nutrient consumption.
Tools range from feeding intermediate metabolites, overexpression or deregulation of key enzymes of a pathway to knock-out and RNA silencing. While conceptionally simple, the latter approaches are either labor intensive or costly to apply at large scale.
Aiming at glycan modulation we added another principle to this toolbox: enzymatic deflection of a biochemical pathway. Fucose is synthesized inside the cell from GDP-mannose via short lived intermediates before it is transported to the Golgi apparatus for attachment to the nascent glycan.
A bacterial enzyme is used to redirect synthesis towards a heterologous activated hexose that cannot be utilized by the cell resulting in depletion of the natural pathway. To our surprise, even lowest level expression of the enzyme completely abolishes fucose synthesis in stably modified cells.
The approach allows producing antibodies that are devoid of core fucose at Fc glycans of the CH2 domain. This modification provides higher flexibility to the Fc-region of IgG1 antibodies and enhances their binding to the FcγRIIIa receptor of NK cells – the dominating effector cells in antibody dependent cytotoxicity (ADCC). Consequently, the potency of antibodies directed against tumor or infected cells is substantially increased.
In contrast to other strategies the approach is easily applied to the starter cell line of choice and, moreover, allows modification of fully developed producer cell lines within weeks.

IMPROVED CELL LINE SELECTION THROUGH *IN SITU* METABOLIC PROFILING AND OFFLINE PRODUCT ANALYSIS

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The ultimate goal of bioprocessing research is the cost effective production of a desired protein. This necessitates the optimisation of cell growth and increase of cell culture productivity in order to obtain the highest amount of protein in a given volume in the least amount of time. However, the selection of high-producing stable mammalian cell lines represents a bottleneck in the production of biopharmaceuticals. Traditional methods are very laborious, time consuming and expensive. These methods rely heavily on achieving high cell growth rates, assessed in terms of confluence, in the early stages of cell line development. However, there are data to suggest that faster growing cell lines are not necessarily the highest producers. In order to improve current techniques, we suggest a new cell line selection method that utilises genetically encoded FRET biosensor technology. The proposed system we will be able to monitor the concentrations of key intracellular metabolites including glucose and glutamine in real time, in a non-invasive way. Glucose and glutamine are key energy sources for cultured cells, and therefore vital metabolites to monitor in cell line selection. Therefore for efficient cell growth to occur glucose and glutamine must be present in a steady supply.

Two biosensors for glucose and one for glutamine, which measure metabolites in the relevant range for healthily growing cells, have been developed and tested. To determine which glucose biosensor to transfect into our mammalian expression system, assays were performed on CHO-S cells grown over a batch overgrow experiment. Both glucose and glutamine biosensors have been successfully stably transfected into CHO-S cells, as confirmed with confocal microphotographs. We have further calibrated the level of biosensor fluorescence against intracellular glucose and glutamine concentration measurements. Our work has therefore been validated *in vivo* and *in vitro* and we are now working towards correlating metabolite measurements with cell growth characteristics. We expect that the proposed system for real-time, *in situ* monitoring of intracellular metabolites will allow us to make informed decisions regarding which cell lines to progress to subsequent stages of selection. Importantly, it will enable the selection of cell lines that are more amenable to growth and production in large-scale bioreactors and therefore exhibit the desired behaviour in terms of manufacturability at an earlier stage than conventional methods.

INFLUENZA VIRUSES AND HOST CELL SIGNALLING – FROM MOLECULAR MECHANISMS TO NOVEL ANTIVIRAL APPROACHES

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Influenza virus infection results in the activation of a variety of intracellular signaling responses. It is a common view that most of these signaling events are initiated as an innate cellular response to defend the invading pathogen. While influenza viruses have evolved strategies to keep these responses in a tolerable limit, the virus also has acquired the capability to exploit some of these activities to support efficient replication.

This dependence of influenza virus propagation on cellular signaling factors provides opportunities for a novel approach of antiviral interventions that targets essential host factors instead of viral components. In the last couple of years we have identified several cell signaling targets that are suitable for antiviral interventions, including the classical mitogenic MAPK cascade, that regulates active viral RNP export, or the NF-kB pathway, that interferes with the apoptotic response. Inhibition of these pathways efficiently blocked virus replication in cells and animals without toxicity or the tendency to induce resistant virus variants.

This promising novel concept that represents a paradigm change in anti influenza therapy will be discussed.

TOWARDS RATIONAL ENGINEERING OF CELLS: RECOMBINANT GENE EXPRESSION IN DEFINED CHROMOSOMAL LOCI

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The strength of recombinant gene expression is a key property of cell lines for biopharmaceutical protein production. In most stable cell lines the expression vector is stably introduced into the host chromosomal DNA. Apart from the copy number and the used expression control elements the performance of recombinant expression vectors is modulated by genetic and epigenetic features provided by flanking host elements. Since targeted integration is very difficult cell clones with high expression of a recombinant vector are created by random integration and large scale screening for gene expression. This allows the isolation of those rare recombinant cells in which gene expression is optimal. This is usually due to locus-specific influences of the chromosomal surroundings.
We have developed an efficient methodology for targeting expression cassettes to specific chromosomal sites. The method (Flp recombinase mediated cassette exchange - RMCE) allows the repeated use of defined loci by targeting constructs for expression of proteins and viruses, thereby allowing to exploit the positive features of a given integration site.
We have systematically evaluated the performance of a set of expression vectors in various chromosomal sites. These vectors differed in the nature and number of promoters. Also, the orientation of the cassettes was varied. As a read out, production of antibodies and recombinant retroviral vectors was used. In this study high performance integration sites, screened for a defined promoter were used. The study shows that high level expression of a given promoter is restricted to defined integration sites, while other sites show only moderate expression. Vice versa, a given chromosomal site is not flexible with respect to the integrated cassette but requires the integration of specific promoters. Moreover, some integration sites are flexible with respect to the orientation of the expression cassettes while others support expression only in one direction. Thus, not the nature of integration site and vector as such but rather the interplay between each other defines the level and stability of expression. To distinguish between locus-specific effects and transacting factor influence, these experiments were carried out in HEK293, NIH3T3 and CHO cells. The results show that the cell type defines the performance of a specific promoter element.
Together, the results show that the definition of favorable combinations of specific integration sites and vector designs allow the rational exploitation of given chromosomal sites. This paves the way for predictable and high expression of biotechnologically relevant products such as antibodies and recombinant viral vectors.

ESTABLISHMENT OF A LARGE SCALE FUNCTIONAL SCREENING METHOD FOR MICRORNAS IN CHINESE HAMSTER OVARY CELLS

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MicroRNAs (miRNA) are a novel category of highly relevant molecules that have recently emerged as negative regulators of transcription, controlling a broad range of physiological as well as pathophysiological functions such as development, regulation of cell proliferation, cellular metabolism, and stress resistance. As such, their use as engineering tools for optimization of cell behavior is a promising new strategy to improve the efficiency of protein production in Chinese hamster ovary (CHO) cells. The goal is to maximize protein yield by accomplishing fast growth and high cell densities, maintaining viability and increasing robustness against stress.

Next-generation sequencing data suggest that several hundred miRNAs are expressed in different host and producer cell lines. Hence, the functional analysis of such large numbers of miRNAs requires an efficient screening method to enable targeted engineering strategies. The aim of this study was to develop a method to perform functional characterization of miRNA effects in CHO host (CHO-K1) and producer (CHO-EpoFc) cell lines regarding different bioindustrially relevant characteristics, such as growth, viability and transgene productivity.

To this end, miRNAs are cloned into the BLOCK-iT™ Pol II miR RNAi Expression Vector (Invitrogen) with EmGFP under the control of a CMV promoter, using oligo based cloning following the instruction manual. When transiently transfected into CHO host (CHO-K1) and producer (CHO-EpoFc) cell lines, the developed constructs are transcribed for 5 days, to enable a functional screen during small scale (50 mL) batch cultivation. To increase the throughput of testing the effects of miRNA mimics or antagomir on cell growth, viability and specific productivity, samples are taken on day 3 (for immediate effects) and day 7 (for overall batch performance results) after transfection. Subsequently, for more detailed characterization of the effect of selected miRNAs, the experiment is repeated with daily sampling, including analysis of GFP expression and transcriptomics, to identify or confirm mRNA targets effects. This way the same method can be used for both high-throughput screening as well as detailed characterization without changing the experimental set-up, thus representing a general platform to be used for identification of likely candidates for cellular engineering.

Poster Prize Session: Short Presentations

Wednesday May 18th, 2011

Chairs: Poster Committee

14:00	Introduction and Explanation of Voting procedure
14:10	Short Presentations
15:20	Coffee Break
15:50	Short presentations
17:00	Collection of Votes

Keynote II:

THE CHEMISTRY OF LARGE NUMBERS

Richard Lerner
The Scripps Research Institute, USA

The Chemistry of Large Numbers

Lerner, R.
The Scripps Research Institute, 10550 N. Torrey Pines Road, La Jolla, CA

Combinatorial Antibody Libraries have allowed the construction of a synthetic immune system that has a repertoire that is al least three orders of magnitude larger than the natural repertoire of animals. These libraries circumvent the tolerance problem and allow one to understand the chemistry of antigen-union. Many important antibodies have been isolated from such libraries. Unlike the usual process in immunochemistry that tells one what **has *happened***, the very large numbers of antibodies in synthetic libraries allow one to determine what ***can happen.*** In this presentation I will give examples to illustrate these principles.

P1.01. HIGH CONTENT ANALYSIS REVEALED REGULATORY MECHANISMS OF CELLULAR SENESCENCE PROGRAMS

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Until now, we have identified key molecules involved in the cellular senescence programs and clarified their molecular network in the induction and maintenance of cellular senescence programs. We used several cellular phenotypes such as senescence-associated b-galactosidase (SA-b-Gal) activity, activation of MAPK p38, augmentation of CDK inhibitor p16/p21, morphological change into flattened and enlarged shape, senescence-associated heterochromatin foci (SAHF) as cellular senescence markers. In this study, we tried to clarify cellular senescence phenotypes, and further molecular networks formed by senescence-associated factors by high-content analysis using imaging cytometer, IN Cell Analyzer1000.
IN Cell Analyzer 1000 is an automated cellular and subcellular imaging system for fast, automated multiwavelength imaging and analysis in fixed and live cells. We used human normal diploid fibroblast cell line, TIG-1 cells, as model cell line. Replicative senescence was induced by serial passagings, and premature senescence was by the treatment with IL-1a and IL-6. We measured several cellular senescence markers in replicatively and prematurely senesced TIG-1 cells. Intracellular reactive oxygen species (ROS) was measured by using BES-H_2O_2. SA-b-Gal activity was measured by using ImaGene Green C_{12}FDG as substrate. Phosphorylated TAK1 and p38 were detected by immunofluorescence staining using anti-phosopho protein antibody.
By using IN Cell Analyzer1000, enlargement of cell and nuclei size, augmented SA-b-Gal activity, and increased accumulation of intracellular ROS were detected in senesced TIG-1 cells, indicating that changes in cellular senescence phenotypes can be traced quantitatively by using IN Cell Analyzer1000. Further, increased accumulation of phosophorylated TAK1 and phosphorylated p38 were also observed in senesced TIG-1 cells, demonstrating that molecular network formed by senescence-associated factors can be analyzed. All these results suggest that IN Cell Analyzer1000 can be a powerful tool for quantitatively investigating cellular phenotypes and molecules of adherent cells.

P1.02. INTEGRATION OF NOVEL SCREENING METHODOLOGIES AND HIGH THROUGHPUT ANALYTICS IN A CELL LINE DEVELOPMENT WORKFLOW

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As demand increases for efficient and streamlined production processes of therapeutic proteins, improvements in the process of cell line screening and clone selection become more important. We have developed an integrated cell line development workflow that incorporates the ClonePixFL workstation and a novel 24-well scale-down cell culture screening platform designed to quickly identify high producing clones in transfected CHO populations expressing biotherapeutic proteins. The ClonePix FL offers significant advantages over traditional limiting dilution and FACS based sorting techniques currently used in for cell isolation and screening.
Furthermore a novel 24-well shaken plate screening platform has been developed that enables high-throughput screening in batch and fed-batch culture modalities. In this format, clones are transitioned early in the process to a suspension culture enabling decisions on clone selection significantly earlier in the process. Lastly, the 24-well screening platform has been integrated with a high throughput analytical platform that enables rapid acquisition of product quality data early in the cell line development workflow. The high throughput analytical platform incorporates an automated liquid handling workstation that prepares samples for both quantitative analysis and conducts 96-well-based affinity purification of proteins in conditioned medium samples. Affinity purified samples are then subjected to glycan analysis (Caliper Lab Chip), functional binding assays, SEC and icIEF analysis. Using this integrated cell line development platform, we have identified clones with acceptable productivity and product quality attributes while significantly reducing cell line development timelines.

P1.03. SELECTION OF CELL LINES FOR INDUSTRIAL PROCESSES: DEVELOPING AN INTEGRATED METHODOLOGY

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One of major technological challenges during development of mammalian cell culture processes is the need for early selection of that clone, which could better fit with the requirements for the future scale-up, because the lack of validated predictors and straightforward methodologies that could render in a short time and with high efficiency cell lines that complain with all desired characteristics, i.e. high and stable expression level, ability to reach and maintain high cell densities in protein free-medium, robustness to stress conditions as mechanical damages and limitations of some nutrients and oxygen.
In this work we have characterized different transfected cell lines in order to establish a selection method that includes some critical parameters for desired phenotypes and from these results we determined the selection criteria that allowed the definition of an integral coefficient (CIS). Moreover we have developed a methodology to select clones with high specific productivity or growth at 96-well plate scale. Several clones of recombinant NSO myeloma cell line with different phenotypes (high productivity, high proliferation) were isolated in protein-free medium combining these integrated selection methodologies. Afterward the influence of different pre-selected phenotypes on overall process yields, measured as value of product integral, was evaluated by kinetic studies in spinners flasks and bench scale bioreactors (5-15 L), both in batch and continuous modes. During these studies growth parameters and metabolic yields for glucose, glutamine and lactate were determined and correlated with productivity for each of selected phenotypes. Further characterization was done attending stability of the expression using intracellular immunoglobulin measurement and level of apoptosis induction after nutrient deprivation.
As main results of this study we obtained that clones with higher CIS showed also higher product integral when culture in bioreactor conditions, as was predicted from spinner flasks studies. This allowed us to propose an integrated selection methodology that includes specific expression levels, stability and cell growth pattern that could be implemented at bench scale and predict best clones in a shorter time schedule, compared with traditional approach.

P1.04. UTILIZATION OF NON-AUG INITIATION CODONS IN A FLOW CYTOMETRIC METHOD FOR EFFICIENT SELECTION OF RECOMBINANT CELL LINES

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Here we describe a method that couples flow cytometric detection with the attenuated translation of a reporter protein to enable efficient selection of CHO clones producing high levels of recombinant therapeutic proteins. In this system, a small cell surface reporter protein is expressed from an upstream open reading frame utilizing a non-AUG initiation ("alternate start") codon. Due to the low translation initiation efficiency of this alternate start codon, the majority of translation initiation events occur at the first AUG of the downstream open reading frame encoding the recombinant protein of interest. While translation of the reporter is significantly reduced, the levels are sufficient for detection using flow cytometric methods and, in turn, predictive of protein expression from the gene of interest since both ORFs are translated from the same mRNA. Using this system, CHO cells have been sorted to obtain enriched pools producing significantly higher levels of recombinant proteins than the starting cell population and clones with significantly better productivity than those generated from limiting dilution cloning. This method also serves as an effective screening tool during clone expansion to enable resources to be focused solely on clones with both high and stable expression. Furthermore, stability of selected clones can continue to be monitored in real time through all stages of cell line development.

P1.05. NOVEL HUMAN PARTNER CELL LINE FOR IMMORTALISATION OF RARE ANTIGEN SPECIFIC B CELLS IN MAB DEVELOPMENT

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Human cross-lineage hybrid cell line was developed as a candidate partner for immortalisation of rare primary human antigen-specific B Lymphocytes using binary electrical cell hybridisation. This novel partner cell line is a tri-hybrid of IL-4 secreting Th2 lymphoblast derived from a patient with acute lymphoblastic leukemia (T), CD20-positive B lymphoblast also derived from a patient with acute lymphoblastic leukaemia (W), and IL-6 secreting peripheral blood derived CD14-positive monocyte (M). The selection of cell phenotypes used in creating tri-hybrid was based on inclusion of factors known to maintain and promote antibody production. The resulting tri-hybrid (WTM) displays characteristics of mixed CD phenotypes with majority of cells being positive for CD20 (95%) with co-expression of CD4 (54%) and CD14 (24%). It secrets IL-4, IL-6, IL-8 and GM-CSF but negative for IL-1A, IL-1B, IL-2, IL-5, IL-10, IL-12, IL-13 and IL-17. The cell line does not express tumour suppressor protein p53. It does neither express on the surface nor secret immunoglobulins (Ig) nor immunoglobulin chains. WTM cells were further used as a fusion partner with primary antigen-experienced B cells in binary electrical cell hybridisation. With 100% success rate in hybridisation, the number of resulting stable hybrids varied from 48% to 78% depending on the phenotype of B lymphocytes used in experiments. 23% to 68% of those stable hybrids secreted immunoglobulins with production ranging between 1.2 to 3.4 □g/10^6 cells. Furthermore, the Ig producing hybrids were generated from the B lymphocyte sample size as small as 50 cells and also eliminated laborious screenings for hybrids and Ig producing clones. In conclusion, when the number of rare antigen specific B cells available is a limiting factor in generating hybridoma, EBV transfection or direct sequencing, binary B lymphocyte hybridisation with WTM cells can provide a very attractive approach for the generation of stable hybrid cell lines producing monoclonal antibodies.

P1.06. GENERATION OF A STABLE CELL LINE FOR REBMAB 200 MAB

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Rebmab 200 (designation for humanized MX35 monoclonal antibody) recognizes the sodium-dependent phosphate transport protein 2b (NaPi2b), expressed at high frequency on the membrane of ovarian tumors and in a limited number of normal tissues. Expression of Rebmab 200 was obtained in Per.C6 human cells (Crucell, Netherlands). The transfection of Per.C6 cells with a vector containing the genes coding for the heavy and light chains of Rebmab 200 generated a stable pool through G418 selection. The cells from the stable pool were cloned by limiting dilution. The screening of the 96-well plates started after 3-4 weeks. A total of 210 clones were transferred to 24-well plates. A titer screen started at this step to eliminate the non- or low-producing clones. The antibody concentration was measured by BIAcore (GE Healthcare, Sweden). A considerable number of clones stopped growing and died during the expansion of the cells. A total of 63 clones could be transferred to T-flasks, and a 3-day pcd was calculated to select 30 clones to be further transferred to shaker flasks. After the 3-week adaptation period of growth under agitation, the clones were assessed for productivity in batch and fed-batch processes. The selected 10 clones were submitted to stability study for 50 generations to determine whether the growth and productivity of lead candidates remained stable, predicting for the successful long-term production of the antibody during a manufacturing process. The plan for the stability test was based on culturing the cells in continuous culture and performing batches. Two out of the 10 tested clones showed instability in the antibody production and were eliminated. Concomitantly to the stability study, the Rebmab 200 was purified by protein A chromatography from the cell supernatants collected at the end of the fed-batch experiment. The purified antibodies were used in FACS and ADCC assays to evaluate, respectively, the binding to the target and the immune-effector function of Rebmab 200 on NaPi2b positive cancer cells. The FACS analysis showed no significant differences between the clones. On the other hand, the ADCC analysis showed higher citotoxic activity for the mAb purified from two clones. All the accumulated data on cellular growth (pdt), overall production, productivity per cell (Qp), performance in batch and fed-batch conditions, binding properties to cancer cell lines (FACS) and immune-effector function in cancer cell lines (ADCC) were put together to choose the 3 lead clones.
Financial support: FAPESP, FINEP, CNPq, RECEPTA-biopharma

P1.07. A STRATEGY TO OBTAIN RECOMBINANT CELL LINES WITH HIGH EXPRESSION LEVELS. LENTIVIRAL VECTOR-MEDIATED TRANSGENESIS

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The first stage of any recombinant protein production is to achieve successful gene transfer and expression in a target cell. There are two general categories of vehicles/vectors delivery employed in protein expression protocols. The first category includes non-viral vectors, ranging from direct injection of DNA to DNA incorporation using cationic lipids, polilisine, etc. The second category comprises DNA and RNA viral vectors.
Viruses have developed specific mechanisms to deliver their genetic material to target cell nuclei. Virus members of the family *Retroviridae*, e.g. retroviruses and lentiviruses, are among the most widely used viral vectors. The use of lentiviral vectors has increased because the vector system presents attractive features. Lentiviruses have an advantage over retroviruses because they can infect both dividing and non-dividing cells and therefore have attracted much attention due to their potential as vectors for gene delivery/therapy. Once integrated into the genome the recombinant cell lines are selected using different selection mechanisms.
Lentivirus particles were produced by simultaneous co-transfection of HEK 293T cells with four plasmids. The packaging construct (pMDLg/pRRE) (Dull *et al.*, 1998), the VSV-G-expressing construct (pMD.G) (Naldini *et al.*, 1996), the Rev-expressing construct (pRSV-Rev) (Dull *et al.*, 1998), and the self-inactivating (SIN) lentiviral vector construct containing the green fluorescent protein (GFP) reporter gene (pLV-PLK-eGFP). After that, the lentiviral particles were added to HEK 293T cells and 96 h post transduction the cells were analized by flow cytometry. Then, the cells were incubated with the puromycin selection agent to obtain stable recombinant cell lines employing two protocols: a) the cells were incubated with 1, 5, 10, 50, 100, 150, 200 and 250 µg/ml puromycin in different plates, and b) from 1 up to 250 µg/ml of puromycin, but the selection agent was gradually changed 7 days each on the same plates. Twenty five days post puromycin addition, the different recombinant cell lines were analyzed by flow cytometry to detect and compare the expression of eGFP (x-mean). Employing the gradual selection protocol (b), it was possible to maintain the cells in culture condition up to 200 µg/ml puromycin and to achieve higher expression levels of the reporter gene, 2 - 5 times depending on puromycin concentration. Contrarily, in the (a) protocol cultures were resistant only up to 50 µg/ml and eGFP expression levels were lower. Simultaneously, the resistant cell lines were cloned by the limit dilution method and the resulting clones were analyzed by flow cytometry. The eGFP expression of each clone was consistent with the respective resistant cell line. Therefore, using this strategy for recombinant cell line selection, it was possible to obtain high eGFP producing stable cell clones without the use of gene amplification systems.

P1.08. RAPID RECOMBINANT PROTEIN PRODUCTION FROM POOLS OF TRANSPOSON-GENERATED CHO CELLS

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Transient gene expression (TGE) is the most commonly used technology for the rapid production of moderate quantities of recombinant proteins for preclinical studies or for analytical assay development. Whereas TGE can provide milligram to gram amounts of recombinant proteins within a time period as short as few days, technical limitations as well as the high costs of plasmid DNA hamper the application of this technology at higher production scale. Here we describe an alternative method for the rapid production of recombinant proteins in mammalian cells. Our technology is based on the use of stable pools generated by *PiggyBac* (PB) transposition. The improved efficiency of stable cell line generation mediated by PB transposition allowed the recovery of stable cell populations within 10 days of transfection. Using stable cell pools expressing an IgG monoclonal antibody, titers up to 600 mg/l were obtained in 14-day batch cultures. Remarkably, the enhanced stability of transgene expression after transposition allows expansion of these cells in non-selective medium for up to 3 months without any observable decrease in productivity. We optimized a protocol for production from transposed cell pools at the 1-L scale starting from a 10-ml transfection. Using stable cell pools expressing an IgG antibody or two different Fc fusion proteins we produced 500-700 mg of recombinant protein within a month after transfection. Our results indicate usefulness of PB transposition generated cell pools as a valuable alternative to TGE for the rapid production of recombinant proteins.

P1.09. CHO CELL LINES GENERATED BY PIGGYBAC TRANSPOSITION

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A major bottleneck in the manufacture of recombinant therapeutic proteins is time and effort needed for the generation of stable, high-producing mammalian cell lines. Conventional gene transfer methods for stable cell line generation rely on random transgene integration, resulting in unpredictable and highly variable levels of expression of the transgene in individual clones. Here we present an alternative method for the generation of high-producing cell lines based on transgene integration mediated by the *PiggyBac* (PB) transposon. Compared to conventional transfection of plasmid DNA, PB transposition resulted in an improvement in the efficiency of stable cell line generation up to 20-fold for both CHO and HEK 293 cells. This enhanced efficiency allowed the recovery of stably transfected cell populations after 5-10 days of selection, considerably reducing the time required for the generation of stable cell lines. To further evaluate the PB system as a transgene delivery tool, Chinese hamster ovary (CHO) cells expressing a tumour necrosis factor receptor:Fc (TNFR:Fc) fusion protein, generated either by PB-transposition or by conventional transfection techniques, were characterized for the level and stability of transgene expression over 3 months in serum-free suspension culture. Transposition increased the frequency of high-producing clones in the transfected population, reducing cell line screening efforts. Furthermore when compared to clones generated by conventional transfection, PB-derived cell lines produced up to 4-fold more recombinant protein and had greater transgene expression stability. Our results indicated that the PB system is a valuable tool to generate at high frequency cell clones with stable and enhanced transgene expression.

P1.10. *PIGGYBAC* TRANSPOSON-MEDIATED INTEGRATION OF TRANSGENES IN CHO-DG44 CELLS

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Transposons mediate stable integration of exogenous DNA elements into a host cell genome and have been successfully used as gene transfer vectors in mammalian cells. Here, we have used the *piggyBac* (PB) transposon to facilitate the integration of three artificial transposons, each bearing a single transgene and the puromycin resistance gene for selection. Enhanced green fluorescent protein (eGFP) and the light and heavy chains of an IgG1 monoclonal antibody were used as the three model proteins. For pools generated by PB transposition, the percentage of cells producing GFP reached 80%, whereas in standard transfections only 30% of cells in puromycin selected pools were GFP-positive. Similarly we observed that the recombinant antibody productivity of the pools obtained by standard transfection was only 5 mg/L in 4-day batch cultures, whereas we obtained titers of up to 120 mg/L from pools generated by PB transposition, corresponding to a 24-fold improvement over the control. The population of cells expressing GFP and the volumetric productivity of antibody were observed to increase with an increase in the stringency of selection pressure. We are further investigating the efficiency of the PB system to co-integrate a higher number of independent transgenes by using plasmids bearing different selection markers in order to further improve the stringency of selection. We will eventually be studying the structure and number of integration sites in transposon-generated clonal cell lines recovered from these pools. Our preliminary data suggest that the *piggyBac* transposon system provides an efficient method for the co-integration of multiple genes, making it particularly attractive for the stable expression of multiprotein complexes.

P1.11. CELL LINE DEVELOPMENT USING THE SEFEX SYSTEM

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Cell lines producing biopharmaceuticals with high yield and high quality in a regulatory compliant environment are a prerequisite for cost effective bioproductions. The development of these production cell lines often include screening strategies combined with gene amplification and limited dilution experiments, a time consuming process. Especially gene amplification tends to interfere with clonal stability. Limited dilution, especially using a serum-free culture environment is prone to failure and low clone yields.
We present here the SEFEX platform technology for the development of non-amplified high yield production cell lines. The strategy is based on a regulatory compliant method for transfection and single cell cloning using a proprietary, fully tested, CHO K1 host cell line adapted to chemically defined medium.
Two case studies for production of humanised monoclonal antibodies exemplify the platform technology. The development comprises serum-free transfection, selection, and single cell cloning and provides high producer cell lines within minimised time frames. Clonal cell lines, as documented using semi-automated photo documentation, are characterised at several culture stages during clonal expansion. After a two-step process optimisation up to 1.3 g/L product were obtained from fed-batch experiments, which is sufficient for early development phases. The other case comprises two serial transfections of the antibody expression vector including regulatory sequences. Employing the platform technology, the product yield achieved was 1.8 g/L without any process optimisation. Specific productivity was in the range of 25 pcd.
The serial transfection cell line development strategy described provides the possibility to develop production cell lines capable of product yields of 2 g/L within minimised time frames. These cell lines can be improved to deliver yields meeting industrial demands employing simplest process development procedures.

P1.12. HIGH-THROUGHPUT CLONEPIX™ FL ANALYSIS OF MAB EXPRESSING CLONES USING THE UCOE SYSTEM

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Creation of high-expressing clones during cell line development remains heavily reliant on stringent selection and gene amplification, which in turn leads to genetic instability and a lack of reproducibility. UCOE (Ubiquitous Chromatin Opening Elements) technology provides improved productivity and stability in transfected mammalian cells lines through modulation of the chromatin structure. UCOE expression technology prevents transgene silencing and gives consistent, stable and high-level gene expression irrespective of the chromosomal integration site and does not require gene amplification.

Clone selection however, remains rate-limiting and relies on traditional cloning methods. In this study we have used high-throughput robotic clone analysis to examine the beneficial effects of the UCOE system. This includes analysing alternate promoters, IgG subtype and UCOE element length. Using the ClonePix™ FL we analysed thousands of clones and found inclusion of the UCOE elements more than doubled the number of antibody-expressing clones. More significantly, the UCOE elements provided for a greater than 3-fold increase in the exterior median fluorescence intensity - a key characteristic used for selecting high-producing clones.

The use of UCOE elements in combination with Clonepix™ FL selection provides a rapid method for selection of stable, high-expressing clones for bioproduction.

P1.13. TOOLBOX APPROACH FOR FAST GENERATION OF STABLE CHO PRODUCTION CELL LINES FROM DIFFERENT HOSTS

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Protein production in CHO cell lines is a highly dynamic process, where the yield limiting step can shift among transcription, translation, and secretion under different cell growth stages and culture conditions. In addition, the choice of CHO cell line has been shown to affect target protein quality and quantity.
Further advancements in titer and specific productivity requires elimination of cellular/process bottlenecks along the generation of stable production cell lines including vector design, clone selection, genetic engineering, and media and feed optimization.
To overcome these limitations, we developed a toolbox for all process phases from transfection to production. This toolbox is based on a chemically defined media platform and includes pre-adapted CHO-K1 or CHO-DG44 cell lines, optimized vectors and protocols, as well as key regulators of multiple cellular pathways.
Here we demonstrate an innovative parallel platform cell line development for CHO-K1 and CHO-DG44 enabling fast and highly reproducible generation of high producer clones stably expressing the transgene for more than 80 pdl without selection pressure and resulting in yields of 3-5 g/L for an Fc-fusion protein drug candidate.

P1.14. DEVELOPMENT OF A HIGH THROUGHPUT IMMUNOASSAY FOR SCREENING PROSAVIN® PRODUCER CELL LINE CLONES

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ProSavin® is an Equine Infectious Anaemia Virus (EIAV) based lentiviral vector for the treatment of Parkinson's disease that encodes three enzymes required for dopamine synthesis; aromatic amino acid decarboxylase (AADC), tyrosine hydroxylase (TH) and GTP-cyclohydrolase 1 (CH-1). Producer cell lines (PCLs) are being developed for the large scale manufacture of ProSavin®. Such cell lines contain a regulatory protein (Tet repressor) and the vector components (EIAV Gag/Pol, VSV-G envelope and the EIAV ProSavin® vector genome) stably integrated into the cellular genomes. To identify cell lines that are capable of producing ProSavin® at high titre a large number of clones have to be screened. This process is labour intensive, involving clonal cell expansion, preparation of a clone cryo-bank, manipulation of clones for screening, and assaying of vector-containing cell culture supernatants to assess titres. For this reason a high throughput titration assay would significantly expedite the selection of high vector producing clones.
Currently, ProSavin® is quantified by transduction of HEK293T cells cultured in 12-well plates. Transduced cells are determined using a flow cytometry based method to quantify fluorescently labelled cells, or by DNA integration assay which uses a quantitative PCR approach. Both methods are time consuming and are not considered high throughput. Here we discuss the development of a number of 96-well plate based ProSavin® titration assays which employ the use of immunodetection for TH. The applicability of these assays as a high-throughput screening strategy will be discussed in greater detail.

P1.15. EFFICIENT PRODUCTION OF RECOMBINANT IgG BY THE GLUT% CO-EXPRESSION SYSTEM

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A fructose containing cell culture medium is suitable for the process control of protein production because of slow sugar consumption rate and low lactate accumulation. The fructose transporter, GLUT5, mediates its incorporation into cells and is required for the fructose-based culture. In this stude, we aimed to develop an efficient production system of recombinant proteins by metabolic control and co-expression with GLUT5 in a fructose-based medium.
As a model, an IgG and GLUT5 co-expression vector was constructed and transfected into the human myeloma derived cell line, SC-01MFP, which has the ability to produce stably recombinant proteins. The cell proliferation in the fructose-based medium was improved by the GLUT5 gene tranfection. The recombinant IgG production of the cells cultured in the fructose-based medium exhibited about two-fold increase of that in the glucose-based medium. Flow cytometric analysis indicated that the GLUT5 protein expression level in cell surface was increased in the fructose-based medium. An exogenous but not endogenous GLUT5 transcription activator remarkably raised IgG productivity in the fructose-based medium when compared to that in the glucose-based medium, suggesting that exogenous GLUT5 expression may be involved in it.
Taken together, the GLUT5 co-expression system worked successfully to increase recombinant IgG production in fructose-based medium. This system may be applicable to CHO cells because other investigators reported that they became proliferative in the fructose-based medium by the GLUT5 gene transfection. Our study may be useful for efficient production of recombinant proteins using the fructose-based cell culture. In particular, the production in SC-01MFP cells is valuable for functional analysis of recombinant proteins with a human glycosylation profile.

P1.16. TOWARDS STABLE PRODUCTION IN CHO CELLS: PREVENTION OF CMV PROMOTER FROM SILENCING WITH CPG ISLAND CORE ELEMENTS

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Generation of cell lines that can maintain productivity during long time culture is of utmost importance for biopharmaceutical manufacturing. Loss in productivity also affects the product consistency, compromising regulatory approval of the product. By using currently available expression vectors, most clones generated are not stable and a large number of clones need to be screened to obtain high-producing cell lines with stable productivity. Our previous study indicated that the loss in productivity was mainly due to transcriptional silencing which involved methylation of CpG dinucleotides within promoters[1]. In this work, we applied the core element of a CpG island from the adenosine phosphoribosyltransferase gene, IE, to protect the human cytomegalovirus promoter (CMV) from DNA methylation with the aim of improving cell line stability. The CMV promoter used in this study consists of an enhancer and a minimal promoter. We found out that the protective effect of IE element depended on the location where it was placed around the promoter and its orientation. One IE element protected about 150 bases in the forward orientation from DNA methylation. Insertion of one IE element upstream of he enhancer in forward orientation, one downstream of the enhancer in reverse orientation, together with one IE upstream of the minimal promoter in forward orientation were sufficient to protect the whole CMV promoter. Moreover, all individual cell clones with fully IE-protected CMV promoters preserved gene expression during an 8-week culture period and exhibited enhanced stability. This strategy of anti-silencing protection by the IE element should be beneficial for generation of cell lines with high stability.

1. Yang Y.S., Mariati, Chusainow J., and Yap M.G.S. (2010). DNA methylation contributes to loss in productivity of monoclonal antibody-producing CHO cell lines. Journal of Biotechnology, 147: 180-185

P1.17. NEUROPROTECTIVE ACTIVITY OF A NEW ERYTHROPOIETIN FORMULATION WITH INCREASED PENETRATION IN THE CENTRAL NERVOUS SYSTEM

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Apart from its hematopoietic effect, erythropoietin (EPO) is a molecule with high neuroprotective potential. However, its prolonged application may cause serious adverse effects due to the erythropoiesis stimulation. Therefore, it is necessary to develop EPO derivates with neuroprotective properties but low hematopoietic activity. For this purpose we developed an alternative purification process for the recombinant hormone produced in CHO cells (rhEPO). The process was designed to obtain a combination of EPO glycoforms called neuroepoetin (rhNEPO). rhNEPO isoforms composition is less acidic (pI 4.2-6.1) and has lower sialic acid content (8.0 ± 1.0 mol/mol protein). This resulted in a very low *in vivo* erythropoietic activity in normocytemic mice (5,034 ± 2,166 IU/mg vs. 131,976 ± 52,324 IU/mg in the case of rhEPO). Despite this, no changes were observed in the affinity with its receptor in *in vitro* UT-7 cells assays.

Additionally, we evaluated the neuroprotective action of both molecules in SH-SY5Y and PC-12 cells (their neural phenotype differentiated with NGF) by subjecting them to different apoptotic stimuli. In proliferation assays using SH-SY5Y cells both variations showed similar neuroprotective action. On the other hand, rhNEPO revealed higher anti-apoptotic activity (evaluated using TUNEL technique) in PC-12 cells. When an intravenous pharmacokinetic study was performed, rhNEPO showed higher *clearance* than rhEPO (31.6 ± 1.9 ml.h^{-1}and 3.3 ± 0.3 ml.h^{-1}, respectively). However, rhNEPO was detected early in cerebrospinal fluid after 5 minutes of injection, while rhEPO was detected only after 30 minutes. These results encourage the study of rhNEPO as a potential drug for the treatment of neurological diseases due to its negligible hematopoietic activity, its neuroprotective capacity and its fast passage through the blood-brain barrier.

P1.18. COMPARISON OF THE ACTIVITY AND PLURIPOTENCY MAINTAINING POTENTIAL OF HUMAN LEUKEMIA INHIBITORY FACTOR (LIF) PRODUCED IN *E.COLI* AND CHO CELLS

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Leukemia inhibitory factor (LIF) is a polyfunctional cytokine with numerous regulatory effects in vivo and in vitro. In murine stem cell cultures it is the essential media supplement for the maintenance of pluripotency of embryonic and induced pluripotent stem cells. To explore if the glycosylation and/or other post-translational modifications are affecting this activity, LIF produced in eukaryotic cells (Chinese hamster ovary (CHO) cells) and prokaryotes (*E. coli*) is compared in this work.

The LIF produced in E.coli is expressed together with a His-tagged thioredoxin as a fusion protein to increase the solubility. The genes for the fusion protein are separated by a TEV cleavage site. For the purification immobilized metal chelate affinity chromatography (IMAC) was used to purify the fusion protein, which was afterwards cleaved. The released hLIF was purified from the protein mixture using ion exchange chromatography. The His-tagged LIF from the CHO cell supernatant was purified using IMAC as well as Heparin affinity chromatography for polishing.

For the comparison adherent as well as suspended growing murine embryonic stem cells and murine induced pluripotent stem cells were used and cultivated with both of the produced LIFs as well as commercial purchased LIF (from *E.coli*) as positive and without LIF as negative control. Finally we determined cell growth, the amount of apoptotic cells and the expression of different pluripotency marker proteins.

P1.19. NEUROPROTECTIVE EFFECT OF 3,5-DI-O-CAFFEOYLQUINIC ACID ON SH-SY5Y CELLS AND SAMP8 MICE THROUGH THE UP-REGULATION OF PGK1

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As aged population dramatically increases in these decades, efforts should be made on the intervention for curing age-associated neurologic degenerative diseases such as Alzheimer's disease (AD). Caffeoylquinic acid (CQA), an antioxidant component and its derivatives are natural functional compounds isolated from a variety of plants. In this study, we determined the neuroprotective effect of 3,5-di-O-CQA on $A\beta_{1-42}$ treated SH-SY5Y cells using MTT assay. To investigate the possible neuroprotective mechanism of 3,5-di-O-CQA, we performed proteomics analysis, real-time PCR analysis and measurement of the intracellular ATP level. In addition, we carried out the measurement of escape latency time to find the hidden platform in Morris water maze (MWM), real-time PCR using senescence-accelerated-prone mice (SAMP) 8 and senescence-accelerated-resistant mice (SAMR) 1 mice. Results showed that 3,5-di-O-CQA had neuroprotective effect on $A\beta_{1-42}$ treated cells. The mRNA expression of glycolytic enzyme (phosphoglycerate kinase-1; PGK1) and intracellular ATP level were increased in 3,5-di-O-CQA treated SH-SY5Y cells. We also found that 3,5-di-O-CQA administration induced the improvement of spatial learning and memory on SAMP8 mice, and the overexpression of PGK1 mRNA. These findings suggest that 3,5-di-O-CQA has a neuroprotective effect on neuron through the upregulation of PGK1 expression and ATP production activation.

P1.20. DEVELOPMENT OF A HUMAN IGE-INDUCING SYSTEM BY *IN VITRO* IMMUNIZATION

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1. Introduction

The immune system, which is the self-defense system of the body, sometimes responds in a way that is harmful to the body. Some of these responses manifest as allergic reactions to cedar pollen, house dust, chemicals, etc. The incidence and severity of allergies partly caused by the surrounding environment are increasing and have recently become a serious social problem. To study various allergic reactions, we report an *in vitro* system for inducing human IgE antibodies specific to a designated antigen[1]. In this study, we tried to develop an *in vitro* IgE-inducing system to stimulate IgE levels in its medium.

2. Experimental

The *in vitro* IgE-inducing system contained E-RDF supplemented with human plasma (final concentration, 10%); FCS (5%); IL-2, IL-4, and IL-6 (10 ng/ml each); and MDP (10 μg/ml), as described elsewhere[2]. Human lymphocytes were cultured in 96- or 24-well plates at a final density of 1×10^6 cells/ml in the medium and incubated in a CO_2 incubator at 37°C for 10 days. After 10 days, approximately 400 ng/ml of IgE antibodies was secreted into the medium.

3. Results and discussion

We used this IgE-inducing system to investigate various factors stimulating IgE production. Adding IL-1 (final concentration, 1pg/ml) and IL-18 (100pg/ml) to the system almost doubled the IgE production in the medium. Furthermore, an increasing number of lymphocytes secreted IgE antibodies into the medium of this system.

4. Conclusions

The concentration levels of the secreted IgE reported in this study may be the highest compared to those reported elsewhere. This improved system for human IgE induction is believed to be of profound use for studying allergy mechanisms and investigating allergy-alleviating materials. This system functions by increasing the number of IgE-secreting lymphocytes as well as IgE levels in its medium.

References

1. H. Kawahara, M. Maeda-Yamamoto and K. Hakamata (2000) Effective induction and acquisition of human IgE antibodies reactive with house-dust mite extracts. J Immunol Methods 233: 33-40.
2. S. Hashizume and H. Kawahara (2007) Inducing of human IgE antibodies by *in vitro* immunization. In Proceedings of the 20th ESACT Meeting (Dresden), in press.

P1.21. IMPROVEMENT OF THE IN VITRO IMMUNIZATION METHOD FOR HUMAN PERIPHERAL BLOOD MONONUCLEAR CELLS: INVESTIGATION OF PATTERN-RECOGNITION RECEPTORS' LIGANDS AS ADJUVANT

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Since 2000, the market for therapeutic antibodies has grown exponentially. Human monoclonal antibodies (mAbs) are one of the main products in biopharmaceuticals. The generation of 'fully' human mAbs contributes to reduction of the immunogenicity of chimeric and humanized mAbs. Beside several methods to produce human monoclonal antibodies (mAbs), we have originally developed a method to generate human mAbs by in vitro immunization (IVI) of peripheral blood mononuclear cells (PBMC) followed by EBV transformation or phage display method. Here, we report the further improvement of the method for efficient production of human mAbs against any antigen. Especially, adjuvant effects of the ligands of pattern-recognition receptors (PRRs) related to the innate immunity on IVI were investigated.
Human PBMC of healthy individuals were used to investigate the expression levels of the PRRs, including Toll-like receptors (TLRs) 1-10, RIG-I-like receptors (RLRs), and NOD-like receptors (NLRs), transcripts in these cells by semi-quantitative RT-PCR analysis. In IVI, PBMC were pre-treated with L-leucyl- L -leucine methyl ester (LLME) to remove suppressing cells and then sensitized with an antigen in the presence of cytokines (IL-2 and IL-4) and adjuvant (TLR 1-9 agonists or MDP). The total amounts of IgM and IgG produced from the in vitro immunized PBMC and their antigen-binding activities were measured by enzyme-linked immunosorbent assay (ELISA). Expansion of antigen-specific B cells after IVI was evaluated by enzyme-linked immunospot (ELISPOT) analysis.
According to the results obtained by semi-quantitative RT-PCR, the gene expressions of most PRRs were detected in PBMC before and after IVI. These results suggested that human PBMC from healthy individuals had the potential to respond to the ligands of PRRs. Further, antigen-specific B cells were efficiently expanded by using MDP (NOD-2 ligand) and CpG-ODN (TLR-9 ligand) as adjuvant. The effects of ligands of other PRRs on IVI are being investigated now.

P1.22. INFLUENCE OF THE NICKEL TITANIUM ALLOY COMPONENTS ON BIOLOGICAL FUNCTIONS

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Introduction: Nickel titanium alloys are applied to biomaterials field because they have great characters such as corrosion-resistance, adequate mechanical strength and shape-memory effect. On the other hand they have a risk of metal allergy because of eluting nickel ion from the nickel titanium alloy. The reason why nickel causes allergy is not clear. Therefore we design biocompatible nickel titanium alloys based only on experiences. For the purpose of the effective and reasonable alloy production, it is necessary to make the relationships between the characters of nickel titanium alloy and biological reactions clearer. In this study, we investigated 1) the physical properties of some nickel titanium alloys, 2) the effect of these alloys on cellular function and 3) the allergy test for these alloys.
Materials and methods: Titanium was added to the nickel matrices to become 5%, 15%, 25% or 50% by weight to produce nickel titanium alloys by arc melting. The components of nickel titanium alloys were determined by X-ray fluorescence analysis. Each nickel titanium alloy was cut into a small sheet of a certain surface area by a shearing machine and then sterilized by 70% ethanol. The sterilized alloys were used for cellular assay. Firstly, the sterilized nickel titanium alloys were immersed in PBS for several hours to make extracts. Then, each extract was added to the culture supernatant of MOLT-3 (Riken bioresource center cell bank, Japan) and the MOLT-3 cells were cultured for 5 or 6 days. After that, the viable cell numbers were counted by a hemacytometer with a phase contrast microscope using the trypan blue exclusion assay. The nickel and titanium concentrations of the extracts were determined by ICP atomic emission spectrometer (Shimadzu, Japan). Sterilized nickel titanium alloys were also tested for an animal allergy test. The nickel titanium alloy was transplanted under dorsal skin of mouse. 28 days later, nickel solution was injected into the auricularis skin and the degree of turgid auriculae was determined.
Results: The nickel concentration of the extract did not correlate with the titanium ratio to nickel simply, but with the structure of nickel titanium alloy. In cellular assay of MOLT-3, the viable number of the cells cultured with the extracts of 5% or 50% titanium content nickel alloy was significantly more than that of the cells done with the extracts of nickel plate. In the allergy test, transplanted 50 wt % titanium - nickel alloy was down-regulated the allergy reaction in mice.

P1.23. SELF-ASSEMBLING HYDROGELS FOR 2D AND 3D CELL CULTURE

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Introduction Stem cells isolated from fat tissue are increasingly used in regenerative medicine. Their easy accessability, differentiation potential and immunomodulatory properties makes them ideal cells for the restoration of damaged or lost tissue. Hydrogels can function as multifunctional scaffolds in various approaches of tissue repair (TR), either as a filler, a slow-release depot for growth factors or a delivery vehicle for (stem) cells. Hydrogels can be prepared from natural ECM proteins or synthetic materials. The advantage of using synthetic materials is that there are no batch-to-batch differences, they have a defined composition and are in general easy to manipulate. Our synthetic self-assembling gels display a nanofibrous structure resembling the *in vivo* ECM structure of collagen fibers. In addition, they allow for easy functionalisation with ECM peptides and cell encapsulation. Therefore, we aim at developing novel (stem) cell-supporting coatings and hydrogels for 2D and 3D cell culture *in vitro* and for (stem) cell-delivery *in vivo*.
Materials & Methods Coatings and hydrogels were prepared from different types of gelator molecules. All gelators share a common core structure consisting of a 1,3,5-cis cyclohexane tricarboxylic acid core. The rigid-core predisposes the arms towards self-assembly resulting in fiber formation and gelation (Fig.1A&B). Gelators with different cell-adhesive properties were obtained by conjugating different peptidic arms to the three arms of the core structure [1,2]. The self-assembling character of the gelators allows for easy encapsultion of structures like micelles or cells[3].

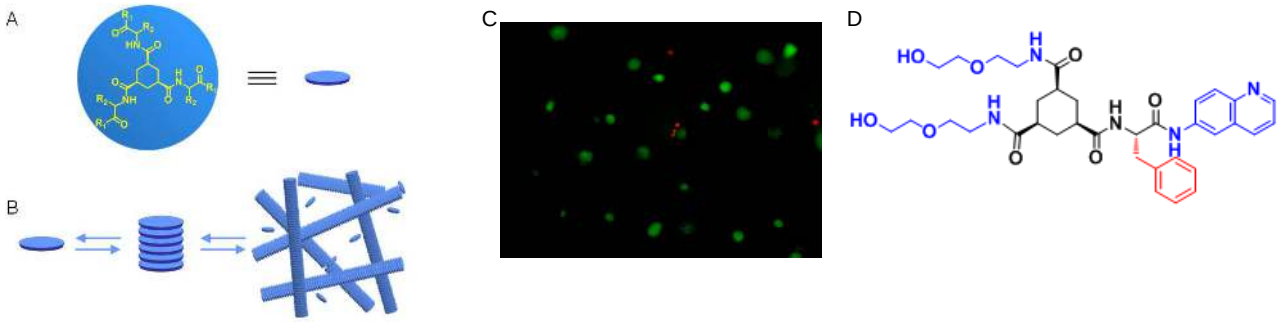

Figure 1. *A) Generic representation of low molecular weight gelator and B) Process of fiber-formation and gelation.* ***C)*** *Microscopy image of live-dead staining of ADSCs cultured in a matrix of D) a low molecular weight gelator.*

Characterization of the cells on the coatings and in the gels is done by assessing 1) the morphology of the cells by light microscopy and staining 2) the viability of the cells by staining and biochemical assays and 3) gene expression by PCR.
Results & Discussion The gelators were either applied as coatings or as 3D-hydrogel matrices in well-plates. The growth of different cell types on scaffolds of these gelators was studied, ranging from cell-lines (*e.g.* HepG2 liver carcinoma, ATDC5 murine chondrocytic cells and CHO-K1 cells) to primary cells like human adipose-derived stem cells (ADSCs), cardiac and neuronal cells derived from human embryonic stem cells and human primary hepatocytes. It appeared that cellular function can be maintained on coatings as well as in hydrogels and that cellular function can be influenced by the choice of gelator.These studies showed that our gelators are suitable materials for 2D as well as 3D cell-culturing. Figure 1C shows a microscopy image of ADSCs in a 3D hydrogel of one of our gelators (Fig. 1D).
Conclusion Cell-culturing on 2D-coatings or in 3D-hydrogels showed that our materials are excellent scaffolds for the growth of different cell-types. Primary cells like human ADSCs, ESCs and hepatocytes as well as different cell lines (e.g. HepG2, ATDC5 and CHO-K1) were growing well on our coatings and in our gels. Therefore, our materials offer unique possibilities for use in e.g. long-term toxicity assays for drug screening (2D) and as cell-seeded scaffolds (3D) in tissue engineering.

[1] K.J.C. van Bommel *et al.*, *Angew. Chem. Int. Ed.* **2004**, *43*, 1663-1667.
[2] A. Brizard *et al.*, *Angew. Chem. Int. Ed.* **2008**, *47*, 2063-2066.
[3] A. Heeres *et al., JACS* **2003**, *125*, 14252-14253

P1.24. APPLICATION OF AUTOMATED IMPEDANCE SPECTROSCOPY FOR MEASURING CELLULAR DIFFERENTIATION

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Cancer cell lines normally have lost their differentiation state in order to proliferate continuously. Therefore they are used as cell lines. Some cancer cell lines however are able to redifferentiate in vitro and form epithelial barriers, like the colon cell line CaCo-2. Here we report on a human airway epithelial cell line, Calu-3, cultured in transfiltersystems to build up a bronchoepithelial cell layer. The formation of the cellular barrier was monitored either by the measurement of transepithelial electrical resistance (TEER) day to day manually with EndOhm Voltohmmeter technology or continuously with automated impedance spectroscopy by CellZscope for two weeks. Finally the cell layers were prepared for transmission, scanning and light microscopy analysis.
Differentiation of the cells turns them from proliferation to build up a cellular barrier. This process was monitored either by measuring increasing transepithelial resistance or reducing impedance values. Continuous measurement of impedance displayed several advantages: The CellZscope device allows monitoring from outside the incubation chamber. So the cultures could remain in the incubator and differentiation was not disturbed by a temperature and pH-shift. When cells had to be removed from the incubator for individual TEER measurements by an EndOhm electrode a considerable decline of TEER was reported after each reading point. In addition the EndOhm procedure was time-consuming. After 4 days in culture, cells in a CellZscope device displayed TEER values of over 250 Ohm per cm^2 and differentiation was confirmed finally by morphological criteria. Cells formed a tight barrier of polarised cells with apical cilia. Tight junctions were formed between adjacent cells forming a tight junction belt visible in freeze-fracture preparations with scanning electron microscopy. These differentiated epithelial cell layers can then be combined with other cell lines in the basal compartment of the transfiltersystems to install coculture assays for cytotoxicity testing.
Preliminary cellular tests were performed using chemical agents like SDS (sodiumdodecylsulphate) or inflammation inducing agents like interleukin-4. Incubation of differentiated cell layers with toxic samples or inflammation inducing agents was monitored and displayed more precise data with CellZscope compared to EndOhm. Differentiated Calu-3 cells can be used to study the influence of drugs, toxins or nanoparticles on the cell layer and differentiation. In cocultures a cross-talk between different cell lines mimics the situation in vivo more detailed than epithelial cells tested alone.

P1.25. MITOGENIC EFFECT OF SERICIN ON MAMMALIAN CELLS

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In *in vitro* cell culture, fetal bovine serum (FBS) or other mammal-derived factors are extensively supplemented as growth factor. However, supplementing mammal-derived factors arouses the concern about the risk of zoonosis. Therefore, serum- and mammal-free culture is strongly required in the industry of antibody therapeutics production and in regenerative medicine including cell therapy. We focused on sericin hydrolysates, originating from silkworm, and reported that sericin is effective as growth factor to various cells and successfully developed a mammal-free medium, Sericin-GIT (Wako Pure Chemical Industries, Ltd., Osaka, Japan).
But it is not elucidated how sericin induces the proliferation and inhibits apoptosis of the cells. In the previous ESACT meeting, we performed cDNA microarray analysis and the involvement of *Src*, *Ras* and *MEK1/2* was suggested. In this study, inhibitor assay was done in order to identify signaling factors involved in sericin effect. Since *Ras* and *MEK1/2* are signaling factors in Map kinase pathway, okadaic acid, a specific inhibitor against PP2A phosphatase involved in Map kinase signaling pathway, was used in order to determine the involvement of them.
From the cDNA microarray analysis, several other genes were also supposed to be affected by sericin treatment. Among the genes, *myc* and *stat1* are the lower signaling factor of *p38* and so SB239063, a specific inhibitor against p38, was tested.

P1.26. THE NEUROPROTECTIVE EFFECTS OF ELECTROLYZED REDUCED WATER AND ITS MODEL WATER CONTAINING MOLECULAR HYDROGEN AND PT NANOPARTICLES

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Human brain is the biggest energy consuming tissue in human body. Although it only represents 2% of the body weight, it receives 20% of total body oxygen consumption and 25% of total body glucose utilization. For that reason, brain is considered to be the most vulnerable part of human body against the reactive oxygen species (ROS), a by-product of aerobic respiration. Oxidative stress is directly related to a series of brain dysfunctional disease such as Alzheimer's disease, Parkinson's disease etc. Electrolyzed reduced water (ERW) is a functional drinking water containing a lot of molecular hydrogen and a small amount of platinum nanoparticles (Pt nps). ERW is known to scavenge ROS and protect DNA from oxidative damage [1]. We previously showed that ERW was capable of extending lifespan of *Caenorhabditis elegans* by scavenging ROS [2]. Molecular hydrogen could scavenge ROS and protected brain from oxidative stress [3]. Pt nps are also a new type of multi-functional ROS scavenger [4] .

In this research, we used TI-200S ERW derived from 2 mM NaOH solution produced by a batch type electrolysis device and model waters containing molecular hydrogen and synthetic Pt nps of 2-3 nm sizes as research models of ERW to examine the anti-oxidant capabilities of ERW on several kinds of neural cells such as PC12, N1E115, and SFME cells. ERW significantly reduced the intracellular ROS and prevented the decrease of mitochondrial membrane potential and ATP production induced by ROS. We also examined the neuroprotective effects of molecular hydrogen and Pt nps and showed that both molecular hydrogen and Pt nps contributed to the neuroprotective effects of ERW. The results suggest that ERW is beneficial for the prevention and alleviation of oxidative stress-induced human neurodegenerative diseases.

References
1. Shirahata S, Kabayama S *et al.*: Electrolyzed-reduced water scavenges active oxygen species and protects DNA from oxidative damage. *Biophys Biochem Res Commun* **234**, 269-274 (1997).
2. Yan H, Tian H *et al.*: Extension of the lifespan of *Caenorhabditis elegans* by the use of electrolyzed reduced water. *Biosci Biotech Biochem* **74**, 2011-2015 (2010).
3. Ohsawa I, Ishikawa M *et al.*: Hydrogen gas as a therapeutic antioxidant by selectively cytotoxic oxygen radials. Nature Med **13**, 688-694 (2007).
4. Hamasaki T, Kashiwagi T *et al.*: Kinetic analysis of superoxide anion radical-scavenging and hydroxyl radical-scavenging activities of platinum nanoparticles. *Langmuir*, **24**, 7354-7364 (2008).

P1.27. SYNTHETIC STIMULUS-RESPONSIVE BIOMATERIALS FOR DIRECTED CELL GROWTH AND DIFFERENTIATION

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Biological systems, such as the skin, the connective tissues, or the bones within the skeleton are complex assemblies, the regeneration and repair of which occurs under spatially- and temporally- controlled orchestration by a myriad of specialized cells and signals. In many of these cases, a complete regeneration cannot be achieved by the organism itself. Therefore, biomaterials are used as carriers or matrices for supportive treatments. They are designed to fill existing cavities and to prevent the loss of tissue. However, efficacy of many current biomaterials is limited by a lack of multi-functional structures to complement the inherent dynamics of these biological systems.

We present a novel platform of stimulus-responsive biomaterials that are able to confer the appropriate dynamic matrix for controllable cell growth and differentiation. In order to integrate the inducible dynamics into our hybrid hydrogels, we combine molecular synthetic biology tools in the form of small-molecule inducible protein interaction domains with hydrogel polymer technologies from the field of material sciences. The combination of both aspects allows for dose-and time-dependent dissolution of the hydrogel and release of incorporated biomolecules such as growth factors, cytokines and antibodies in response to a small-molecule inducer.

The presented biomaterial acts as a matrix that mimics the dynamics present in vivo to promote directed cell growth and further differentiation. The setup allows for incorporation and dose- and time-adjustable release of specific growth factor combinations tailored to the respective treatment leading to possible applications in tissue engineering, regenerative medicine and stem cell research.

P1.28. STANDARDISATION OF ADCC ASSAYS BY USE OF A NK CELL LINE MODIFIED TO EXPRESS CD16

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ADCC (antibody dependent cellular cytotoxicity) is a major mode of action of monoclonal antibodies and therefore a necessary part of their pharmaceutical development. Current ADCC assays depend on primary NK effector cells which limit the number of samples that can be analyzed in parallel. Moreover, successful monoclonal antibody testing using primary NK effector cells is strongly dependent on the frequency and activity of the isolated effector cells. We therefore devised a novel human transgenic NK cell line as substitute for primary effector cells in ADCC assays.
In several *in vitro* test systems human tumor target cell lines with a high expression level of tumor antigens (HER-2/neu, CD20) are mixed with transgenic NK effector cells and serial dilutions of Trastuzumab (anti-HER-2/neu) or Rituximab (anti-CD20) in 96-well format. Tumor target cell killing is measured after four-hour incubation by XTT. The loss of target cell viability indicates killing of the target cells by the transgenic NK cell line. Alternatively tumor target cells are loaded with calcein-AM and killing activity is indicated by calcein release. Furthermore, we compare the ADCC assay performance using either the transgenic NK cell line or primary NK cells freshly isolated from whole blood or aphaeresis under standardized conditions. The transgenic NK cell line is thawn from primary or secondary cell banks and kept under standardized cell culture conditions until use.
The transgenic NK cell line shows phenotypical and functional stability. Moreover, an antibody dose-dependent, effector-to-target cell ratio dependent and time dependent killing of tumor target cells with high precision and accuracy is found. In conclusion, the high reproducibility, precision and accuracy of ADCC assays using our transgenic human NK cell line as effector cells isolates the potential of antibodies to mediate ADCC from all other variables.

P1.29. DAPHNANE DITERPENE HIRSEIN B DOWNREGULATES MELANOGENESIS IN B16 MURINE MELANOMA CELLS BY CAMP PATHWAY INHIBITION

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Skin pigmentation serves as protection against ultraviolet (UV)-induced skin damage through its optical and chemical filtering properties. Although melanin plays and important role in skin protection, excessive melanin production or hyperpigmentation may lead to skin cancer. Recently, the inhibition of melanogenesis has been considered as a valid therapeutic target for the management of advanced melanotic melanomas which increases the need for melanogenesis inhibitors that are of plant origin and are not cytotoxic to mammalian cells. The biosynthesis of the pigment melanin is catalyzed by the melanogenic enzymes tyrosinase, tyrosinase related protein 1 and the dopachrome tautomerase, the transcriptional regulation of which is being regulated by the microphthalmia associated transcription factor (*Mitf*).

We have previously reported that daphnane diterpene hirsein B or 5β-hydroxyresiniferonol-6α,7α-epoxy-12β-coumaroyloxy-9,13,14-*ortho*-decanoate from *Thymelaea hirsuta* has antimelanogenesis effect (without cytotoxicity) on B16 murine melanoma cells by downregulating the expression of the *Mitf* gene and the melanogenic enzymes' genes. Although the effect of hirsein B on the *Mitf* gene is already known, the exact mechanism by which hirsein B inhibited the *Mitf* gene expression has not yet been determined. In melanogenesis, the *Mitf* gene expression can be regulated through the cAMP pathway or the Wnt signaling pathway.

DNA microarray analysis was performed to determine the transcriptional response of B16 murine melanoma cells to hirsein B treatment. DNA chips of 528 spots loaded with 265 genes prepared by Genopal™ (Mitsubishi Rayon Co., Ltd, Tokyo, Japan), were used to determine the expressions of genes for melanogenesis, membrane-bound receptors, tyrosine kinase regulation, melanosome transport, and other cell signal regulation-related genes (including the housekeeping and negative control genes). Results show that the expression of the *Mitf* gene and the melanogenic enzymes' genes were downregulated, verifying our previous report. In addition, the expression of the gene for melanocortin 1 receptor (*Mc1r*) of the cAMP pathway was downregulated while most of the genes that were upregulated are those involved in the Wnt signaling pathway. The results obtained suggest that the significant antimelanogenesis effect of hirsein B is through the inhibition of the cAMP pathway.

P1.30. THE MECHANISM OF APOPTOSIS INDUCTION IN MCF-/ HUMAN BREAST CARCINOMA CELLS BY LOW MOLECULAR WEIGHT FUCOIDAN EXTRACT

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Fucoidan, known as a fucose-rich sulphated polaysaccharide, is an active component extracted from brown seaweed and das a variety of physiological functions. Accumulative evidences have supported that low molecular weight fucoidan extract (LMFE) prepared by enzymatic digestion has preventive effects against various types of cancer both *in vitro* and *in vivo*. Here, MCF-7 human breast carcinoma cells were used to investigate the action mechanism of LMFE as an apoptotic cell death inducer. To this aim, we have examined caspoase family activation as well as mitochondira-dependent apoptotic factor expression and ROS-dependent phosphorylation of MAPK kinases in response to LMFE.
Our results showed that LMFE induced caspase-independent apoptosis in MCF-7 cells, which was accompanied by DNA fragmentation, nuclear condensation and phosphatidylserine exposure. Furthermore, LMFE induced the loss of mitochondrial membrane potential by regulating the expression of Bcl-2 family members. Loss of □□m led to the mitochondria release of cytochrome C and apoptosis-inducing factor (AIF) which subsequently translocated into the nucleus and induced chromatin condensation and DNA fragmentation. Additionally, LMFE induced phosphorylation of JNK, p38 and ERK1/2 MAPKs and apoptotic cell death was attenuated by the inhibition of JNK MAPK. Finally, LMFE mediated apoptosis involving ROS generation which was responsible for the decrease of □□m and phosphorylation of JNK, p38 and ERK1/2 MAPKs. Taken together, these results suggest a model for the caspase-independent apoptosis induced by LMFE in MCF-7 human reast carcinoma cells based on sequential of ROS generation, MAPKs activation, bcl-2 family mediated-mitochondrial dysfunction and AIF release.

P1.31. CHARACTERISATION OF CULTIVATION OF THE HUMAN CELL LINE AGE1.HN

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Human cell lines are an interesting alternative to CHO cells for the production of recombinant proteins and monoclonal antibodies, because of their ability to produce genuine human posttranslational modifications. The alpha-1-antitrypsin producing human cell line AGE1.HN (ProBioGen, Berlin, Germany), that originated from human neural precursor tissue, has been adapted to serum-free conditions and cultivated in many different systems. In this work we present our experience with this cell line in a scale-up of batch cultivation from 50 mL vented polypropylene tube on a shaking platform, polycarbonate shaking-flask (cultivation volume from 50 mL up to 300 mL), a 2 L glass vessel stirred-tank-reactor and a 20 L stainless steel stirred-tank-reactor (both Sartorius Stedim, Goettingen, Germany).
Cell growth in a batch-cultivation in a 2 L glass vessel is very similar to a 20 L stainless steel reactor, with a maximum viable cell density from 3.5 to 5 E6 viable cells per milliliter, while using chemically-defined and animal component-free 42MAX-UB media (Teutocell AG, Bielefeld, Germany). Furthermore, chemostat cultivation could be established with these cells in 0.5 L bioreactor (DasGip, Juelich, Germany) with a media exchange rate of 7 mL/h. The process exhibited constant growth over two weeks with a nearly constant viable cell density of 2.5 E6 cells per milliliter. As a further cultivation system a dialysis reactor (Bioengineering, Wald, Switzerland) was established, with a 1.3 L cell-containing inner chamber and a 4 L media reservoir in the outer chamber, separated by a semipermeable dialysis membrane. Batch cultivation without media-exchange in the outer chamber showed maximum cell density up to 1.6 E7 viable cells per milliliter in the inner chamber.
The above mentioned approaches prove the possibilities for successful cultivation of the human cell line AGE1.HN, including a scale up to 20 L cultivation volume making it a relevant alternative to other established production cell lines.
This work is a part of the SysLogics Project: Systems biology of cell culture for biologics, founded by German Ministry for Education and Research (BMBF).

P1.32. ADVANTAGES OF THE PER.C6® HUMAN CELL LINE AS THE HOST FOR MANUFACTURING THERAPEUTIC PROTEINS

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PER.C6® cells have produced 10 gram/liter of several monoclonal antibodies in our platform fedbatch process. This platform fedbatch process and another platform process that we used to generate and select clones can be readily applied to other monoclonal antibodies. Given the human cell origin, PER.C6® cells may NOT produce proteins with immunogenic antigens, such as alpha-Gal and N-glycolylneuraminic acid that are found in the traditional murine expressions systems (e.g. CHO, NS0 and SP2). FDA has shown increasing concerns about the presence of these two antigens on the therapeutic proteins; therefore creating proteins without these antigens becomes increasingly important. PER.C6® cells provide a solution to this problem and in the mean time give you a superior protein yield. Examples of a number of expressed molecules and advantages over traditional expressions systems, such as CHO, will be presented.

P1.33. CAP TECHNOLOGY: PRODUCTION OF BIOPHARMACEUTICALS IN HUMAN AMNIOCYTES

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Addressing the needs of biopharmaceutical manufacturing, CEVEC has developed a human cell expression system for the production of safe and effective biopharmaceuticals. Although the current animal cell-based expression technologies for proteins are commercially proven platform with a widely recognized robustness and productivity, a human cell-derived expression technology offers significant advantages in quality, serum-half life and safety. Whereas the expression of very complex proteins demand authentic human glycosylation patterns, also antibody expression technologies rise to the challenge for high titers and for avoiding non-human glycan structures.
Based on primary human amniocytes, the CAP technology has been generated in order to reach competitive yields and superior posttranslational modification patterns. The stable producing CAP and the new transiently producing CAP-T cells meet all regulatory guidelines, they are of non-tumor origin and from ethically accepted source.
CEVEC is routinely using its CAP technology for expression of biotherapeutics and we will present results exemplarily from a very complex, highly glycosylated protein but also from antibodies. We have optimized expression plasmids in order to achieve high levels and ensure consistent performance. We have developed defined protocols for cell line development in order to simplify and shorten the development time lines. Applying these improvements, we have produced very high titers for both antibodies and very complex proteins, with authentic human glycosylation patterns, very short development time lines and thus optimized cost efficiency.
The combined use of the CAP-T cell, a new version of CAP for transient protein expression for very early discovery or preclinical evaluation with the CAP cells for stable expression up to clinical supply offer the advantage of a one stop protein expression solution with identical genetic origin, high titers, authentic human glycosylation and shortened time lines for production of safe and effective therapeutic proteins.

P1.34. CAP-T CELL EXPRESSION SYSTEM – A NOVEL RAPID AND VERSATILE HUMAN CELL EXPRESSION SYSTEM FOR FAST AND HIGH YIELD TRANSIENT PROTEIN EXPRESSION

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Human CAP (CEVEC's Amniocyte Production) cells show high expression levels even for very complex proteins, they are mainly used for stable production of recombinant proteins with excellent biologic activity and therapeutic efficacy as a result of authentic human posttranslational modification.
Based on human CAP cells for stable production, a new transient expression system CAP-T has been developed. CAP-T cells stably express the SV40 large T-Antigen and thus transfection of plasmids containing the SV40 origin of replication results in very high plasmid copy numbers. CAP-T cells show fast grow in serum-free suspension culture, show very high transfection efficiencies with very high viability, and outperform currently used HEK293 cells and variants in expression levels and process times. Moreover, high levels of very complex proteins with authentic human glycosylation patterns are additional key features of the CAP-T expression system.
The major bottlenecks in transient protein expression are low specific productivity, low transfection efficiency, upscaling, the use of serum-containing media, and either impaired or non-human glycosylation. In order to overcome these limitations we have tested different commercial transfection reagents and optimized parameters for transfection and for the plasmids used. We will present data on upscaling of transfections in bioreactors with optimized medium and feeding strategies. These optimizations enable fast, easy and scalable high titer expression of antibodies and even complex proteins with authentic human glycosylation pattern.
In combination, CAP-T cells for transient and CAP cells for stable expression of proteins offer the advantage of a single platform from transient expression for preclinical evaluation up to stable expression of biotherapeutics for clinical supply.

P1.35. DEVELOPMENT AND VALIDATION OF A MALDI-TOF MS BASED CELL LINE IDENTIFICATION PROTOCOL

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Current methods for the testing of cell line identity are based on molecular approaches such as STR (short tandem repeat)-typing or sequencing technologies. These methods are time consuming and rather expensive.

MALDI-TOF mass spectrometry is an emerging tool for fast routine identification of microorganisms. Identification of such organisms depends upon a validated and comprehensive database of protein mass fingerprints. We have developed an adapted protocol for the reliable identification of insect and mammalian cell lines within minutes. In addition, MALDI-TOF mass spectrometry allows the direct characterization of recombinant expressed proteins in the mass range of m/z 2 – 50 kDa within the same experiment.

We propose this comparatively simple and inexpensive method to be used routinely for ensuring the identity of cell lines employed in the area of biotechnological R&D.

P1.36. A HUMAN SOMATIC HYBRID CELL LINE, F2N, FOR RECOMBINANT PROTEIN PRODUCTION: COMPARISON WITH CHO K1

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Recently, there are many attempts to produce recombinant protein-based pharmaceuticals using human-origin host cell lines. Human cell lines receive attention lately because they can produce recombinant proteins with human glycosylation profiles and produce recombinant proteins which are difficult to be expressed in CHO cells. Among these human host cell lines, F2N, generated from fusion of a human embryonic kidney-derived cell, HEK293, and a human B-cell-derived cell, Namalwa, is one of prospective host cell lines. In this research, we characterized F2N as a host cell line to produce recombinant protein. The characterization of F2N was progressed through comparison with CHOK1. F2N and CHOK1 were cultured in CD Opti CHO medium supplemented with 4mM glutamine. In batch cultures, a maximum cell density of F2N was 5.6 x 10^6 cells/mL, while that of CHOK1 was 9.9 x 10^6 cells/mL. In addition, batch cultures with different osmolalities (270, 330, 390, 450, 510 mOsm/kg) were performed. Among the two cell lines, CHOK1 was more tolerant against hyperosmolality than F2N. In comparison with CHOK1, we observed the advantage of F2N for transient transfection. F2N showed higher transfection efficiency than that of CHOK1. Furthermore, glycosylation patterns of the product protein derived from F2N were different from CHOK1. Taken together, F2N is an efficient host cell line for the transient gene expression system and is a prominent cell line to produce various recombinant proteins as other human-origin host cell lines.

P1.37. THE EB66® CELL LINE: A DUCK-DERIVED EMBRYONIC STEM CELL FOR THE INDUSTRIAL PRODUCTION OF HIGH POTENCY, ADCC ENHANCED MONOCLONAL ANTIBODIES

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Monoclonal antibodies (mAb) represent the fastest growing class of therapeutic proteins. The increasing demand for mAb manufacturing and associated high production costs call for the biopharmaceutical industry to improve its current production processes or develop more efficient alternative production platforms. The experimental control of IgG fucosylation to enhance antibody dependent cell cytotoxicity (ADCC) activity constitutes one of the promising strategies to improve the efficacy of monoclonal antibodies and to potentially reduce therapeutic costs. We report here that the EB66 cell line, derived from duck embryonic stem cells, can be efficiently engineered to produce mAbs at yields beyond 1g/L, in serum-free suspension culture. EB66 cells display additional attractive characteristics such as a very short population doubling time of 12 - 14 hours, very high cell densities (> 30 million cells/mL) and a unique metabolic profile resulting in low ammonium and lactate accumulation with low glutamine consumption, even at high cell densities. Furthermore, mAbs produced in EB66 cells display a naturally reduced fucose content resulting in strongly enhanced ADCC activity. EB66 cells therefore have the potential to evolve as a novel cellular platform for the production of high potency therapeutic antibodies.

P1.38. OPTIMIZATION OF ANTIBODY PRODUCTION PLATFORM BASED ON THE HUMAN CELL LINE HKB11

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MorphoSys' Human Combinatorial Antibody Library (HuCAL®) has proven to be a robust and flexible platform for the selection of high-quality human antibodies. In the process of selecting therapeutic lead antibody candidates, production platforms have to serve multiple requirements from the discovery phase to pre-clinical development.
Various transient expression platforms have been established in house based on the suspension growing human cell line HKB11 allowing fast track production of a large number of different IgG candidates from microgram to gram amounts.
We have improved the transient gene expression platform by systematic optimization of transfection and culture conditions. This resulted in the implementation of a simplified transfection protocol with shortened timelines and increased throughput. In summary, an effective, robust and easy to handle platform for the delivery of high quality recombinant HUCAL antibodies has been developed.

P1.39. PRODUCTIVITY AND STABILITY FROM REGULATED-EXPRESSION CELL LINES

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Clone productivity and stability are the foundation of commercial cell line development, but, high cell productivity is often associated with low clone stability because the constitutive high-level expression of a secreted recombinant protein can result in a significant burden on normal cellular processes leading to a reduced growth rate. This impact on growth rate provides the means by which cells with reduced expression can increase in proportion relative to high-expressing cells as observed by the decrease in average productivity of the culture. We developed a novel inducible cell expression system that facilitates isolation of clones with high specific productivities and ensures stability by inhibiting recombinant gene expression during culture expansion. The system is amenable to large-scale production, as it does not require any additional medium component or unusual growth condition.

P1.40. GROWTH CHARACTERIZATION OF CHO-DP12 CELL LINES WITH DIFFERENT HIGH PASSAGE HISTORIES

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For industrial pharmaceutical protein production fast growing, high producing and robust cell lines are required. To select more pH shift permissive and fast growing sub-populations, the CHO-DP12 (ATCC clone# 1934) cell line, an anti-IL8 antibody producing CHO K1 (DHFR-) clone, was serially subcultured at high viability (>90 %) for more than four hundred days in shaker flasks. Initial adaption to growth in suspension was carried out in a chemically defined medium without Hypoxanthine and Thymidine (HT), while the medium used for long term cultivation contains HT. Cell samples were cryopreserved at four different time points, after 21, 95, 165 and 420 days. Cultivations of these four sub-populations (SP) in shaker flask and bench-top bioreactor experiments revealed distinct differences in specific growth rates, product formation and metabolism.
During the long-term cultivation the CHO-DP12 cells showed an enhancement of the specific growth rate from 0.18 ± 0.04 d^{-1} after initial suspension adaption to 0.82 ± 0.12 d^{-1} after 100 days in culture. Though the sub-populations SP95, SP165 and SP420 seem to possess equal growth rates, they differ remarkably in their maximum cell density. For SP21 and SP420, maximum cell densities of about $1.1 \cdot 10^7$ cells/mL and $2.1 \cdot 10^7$ cells/mL, respectively, were determined. The highest specific production rate was obtained in the sub-population SP95 with 10.5 pg/(cell·day) resulting in a final antibody titer of 334 mg/L.
Considerable differences between the four sub-populations could be observed in terms of the consumption and formation of glucose, lactate and several amino acids. In bioreactor cultivations a twofold increased lactate formation for SP420 compared with SP21 was found. Furthermore, an increased passage number leads to decreasing growth performance in controlled cultivation system. This effect can be explained by the adaption of subcultivated cells to the pH-shift that occurs during cultivation under uncontrolled conditions.
For the elucidation of the intracelluar mechanism behind these alterations additional samples for proteomic and metabolomic analyses were generated*.

P1.41. PROTEOMIC AND METABOLOMIC CHARACTERIZATION OF CHO-DP12 CELL LINES WITH DIFFERENT HIGH PASSAGE HISTORIES

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For industrial pharmaceutical protein production fast growing, high producing and robust cell lines are required. To select more pH shift permissive and fast growing sub-populations, the CHO-DP12 (ATCC clone #1934) cell line, an anti-IL8 antibody producing CHO K1 (DHFR-) clone, was serially subcultured at high viability (>90 %) for more than four hundred days in shaker flasks. Initial adaption to growth in suspension was carried out in a chemically defined medium without hypoxanthine and thymidine (HT), while the medium used for long term cultivation contains HT. Cell samples were cryopreserved at four different time points, after 21, 95, 165 and 420 days. Cultivations of these four sub-populations (SP) in shaker flask and bench-top bioreactor experiments revealed distinct differences in specific growth rates, product formation and metabolism.*
Beside the comparison of cultivation characteristics (e.g. μ, q_P, q_S, metabolic shifts) samples for proteomic and metabolomic analyses were taken from parallel bioreactor cultivations of SP21, SP95, SP196 and SP420. For the analysis of intracellular metabolites we used our in house developed fast-filtration quenching procedure to generate the samples for GC-MS and LC-MS measurements. Changes in the protein expression were analyzed by differential two-dimensional gel electrophoresis (DIGE). The proteomic experiment revealed 43 out of 1377 overall reliable detected protein spots with a different protein expression for at least one sub-population (ANOVA, $n = 4$, $\alpha \leq 0.05$, false significant proportion: 0.01 or less). The detected differences between the analyzed groups increase with their number of passages. Between SP21 and SP95 there were only three protein spots detected with a fold change higher or equal to two. For SP196 and SP420 seven and 41 spots show a ratio with an absolute value of two in comparison with SP21, respectively. By merging the proteomic findings with the measurements of intracellular metabolite pools we gained a better understanding of factors which make a production cell line faster growing and more robust against pH shifts.

P1.42. EVALUATION OF THREE COMMERCIAL KITS FOR MYCOPLASMA NAT ASSAYS: SELECTION AND QUALITY IMPROVEMENT

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Mycoplasma testing of cell lines and biological products was performed for a long time with classical methods including culture medium and indicator cell culture. However, these methods required long incubation period and are sometimes not adapted for the detection of Mycoplasmas in particular samples, like vaccine viruses which can not completely be neutralize or products with short shelf life. The European Pharmacopoeia updated few years ago the 2.6.7 section by adding the detection of Mycoplasma with NAT methods. Nat assays have several advantages including rapid-time to results, robustness and sensitivity.
Texcell's offers for Mycoplasma testing, that already included classical methods, were incremended with NAT assay. For this purpose, we evaluated 3 commercial kits based on NAT assay:

MycoDtect™ from Greiner bio-one
MicroSeq[R] from Life Technologies
MycoTool from Roche

These 3 commercial kits were selected based of their claim to meet the European Pharmacopeia guidance for nucleic acid amplification techniques for Mycoplasma testing, including sensitivity and range of detection.
Results were obtained at Texcell by comparing the detection of different Mycoplasma species with low spiking level. One kit was selected and we report its successful used with biotechnological samples. For GMP and GLP services, an additional control consisting of viable Mycoplasmas was included to improve quality control.

P1.43. PHYSICAL METHODS FOR SYNCHRONIZATION OF A HUMAN PRODUCTION CELL LINE

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The study of central metabolism and the interaction of its dynamics during growth, product formation and cell division are key tasks to decode the complex metabolic network of mammalian cells. For this purpose, not only the quantitative determination of key cellular molecules is necessary, but also the variation of their expression rates in time, e.g. during cell cycle dependent gene expression. Thus, synchronization of cultured cells is a requisite for almost any attempt to elucidate these time dependent cellular processes. Synchronous cell growth can help to gain deeper insight into dynamics of cellular metabolism.
In this work, physical methods for synchronization of the human production cell line AGE1.HN (ProBioGen AG) have been experimentally tested. Cell size distribution, DNA-content and the number of synchronous divisions after resumption of growth conditions have been used for comparison of the methods.
According to our results, the enrichment of an AGE1.HN cell population within a cell cycle phase is possible. Currently, conditions needed for optimal cell growth resumption after synchronization are being studied.
This work is a part of SysLogics: Systems biology of cell culture for biologics, a project founded by the German Ministry for Education and Research (BMBF).

P1.44. STABILITY OF RECOMBINANT PROTEIN PRODUCTION IN AMPLIFIED CHO CELLS

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Therapeutic proteins require proper folding and post-translational modifications (PTMs) to be effective and biologically active. Chinese hamster ovary (CHO) cells are the most frequently used host for commercial production of therapeutic proteins and DHFR-mediated gene amplification is extensively applied to generate cell lines with increased protein production. However, decreased protein productivity is observed unpredictably during the time required for scale-up with consequences for yield, time, finance and regulatory approval. Ubiquitous Chromatin Opening Elements (UCOEs) are DNA elements naturally found upstream of specific housekeeping genes, which are proposed to maintain open chromatin structure, supporting stable and high-level transgene expression by prevention of transgene silencing. In this study we have examined the interaction between UCOE and DHFR-linked amplification in relation to cell expression stability. CHO-DG44 cell lines were engineered to express erythropoietin (EPO) or a green fluorescent protein (GFP) from constructs with or without the inclusion of a UCOE. Cell lines were amplified in the presence of 250 nM metotrexate (MTX)and were then grown continuously for 70 days in the presence and absence of MTX . Growth characteristics, protein expression, plasmid copy numbers, mRNA expression, karyotype and recombinant gene localisation (by fluorescent in situ hybridisation - FISH) were assessed for cells at stages throughout the period of long-term culture. In summary the inclusion of UCOE elements generated cells that;

- achieved higher cell densities and exhibited increased production of recombinant mRNA/cell and protein yield
- allowed isolation of greater numbers of high producing clones
- resulted in greater mRNA recovery/recombinant gene copy
- retained stable mRNA and protein expression after amplification provided MTX was present (but not in the absence of MTX when instability was observed)
- exhibited a different pattern of telomeric and non-telomeric recombinant gene FISH localisation

We conclude that the inclusion of UCOEs within expression constructs offer significant advantages for certainty of cell line generation (and the number of recovered clones for more detailed characterisation/optimisation) and that UCOEs are compatible with DHFR amplification protocols. Our data identify that selective integration may relate to the favourable transcriptional activity of UCOE-containing vectors and that environmental effects allow enhanced cell line recovery by transcriptional enhancement of selection markers, such as DHFR.

P1.45. A NEW REPORTER CELL CLONE TO DETERMINE THE BIOLOGICAL ACTIVITY OF TYPE I INTERFERONS

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Interferons (IFNs) are potent biologically active proteins synthesized and secreted by somatic cells of all mammalian species. They play an important role in the immune response and defence against viruses because they have an antiproliferative, antiviral and immunomodulatory activity. They are widely used as biopharmaceuticals, so their potency must be correctly identified. Usually, the biological activity is quantified by a bioassay based on its capacity to induce an antiviral state in target cells. Antiviral assays may be subject to some variability and the virus must be titrated. The elucidation of the mechanisms of cytokine-induced gene transcription led to the setting up of highly specific and sensitive bioassays, called reporter gene (RG) assays. The RG assays rely upon a cell bearing the receptor for the cytokine of interest, transfected with a plasmid carrying a reporter gene under the control of a cytokine inducible promoter. In the last few years, several specific and sensitive RG assays have been developed.
In this work we generated WISH-Mx/eGFP reporter cell line to determine type I IFNs activity. WISH cells were stably transfected with the enhanced green fluorescent protein (eGFP) gene under the control of type I IFN-inducible Mx promoter. The percentage of eGFP expressing cells accurately correlates to the amount of type I IFN added to the culture and can easily be monitored. This system has several advantages when compared to antiviral activity assays and other reporter genes systems: it can determine the potency of all type I IFNs using only one cell line; it is a very fast assay, showing the highest expression of eGFP after 52 h; the expression of the eGFP reporter gene in WISH-Mx/eGFP cells is observed only in cells treated with type I IFNs, proving the specificity of the Mx promoter. It is a sensitive and safe assay, as the cell line showed a reproducible response in a dose-dependent manner between 0.32 and 750 IU/ml and 1.2 and 1,400 IU/ml for hIFN beta and alpha, respectively. The clone showed the same response along fifty generations, confirming the stability of the Mx/eGFP constructs incorporation into the cells. The inter- and intra-assay variation coefficient was lower than 20%, demonstrating the reproducibility of the assay. In conclusion, we have developed an alternative reporter system for the analysis of type I IFNs, in which the performance of the assay using WISH-Mx/eGFP line, together with its simplicity, speed, low cost, precision, sensitivity and safety make it a suitable candidate to replace conventional bioassays that are currently employed to measure IFNs potency.

P1.46. FAST AND FLEXIBLE GENERATION OF CELL LINES FOR PRIMARY DRUG SCREENING

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G-protein coupled receptors (GPCRs) are the most important drug targets today. In large-scale drug screening campaigns compounds are screened for their ability to modulate the activity of the GPCR. For this purpose appropriate cell lines which stably express the GPCR are required. The downstream signaling of a GPCR is complex and is influenced e.g. by the type of g-protein, by heterodimerization events or by desensitization through phosphorylation. Therefore it is desired to introduce into a drug screening cell line more than one component of the GPCR signaling.
We used our SCREEN*flex* "master cell lines" for the generation of stable cell lines suited for drug screening campaigns. The master cell lines are optimized for the expression of membrane-bound proteins and can be used to integrate any target by a molecular "cut and paste" process (recombinase mediated cassette exchange). This process is rapid which allows the generation of stable cell lines within 2-4 weeks. The high efficiency of this technology was proven by the establishment 15 different GPCR expressing cell lines within six months. In addition, we demonstrated that the SCREENflex technology can be successfully used for the establishment of stable cell lines expressing two components (GPCR and reporter system).
Therefore the SCREENflex technology allows generating sophisticated screening cell lines with little effort and within a short timeframe.

P1.47. GENERATION OF NOVEL CELL LINES FROM BONE MARROW STROMA FOR THE INVESTIGATION OF NORMAL AND LEUKEMIC HAEMATOPOIESIS

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A well-regulated haematopoiesis is dependent on the direct interaction of a haematopoietic stem (HSC) or progenitor cell with stromal cells in the bone marrow (BM) microenvironmental niche and the exchange of soluble mediators. Among other cell types mesenchymal stem cells (MSC) are found in the BM in a considerable frequency. These adult stem cells are able to differentiate into various cell types such as osteoblasts, adipocytes, and chondrocytes and can self-renew. In murine systems MSC have been shown to represent a central component of the stem cell niche for appropriate HSC function. Cumulative evidence indicates that in haematopoietic malignancies the BM stroma is altered in a way to support neoplastic cells at the expense of normal haematopoietic differentiation. Thus, comparative study of normal vs. leukemic stroma with more standardized tools might contribute to the understanding of mechanisms of malignant transformation and, at best, define novel therapeutic targets in the stroma compartment.
To this end, we established novel cell lines from BM stroma of two patients suffering from acute myeloid leukemia, two lymphoma patients without malignant infiltration of the BM, and, in addition, from the BM of a healthy donor. Plastic-adherent primary BM cells were cultured under conditions favouring outgrowth of fibroblasts. After transduction with different immortalizing genes we established five independent polyclonal cell lines. These cell lines showed a robust proliferation and expressed surface markers reminiscent of MSC. Two of the cell lines were also able to differentiate into osteoblasts, adipocytes, and chondrocytes. With regard to cell lines derived from leukemic and non-leukemic stroma a differential expression pattern was observed for membrane-anchored and soluble molecules HLA-G, MICA, MICB, ULBP2, ILT-4 known to be implicated in the suppression of the innate and adaptive immune system. These novel bone marrow stroma cell lines are considered as a robust and reliable *in vitro* test system to investigate the microenvironment required for the comparative investigation of leukemic vs. normal haematopoiesis.

P1.48. CULTIVATION STRATEGIES OF A BA/F3 CELL LINE FOR FUNDAMENTAL CELL RESEARCH

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For fundamental studies often a high number of cells is required, which is difficult to obtain reproducible with flask cultures or roller bottles. The aim of this project was to develop and establish a reproducible bioreactor cultivation of murine suspension cell lines (BA/F3 p210), which yields a total cell number close to $1 \cdot 10^{10}$ during exponential growth at constant culture conditions.
A small stirred tank bioreactor with a working volume of 150 ml (Vario1000, Medorex, Germany) was used to study and compare different operation modes: batch, fed-batch and continuous. Cell growth and glucose consumption were followed as main culture parameters. Compared to t-flasks, glucose uptake during bioreactor cultivation was much higher, which led to minor final cell density yields. Fed-batch and continuous modes were firstly favored due the theoretical final cell numbers reached during culture. However, the difference in growth, limitation of bioreactor volume and the need of a special formulation for higher cell densities during fed batch limited the final yield. Continuous mode with temperature reduction of harvested cells allowed for constant cell production in exponential phase. On the other hand, intact cell storage was limited probably due to protease action. The 150 ml batch cultivation was scaled up to 5 L in a stirred bench top bioreactor (Biostat B, Sartorius). Regarding the required reproducibility for cultivation, the 5 L batch mode was preferred over t-flasks due to the possibility for control of process variables like pH and pO_2.

P1.49. COMPARISON OF DIFFERENT MEMBRANE SUPPORTS FOR MONOLAYER CULTURE OF BOVINE OVIDUCT EPITHELIAL CELLS

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Introduction: The oviduct epithelium consists of ciliated and secretory cells which play an important role in key reproductive processes such as sperm capacitation, fertilization and early embryonic development [1,2]. Bovine oviduct epithelial cells have been widely used in co-culture experiments to condition culture media and improve early embryonic development [3]. However, these cells dedifferentiate in monolayer culture and manifest alterations like loss of cilia and secretory granules, reduction of cell height and flattening on the culture surface [4,5]. The aim of this study was to compare three different membrane supports for their potential to maintain ultrastructural features and monolayer integrity during *in vitro* culture of bovine oviduct epithelial cells.
Methods: Oviducts were excised from the genital tracts of cows slaughtered in abattoir. After mechanical isolation, the oviduct epithelial cells were cultured on three different membrane supports in the presence of TCM-199 medium supplemented with 10% fetal bovine serum. In this study, we compared three different collagen coated membrane supports i.e. polyester membrane support (Thincert™, Millipore Inc. USA), polytetrafluoroethylene membrane support (Transwell™, Corning Inc. USA) and cellulose ester membrane support (Millicell™, Millipore Inc. USA). The ultrastructural features of oviduct cells in monolayer were examined by scanning electron microscopy while the integrity of monolayer was confirmed by transepithelial electrical resistance and permeability to radiolabeled ^{14}C-Mannitol.
Results: Scanning electron microscopy revealed presence of an intact monolayer of polygonal epithelial cells on polyester (Thincert™) and cellulose ester membrane supports (Millicell™) while no such monolayer was seen in polytetrafluoroethylene membrane support (Transwell™). The confirmation of monolayer integrity by transepithelial electrical resistance showed no significant difference between the three membrane supports ($P>0.25$, Kruskal-Wallis Test). However the test for permeability to radiolabeled ^{14}C-Mannitol showed a tendency for lowest permeability in polyester membrane support (Thincert™).
Conclusion: The potential of polyester membrane supports (Thincert™) to maintain ultrastructural features and ensure monolayer integrity during *in vitro* culture may serve as a model to study the secretory capacity of oviduct cells as well as embryo-maternal interactions.
References: 1) Leese 1988. J Reprod Fertil 82 (2):843-56. 2) Hunter et al., 1991. Tissue Cell 23(5):641-56. 3) Thibodeaux et al., 1992. Arch Pathol Lab Med 116(4):364-72. 4) Walter 1995. Anat Rec 3:347-56. 5) Reischl et al., 1999. Cell Tiss Res 296(2):371-383. 6) Rottmayer et al., 2006. Reproduction 132:637-648.

P1.50. CYANIDIN 3-GLUCOSIDE, ANTHOCYANIN FROM BLACK BEANS PROTECTS INSULIN RESISTANCE ON 3T3-L1 ADIPOCYTES BY INHIBITING TNF-A RELEASE

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Cyanidin 3-glucoside (Cy-3-G), a typical anthocyanin most abundant in black bean was examined for its protective effect on insulin sensitivity in 3T3-L1 adipocytes. Glycerol-3-phosphate dehydrogenase (GPDH) activity and triglyceride (TG) content were increased by Cy-3-G treatment in 3T3-L1 adipocytes. However, Cy-3-G prevents the hypertrophy adipocytes and decreased the amount of reactive oxygen species (ROS) and TNF-α release. Furthermore, the mRNA expression of peroxisome proliferrator-activated receptor-γ (PPARγ), CCAAT/enhancer binding protein α (C/EBPα), fat acid binding protein 4 (aP2), glucose transport 4 (GLUT4) were enhanced by Cy-3-G treatment. In addition, the release of adiponectin, important modulator of insulin sensitivity, was improved by Cy-3-G treatment in 3T3-L1 adipocytes. These findings indicate that Cy-3-G reduced the intracellular ROS level through inhibiting TNF-α release. The decrease of intracellular ROS level contributes the maintenance and improvement of insulin resistance.

In conclusion, we suggest that Cy-3-G prevent insulin resistance in 3T3-L1 adipocytes through the inhibition of TNF-α release, which is in part due to a down-regulation of intracellular ROS and an up-regulation of GLUT4 and adiponectin expression. These effects of Cy-3-G can contribute to the improvement and prevention of metabolic syndrome, type II diabetes, cardiovascular disease, and osteoarthritis.

P1.51. ADCC POTENCY ASSAY: INCREASED STANDARDIZATION WITH MODIFIED LYMPHOCYTES

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For few years now, the use of monoclonal antibodies represents a significant progress in different therapeutic applications. In addition to commercialization of new products, important efforts in research and development have been made to launch new therapeutic antibodies. Many antibodies act through a mechanism of Antibody-dependant cell cytotoxicity (ADCC). National Health Agencies recommend or require use of biological activity assays (potency) in order to characterize those pharmaceutical products. The ADCC assay combines the following 3 elements:

1. The antibody of interest that is specific to a given antigen;
2. The targeted cells that express the antigen of interest at their surface;
3. The effector that can trigger the lysis the targeted cell when the antibody is linked to the antigen.

Given that the usually met ADCC assays use Natural Killer cells, isolated from healthy donors, as effectors they are hardly reproducible. Thus, those assays can barely be validated when lots of pharmaceutical products are released. Furthermore, the rare NK cell lines established in culture don't express the CD16 receptor needed for the ADCC function. In this context, the use of standardized effectors should improve significantly the ADCC assays. Previous work has highlighted that human lymphocytes, modified to express the CD16 receptor, have acquired the ADCC functions. Thanks to our specific knowhow, clones of CD16+ lymphocytes have been produced on a large scale (10^9 cells). The results obtained have pointed out that cells stored in liquid nitrogen and used when they were thawed, were usable for ADCC assays on a reproducible basis. Thanks to that approach two models of ADCC measurement are characterized in the present presentation: CD20 and Her2neu. The produced effector cells constitute a relevant alternative to replace the use of NK cells when the standardization of ADCC potency assay is needed.

P1.52. USE OF CACO-2 CELLS BASED CULTURE SYSTEMS FOR IMPROVED BENEFIT/RISK ASSESSMENT IN PHARMACOLOGY AND HUMAN FOOD

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Caco-2 cells are widely used for both mechanistic research in molecular cell biology as well as in studies aiming at estimating, *in vitro*, the bioavailability of drug candidates. This results largely from the differentiation of these human colon adenocarcinoma cells into enterocytes in classical culture conditions, as well as from the use of bicameral culture inserts including porosity calibrated filters. Developing a model as close as possible to the intestinal epithelium is of high interest for toxicological studies of pharmaceutical compounds. M cells for example have become a promising research field, offering a putative way for oral delivery of nanoencapsulated therapeutic peptides and vaccines The benefit/risk assessment of food substances (*i.e.* nutrients, food supplements, mycotoxins, nanoparticles, microorganisms,…) appears however more complicated than for drugs, partly due to the presence of complex matrices that may decrease their bioaccessibility, but also contain various substances able to interfere with their absorption (*i.e.* nutrients, mucus layer, transport systems, biotransformation enzymes, tight junctions,…) either directly (protein level) or indirectly (gene expression). We currently use or develop different cell culture systems designed for these particular applications:

- monocultures of Caco-2 cells to evaluate intestinal absorption after *in vitro* digestion of the food matrix by salivary, gastric and pancreatic enzymes;
- cocultures of Caco-2 cells and RAW264.7 macrophages to better mimic the interactions involved in an inflammatory process;
- cocultures of Caco-2 and Raji cells to include the effect of M cells in the uptake of e.g. nanoparticles;
- cocultures of Caco-2 and HT29-5M1 cells to circumvent the absence of a mucus layer;
- cocultures of Caco-2 and HepG2 cells to estimate the intestinal and hepatic effects in presystemic biotransformations.

Results will be presented to illustrate the advantages of these culture systems in different situations: effects of phenolic compounds from vegetal extracts or mycotoxins on the intestinal inflammation; effects of fungicides on intestinal biotransformation activities, effects and transport of nanoparticles.
Both the multiplicity and complexity of the interactions that take place at the intestinal level make mandatory the use of *in vitro* models in order to screen potential benefits or risks for human health, but probably more importantly, to elucidate the cellular and molecular mechanisms involved in these phenomena.

P1.53. Long term maintenance of liver-specific phenotype in primary bioreactor cultures of human hepatocyte spheroids

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Maintaining the phenotype of human hepatocytes in long term cultures is a yet unmet need; in this work, we have assembled and validated a bioreactor perfusion system to allow long term culture of functional human hepatocytes; due to its easy sampling, it is possible to follow the time course of an hepatocyte population subject to any hepatotoxic agent and test short term drug interactions (such as CYP450 induction) in a higher throughput format by taking a larger sample and sub culturing it in microtiter plates.
For the last 20 years a considerable amount of literature was published suggesting that 3D primary cultures of hepatocytes outperform 2D culture methods. However, these studies are mostly made using rat hepatocytes and the response of the cells to typical CYP450 inducers (phase I enzymes) is only maintained for approximately 1 week. Human hepatocytes, cultured as spheroids (<200 micrometer diameter) in a fully controlled perfusion bioreactor with easy sampling (working volume 300 mL), maintained Albumin production up to 30 days and CYP450 activity (measured by ECOD biotransformation and gene expression analysis) was inducible up to 2 weeks. Cytokeratin-18, Albumin and CYP450 3A were detected by confocal and 2-photon immunofluorescence microscopy. The data presented herein constitutes a validation of multicellular human hepatocyte spheroids, cultured in perfusion bioreactor, as a novel valuable tool for long-term in vitro drug induction tests; moreover, the possibility of inducing the human hepatocytes either in the perfusion bioreactor or by taking a sample and sub-culturing in 24 well plates proves the flexibility of our system for drug testing studies.

P1.54. 3D-BIOREACTOR CULTURE OF HUMAN HEPATOMA CELL LINE HEPG2 AS A PROMISING TOOL FOR „*IN VITRO* SUBSTANCE TESTING“

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New challenges in drug development and toxicity testing arise from regulatory requirements. Animal trials have to be replaced by cell culture assays, preferably by test systems with human material. Standard 2 D monolayer cultures are often unsatisfactory and therefore long term tissue-like 3 D cultures are suggested as an alternative. In this study in vitro assessment of hepatocyte xenobiotic metabolism was performed in a flow chamber and a miniaturized fixed bed bioreactor. Both reactor systems were characterised by with respect to fluid dynamics.
Cultivation of hepatoma cells (HepG2) cells was carried out in static culture (12-well plate) or dynamically in a flow chamber or a fixed bed bioreactor. Static and dynamic cultivations ofHepG2 cells were compared for their xenobiotic metabolising capacity determined by measuring the EROD (Cytochrome P450 1A1 and 1A2) activity.
Bioreactor cultivation of HepG2 cells was carried out for 2 weeks. Staining with acridine orange and propidium iodide revealed cell clusters of viable cells on the ceramic carriers (Sponceram, Zellwerk) in the fixed bed reactor and on polymeric meshes (Fibracel, New Brunswick Scientific) in the flow chamber. Cell specific activities in functional assays were dependent on type of carrier, time point and cultivation system.
Cell specific activities in functional assays were dependent on type of carrier, time point and cultivation system. The cell line HepG2 is not optimal, but a valuable reference system for further studies. Extended cultivation on 3D carrier systems, especially in perfused systems, is feasible and appropriate for long term studies.

P2.01. ADVANCES IN CRYOPRESERVATION OF ENCAPSULATED STEM CELLS AND NEUROSPHERES

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Cryopreservation of mammalian cells provides unique benefits for many biomedical applications, such as regenerative and reparative medicine, artificial insemination and *in vitro* fertilization. Such applications demand the development of effective cryopreservation methodologies that can cope with the production of banks of well-characterized and safety-tested stocks of a large number of samples. Although effective methods are established for a wide variety of cell types, which are frozen as single-cell suspensions, such currently used techniques fail to assure the retention of high degrees of recovery in complex systems such as three-dimensional (3-D) aggregates, or more sensitive systems such as primary cultures or human embryonic stem cells (hESC).
Previous studies have demonstrated that cell entrapment within a polymeric matrix is a promising approach for the cryopreservation of cell aggregates through protection against mechanical damages during ice crystallisation and reduced disruption of cell-cell contacts [1,2]. Our research has focused on the development of effective, scalable cryopreservation strategies for primary cultures of 3-D brain neurospheres and hESC, based on cell entrapment in clinical-grade, highly purified, ultra-high viscous (UHV) alginate uniformly cross-linked with Ba^{2+} [3], as the main strategy for avoiding the commonly observed loss of cell viability and specific function and disruption of cell-cell/matrix interactions.
Our results have shown that the combination of different strategies based on cell entrapment in UHV alginate and serum-free CryoStor™ solution lead to optimized protocols for the cryopreservation of brain neurospheres and hESC clumps, which enabled the retention of cell membrane, metabolic activity and cell specific function. On the other hand, for the cryopreservation of intact, adherent hESC colonies a novel, surface-based vitrification method [4] has proven to be the best strategy, yielding significantly higher recovery yields, reduced differentiation rates and maintenance of pluripotency when compared with the slow-rate freezing approach.

References

[1] Malpique, R. et al. Tissue Engineering Part C: Methods 15(3): 373-386 (2009).
[3] Zimmermann, H. et al. J. Mat. Sci.: Mat. Med. 16(6): 491 - 501 (2005).
[4] Beier, A.F.J., et al. (2010) submitted.

P2.02. APPLICATION OF JELLYFISH COLLAGEN TO THE SCAFFOLD OF MESENCHYMAL STEM CELLS

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Mesenchymal stem cells (MSCs) can act as a source of reparative cells during injury *in vivo* and therefore have great potential in regenerative medicine. In the culture of MSCs, scaffold play important role for cell behavior and as the scaffold, mammalian-derived collagen has mainly been used. However, those factors have several problems, such as high cost and the concern about the risk of contamination with viruses, abnormal prion and so on. Therefore, novel scaffold as a substitute for mammalian collagen is desired. We focused on jellyfish collagen and investigated the effects of jellyfish collagen on the proliferation and the differentiation of MSCs.

Collagen tested were isolated from jellyfish proteins and separated into hydrosoluble fraction and insoluble fraction. MSCs were isolated from rat bone marrow and the proliferation and the potential to differentiate into osteocyte were investigated using the trypan blue dye exclusion method and Alizarin red O staining. MSCs were significantly proliferated and maintained the potential to differentiate into osteocyte on jellyfish collagen. The proliferation on hydrosoluble fraction of collagen was different from those on insoluble fraction of collagen.

Moreover, the osteogenic differentiation of MSCs was investigated. MSCs were differentiated into osteocyte on jellyfish collagen and the expression level of bone marker genes and the calcium accumulation were evaluated by real-time PCR and Alizarin red O staining, respectively. Osteogenic differentiation was accelerated on insoluble fraction of jellyfish collagen, superior to on pig collagen. These results suggest that jellyfish collagen was not only a substitute of mammalian collagen but also more effective scaffold for stem cells.

P2.03. BIOPROCESS SCALE-UP AND AUTOMATION OF MESENCHYMAL STEM CELL PRODUCTION – A NEW APPLICATION FOR OLD TOOLS

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Allogeneic mesenchymal stem cells (MSC) have shown great promise for treatment of a number of human diseases including improvement of cardiac function after myocardial infarct, treatment of inflammatory and autoimmune disease, and improved healing of musculo-skeletal injuries. For this promise to be realized on a routine clinical basis, it will be necessary to produce large numbers of cells. Current methods (manual processing in clean room facilities) are inappropriate for this purpose, being time consuming, costly, variable, and susceptible to contamination. Furthermore, cultivation in static tissue culture flasks dictates that large working areas are required with little geometric flexibility.
The scale-up of adherent cell culture processes is by no means a new challenge, the protein and vaccine production industries successfully dealt with the problem decades ago through the use of microcarriers. Providing large amounts of surface area in liquid suspension, microcarriers permit bulk culture of anchorage-dependent cell lines in agitated bioreactor systems. However, the production of MSC presents several new challenges. Here the product is cells, and thus the objective is to maximize cell growth on what is still a finite surface rather than maximizing cellular productivity. As product potency is a function of cell phenotype, maximum cell growth must be achieved whilst also maintaining phenotype. In addition to the challenge of culture scale-up, a suitable process for harvest of the starting cell population from donor tissue is required. In our case, we obtain these cells from placenta, a large complex human tissue consisting of many cell types.
We have adapted off-the-shelf technologies for closed processing of the initial donor material and subsequent expansion of cells. Employing industry proven single-use technologies, this process is readily scaled and automated, and is capable of producing 1000's of cell doses from a single placenta donation. Such a platform technology could enable widespread therapeutic application of MSC therapies at relatively low cost to the health care system.

P2.04. CARBON MONOXIDE IN NEURONAL DIFFERENTIATION – NOVEL APPROACHES FOR CELL THERAPY APPLICATIONS

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Ischemic injuries and neurodegenerative disorders lead to death or impairment of neurons in the central nervous system. Application of stem cell based therapies, namely stimulation of endogenous neurogenesis or cell transplantation, are promising strategies and currently under investigation.
The human embryonic teratocarcinoma stem cell line, NT2, is reported as model of neuronal differentiation. NT2 cells are pluripotent, characterized by high proliferation yields *in vitro* and neuronally committed progenitor cells. Carbon monoxide (CO) is an endogenous product of heme degradation by heme-oxygenase. Administration of CO at low concentrations produces several beneficial effects in distinct tissues, such as anti-inflammation, anti-proliferation, anti-apoptotic or neuroprotection. Although there is no data reporting CO as a factor involved in stem cell differentiation, several evidences support this hypothesis. This gasotransmitter is antiproliferative in smooth muscle cells and induces mitochondrial biogenesis in cardiomyocytes. These two cellular processes are broadly described to be involved in cell differentiation.
In order to assess the effect of CO in neuronal differentiation, NT2 progenitor cells were treated in the presence or absence of CO. In the presence of CO, post-mitotic neurons were obtained by treatment of NT2 with retinoic acid (RA) at 10μM supplemented with 50 or 100μM of CO. While, as control, neurons were generated by treatment with RA only. After differentiation procedure (5 weeks of treatment) and isolation of post-mitotic neurons, neuronal quantification was performed by microscopic counting and immunofluorescent microscopy assays.
CO does increase the final yield of post-mitotic neurons presenting similar morphology as RA-treated NT2 neurons. Thus, one can speculate that CO improves the final yield of neuronal differentiation by increasing mitochondrial biogenesis, modulating apoptosis and/or preventing proliferation. In conclusion, CO appears as a promising therapeutic molecule to stimulate endogenous neurogenesis or to improve *in vitro* neuronal production for cell transplantation.

P2.05. CHARACTERISATION OF HUMAN EMBRYONIC STEM CELL (HESC) CULTURE

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The potential for hESCs to address clinical needs, for replacement of non-functioning tissues within the human body, cannot be overstated. The aim of the project which encompasses this study is to investigate two possible routes to scaling up hESC culture; automation & microcarrier culture. The work presented here compares growth of cells within the control paradigm (cells growing in medium that has been "conditioned" by mouse fibroblasts) with that of a synthetic, fully-defined, medium (StemPro; Life Technologies). During this time, the expression of a minimum of 2 pluripotency factors, a vital quality parameter, has been assessed along with daily determination of 11 different metabolites, including glucose, lactate, ammonium and dissolved oxygen. It has been established that a significant proportion of the cells in culture (> 60 %) contain an abnormal karyotype, and that this change is not obviously detectable from any of the routine analyses being performed. The majority of the abnormal cells exhibit alterations which are consistent with those detected in embrycarcinoma cells (transformed embryonic stem cells), and would not be suitable for implantation into a living patient, following differentiation. This has important ramifications concerning the extent of heterogeneity within a small-scale system, and its impact on the production of these cells. As a result, any generic hESC production process must take into account both the presence of differentiated cells, as well as this karyotypically altered population. At present most of the focus is centred on removing the heterogeneity during the growth phase, however, if these heterogeneities cannot be completely eradicated, the purification process will have to separate all non-hESC elements from the process stream.

P2.06. CHARACTERIZATION OF AN IRRADIATED GENE-TRANSFER MEDICINAL PRODUCT CONSISTING OF A PROSTATE CANCER DERIVED CELL LINE CONSTITUTIVELY SECRETING INTERLEUKIN-2 AND INTERFERON-GAMMA

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In a first in man Phase I/II study, a prostate cancer cell line constitutively secreting the pro-inflammatory cytokines interferon-gamma (IFN-gamma) and interleukin-2 (IL-2) has shown anti-tumour response (median PSA doubling time prolonged from 63 days to 114 days, p = 0.0035; Brill et al., J Gene Med 2007 and Hum Gene Ther 2009). Several preclinical *in vitro* studies were performed with prostate cancer vaccine cells in order to characterize the product, shed light on the proposed Mode of Action and develop assays to be applied in manufacture of an IMP and furthermore to rectify the limitations of homologous animal models. A clinical scale manufacturing process including centralized irradiation was developed in parallel.
Considering safety and stability of the tumour vaccine we tested the viability, colony formation, phenotype (surface marker expression of the prostate specific antigens PSA, PSMA, EpCAM), the release and bioactivity of IL-2 and IFN-gamma, vector integrity and the release of replication competent retrovirus besides the generic tests stipulated for such products.
The absence of tumourigenicity, e.g. outgrowth of potentially remaining replication competent cells, of the finally irradiated cells was controlled by the assessment of proliferating cells in long term cultures and proliferation assays. In order to show the induction of an immunological reaction which is considered as a prerequisite to induce an anti-tumour response we evaluated the allogenic response towards the irradiated cells, analysed the deposition of complement and the uptake of cellular particles by phagocytes.
The stability and safety as well as the functionality and biologic activity of the cellular product could be reliably demonstrated. It was shown to be highly active in inducing immune responses. The developed assays, first of all the bioassays, ELISAs and flow cytometry for the cytokines and the tumour antigens, are ready to be validated and used in routine batch release analytics.

P2.07. CHARACTERIZATION OF POLYELECTROLYTE CAPSULES IN TERMS OF THE DEVELOPMENT OF A MICROENVIRONMENT FOR PRIMARY T – LYMPHOCYTES

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An important method for future applications in biotechnology and medical research is the encapsulation of mammalian cells. Based on the protection of cells from sheer forces, we observed that high cell densities and viabilities can be reached. Up to now, the reason for that trend is not really understood. Therefore, the investigation of the microenvironment within the capsules is an important issue in the Tissue Engineering field.
The aim of this study is the analysis of the microenvironment inside polyelectrolyte capsules in terms of cell physiology and material properties. The Jurkat cell line and human T lymphocytes are encapsulated in sodium cellulose sulfate (NaCS) with an outer membrane consisting of the polycation polydiallyldimethylammoniumchloride (PDADMAC). These capsules show long-term stability and the capsule materials a good biocompatibility.
The Jurkat cell line was used as a model for T lymphocytes in order to optimize and compare different cultivation methods. This included growing of cells in suspension and after encapsulation using the identical conditions in spinner flasks and in T-flasks. We observed tissue like cell densities inside a capsule, yielding up to 100 x 10^6 cells/ml. The results were used for the establishment of cultivation of encapsulated T lymphocytes. In an attempt to identify the factors responsible for the high cell density within the capsules, production of cytokines (e.g., IL-2), expression of activation (e.g., CD25) and cell surface T lymphocyte markers (e.g., CD3, CD4, CD8) were analyzed by standard immunological methods. Moreover, putative influence of the capsule environment on proliferation of T lymphocytes sub-populations was also investigated.
This contribution presents the first step towards a strategy for high density proliferation of primary T lymphocytes in polyelectrolyte capsules and the idea to recreate the *in vivo* microenvironment.

P2.08. DEVELOPMENT OF 3-DIMENSIONAL SKIN EQUIVALENTS FOR PHARMACEUTICAL AS WELL AS MEDICAL APPLICATIONS

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The development of 3-dimensional skin equivalents is crucial not only due to the OECD guidelines but also due to the growing medical demand of autologous skin transplants. Skin equivalents that are histologically and physiologically comparable to the in vivo state can for example be used for penetration assays or to test the efficacy of glucocorticoids including monitoring of adverse reactions as skin thinning. Those equivalents can also be applied for the treatment of burn injuries or hardly healing wounds. Whereas the length of the pre-cultivation time of skin equivalents for pharmaceutical or cosmetical causes is not essential, it is crucial that the pre-cultivation time of skin equivalents for medical causes is minimal. The impaired skin barrier has to be restored as fast as possible to decrease fluid loss and the risk of infection.
To create the skin equivalents primary fibroblasts and epidermal cells were enzymatically isolated from 1cm^2 split skin pieces or foreskin. After ex vivo expansion the cells were seeded into scaffolds and cultivated according to common protocols. Characteristically fibroblasts inhabited the matrix and keratinocytes formed a stratified epidermis containing stratum basale, -spinosum, -granulosum and -corneum at the air-liquid-interface. It could be shown that the skin equivalents responded to inflammatory stimuli and glucocorticoids as it is described for skin in vivo. Skin equivalents offer a nearly inexhaustible array of utilizations.

P2.09. FABRICATION OF BONE MICROTISSUES USING MESENCHYMAL STEM CELLS AND MICROCARRIERS *IN VITRO*

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Recently, tissue engineering via modular microtissues holds great promise for engineering tissue constructs with large dimensions. Here, we investigated the feasibility of using Cultispher S microcarriers seeded with hAMSCs to fabricate bone microtissues, which may mimic the natural formation of bone tissue micro-structure. Microcarriers were found to assemble into small aggregates with homogenous cellularity and abundant ECM production and most importantly, conductive to osteogenic differentiation of seeded hAMSCs. Our results provide promising basis to engineer bone tissue replacements with large dimensions ex vivo in future.

P2.10. CONTROLLED EXPANSION AND DIFFERENTIATION OF MESENCHYMAL STEM CELLS IN A MICROCARRIER BASED STIRRED BIOREACTOR

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Cell based therapy necessitates a large amount of cells in a functional state permitting their *in vivo* implantation for the restoration of tissue homeostasis. Three main parameters are believed to be essential for such a purpose: an adapted cell population, an adapted scaffold and adapted physico-biochemical factors enabling proper expansion and *in vitro* cell differentiation. In recent years, mesenchymal stem cells (MSCs) have been attracting a lot of interest in this field, because of their differentiation potential and their trophic factor secretion abilities. The aim of this work is to perform a rational analysis of key factors involved in the efficient proliferation and differentiation of MSCs, in the context of a stirred microcarrier based bioreactor.
First, E-MSC (ear mesenchymal stem cells) was compared to the "golden standard" BM-MSCs (bone marrow mesenchymal stem cells) on the basis of their proliferative properties. E-MSCs bear characteristics of progenitor cells: expression of CD73, Sca-1 and Notch-1, and also *in vitro* differentiation potential into mesodermal cell types such as adipocytes, chondrocytes and osteoblasts. Thus, these cells are *in vitro* functionally analogous to BM-MSCs. This cell population was further selected on the basis of its high intrinsic proliferation potential, a clear advantage in the field of MSC bioprocessing.
Secondly, in order to establish rational criteria for the choice of scaffolds, such as microcarriers (MCs), for use in bioreactor-based processing of adult mesenchymal stromal cells, we first analyzed their behavior on various types of microcarriers (MCs). Interestingly, both cell types (E- and BM-MSCs) had similar proliferation profiles under all the conditions tested, validating that E-MSCs are a valuable model for MSCs proliferation on solid supports, given their faster growth and easier handling compared to BM-MSCs. Using confocal microscopy to probe E-MSC behaviour on deacetylated chitosan beads, cellulose-grafted DEAE MCs (Cytopore 2) and on gelatin-based MCs (Cultispher-S) and Cytodex-3 (a solid collagen coated MC), we found that initial actin organization correlated with E-MSC proliferation potential. Due to the good actin organization and proliferation profile, Cultispher-S turned out to be the most adapted MCs for MSC proliferation. On the basis of the analysis of actin conformation on MCs, we sequentially differentiated in a stirred bioreactor E-MSCs after expansion, along the adipogenic, chondrogenic and osteogenic pathways.
Thirdly, in testing various cell culture modes for nutrient supply, bead concentration, and oxygen supply with no major impact on E-MSC growth, we concluded that a critical parameter for optimizing E-MSC yield was the medium's content of growth factors. It was shown that the apparent growth rate of E-MSCs was correlated with the percentage of cells in the S phase of the cell cycle. Moreover, this percentage was directly linked with the fraction of growth factor/receptor complexes. Thus, controlling the percentage of E-MSCs in S phase with suitable growth factor feeds led to an increase of their growth span. After this optimized expansion protocol, cells maintained their *in vitro* differentiation potential into adipogenic, chondrogenic and osteogenic pathways as well as their MSC marker expression. Taken together, these results open the way toward mass scale production of MSCs suitable for future *in vivo* applications.

P2.11. NOVEL XENO-FREE, SERUM FREE CULTURE SYSTEM FOR HUMAN MESENCHYMAL STEM CELL

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Human MSC are multipotent cells with the ability to differentiate into cells of connective tissue lineages, including mainly adipocytes, osteoblasts and chondrocytes. hMSC have advantages over other stem cells types, due to the broad variety of their tissue sources and for being immuno-privileged. These traits have led hMSC to become desirable tools in regenerative medicine and cell therapy. Application of hMSC in cell therapy needs the elaboration of appropriate culture media and defined culture conditions in order to minimize the health risk of using xenogenic compounds and to limit the immunological reactions once MSCs are transplanted while maintaining multi-potentiality, self-renewal, and transplantability. To date, the most common culture media for growth and expansion of hMSC include serum. In addition, the common auxiliary medium supplements and solutions (for attachment, freezing and dissociation) required for long term growth and maintenance of hMSC, are mostly animal-derived.
This study addressed the ability of a developed system which is serum-free and xeno-free, to support hMSC expansion under xeno-free culture conditions, suitable for medical applications. The xeno-Free culture system includes specially developed solutions for attachment, dissociation and freezing as well as a culture medium that enable long-term growth of hMSC with retention of multi-lineage differential potential.

P2.12. NON-INVASIVE DERIVATION OF HUMAN INDUCED PLURIPOTENT STEM CELLS FROM URINE

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The transformation of somatic cells into induced pluripotent stem cells (iPSCs) using exogenous factors, also termed reprogramming, may be used for personalized medicine in the future and can produce valuable *in vitro* models of human diseases. So far, human iPSCs have been generated from skin, amniotic fluid, human umbilical vein endothelial cells, extra-embryonic tissues, cord blood, periosteal membrane, dental tissue, adipose tissue, neural stem cells, hepatocytes, and peripheral blood cells. The reprogramming from these tissues has been achieved with varied frequencies, indicating that the cells of origin are an important determining factor. In addition, there is intense debate regarding whether human embryonic stem cells (ESCs) and iPSCs are equivalent, and donor cell heterogeneity may further complicate this due to difficulties in setting standards for performing such comparisons. The ideal cell source for reprogramming should be easily accessible, easily reprogrammed, and universal (any age, sex, ethnic group, and body condition). Here, we report the generation of human iPSCs from cells obtained non-invasively from human urine. Urine-derived cells from 12 donors yielded iPSCs with excellent differentiation ability. Therefore, we propose urine to become the preferred source for generating iPSCs in many instances. The ease of this method may facilitate the standardization of iPSC technology, will boost the generation of cell based disease model systems and is also an advance in the direction of clinical use of iPSCs.

P2.13. PRODUCTION OF EMBRYONIC STEM CELLS IN STIRRED BIOREACTORS

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Pluripotent embryonic stem cells (ESC) derived from the inner cell mass of blastocysts have been considered as a valuable cell source since they can be kept in culture as undifferentiated cells, maintaining an unlimited self-renewal capacity, and are capable of differentiating into all somatic cell types and germ cells. In addition to their utility for developmental biology studies, ESC may be useful for novel regenerative cell therapies and as in vitro diagnostic tools. The aim of this study was to develop a quality controlled and reproducible large-scale culture system using serum-free (SF) medium for ESC production in order to surpass the limited cell productivity and lack of control of the commonly used static culture systems (e.g. culture flasks), as well as laboratory-scale bioreactors (*e.g.* spinner flasks). Herein, a fully controlled, microcarrier-based, stirred bioreactor with a working volume of 700 mL was used and the initial operational parameters were set to: pH 7.2, DO = 20%, T = 37ºC, agitation = 60 rpm and 50% medium renewal everyday. The cells attained a maximum concentration of $(4.3\pm0.5)\times10^6$ cells/mL after 11 days, which represents a 80±15-fold increase in total cell number. These results showed that the growth kinetics of ESC grown in a large-scale bioreactor is comparable to the one observed in the spinner flasks in our previous work. Furthermore, ESC were successfully cultured in the bioreactor under continuous mode using medium perfusion and cell retention. The influence of different residence times has been also evaluated. Importantly, ESC expanded under stirred conditions using SF medium, retained the expression of pluripotency markers such as OCT4, NANOG, and SSEA1 and their differentiation potential into cells of the three embryonic germ layers as assessed by flow cytometry and RT-PCR. Our results demonstrate the feasibility of using a microcarrier-based stirred bioreactor culture system for the scalable and controllable production of undifferentiated ESC cells for use in multiple settings.

P2.14. HIGH-CAPACITY ASSAY TO QUANTIFY THE CLONAL HETEROGENEITY IN POTENCY OF MESENCHYMAL STEM CELLS

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The regenerative capacity of mesenchymal stem cells (MSCs) is contingent on their content of multipotent progenitors. This research addresses a basic prerequisite to realize the therapeutic potential of MSCs by developing a novel high-capacity assay to quantify the clonal heterogeneity in potency that is inherent to MSC preparations. The in vitro assay utilizes a 96-well format to (1) classify MSCs according to colony-forming efficiency as a measure of proliferation capacity and trilineage potential to exhibit adipo-, chondro-, and osteogenesis as a measure of potency and (2) cryopreserve a frozen template of MSC clones of known potency for future use. The heterogeneity in trilineage potential of normal bone marrow MSCs is more complex than previously reported: all eight possible categories of trilineage potential were detected, suggesting a convoluted hierarchy of lineage commitment. The assay was employed to resolve the relationship between the regenerative properties of MSCs and their potency. Greater cell amplification, colony-forming efficiency, and colony diameter for tri- versus unipotent clones suggest that the proliferation potential of MSCs is a function of potency. The cell adhesion molecule CD146 may be a biomarker of potency, with a 2-fold difference in mean fluorescence intensity between tri- and unipotent clones. The significance of these findings is discussed in the context of the efficacy of MSC therapies. The in vitro assay described herein will likely have numerous applications given the importance of heterogeneity to the therapeutic potential of MSCs. This work was funded by the National Institutes of Health and National Science Foundation.

P2.15. IMPROVEMENT OF STEM CELL PERFORMANCE BY SUPPLEMENTATION WITH METABOLIC ENHANCERS

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Every cell-based application is unique and poses is own set of challenges to the process development scientist. It is therefore useful to identify tools that can be applied across a variety of media strategies, be them defined or undefined in nature. It is also of unique advantage to utilize components which act through different mechanisms than traditional media components and supplements. Here, we present data demonstrating the application of novel, non-nutritive metabolic enhancers, which act on ubiquitous cellular pathways to affect positive outcomes in a variety of cell types. These supplements are chemically defined and comprised of mixtures of small organic molecules. Data demonstrate unique improvements in performance of MSC and ESC, promoting expansion while limiting differentiation. In one experiment, human bone marrow cells were cultured in plates with several media. On day 5, non-adherent cells were washed off, remaining MSC were counted and cultured in α-MEM medium with 10% FBS (control) or in GEM-Novo medium containing 1x RS-Novo metabolic enhancer and 2.5%, 5% or 10% FBS. Cultures were monitored daily for viability and morphology and fed every other day. MSCs were collected, counted and passaged on day 14 and collected and counted on day 21. Results from 2 bone marrows are presented. Cultures containing the GEM-Novo medium containing 1x RS-Novo and reduced serum levels (GEM-RS-2.5 & GEM-RS-5) had similar cell numbers as control medium containing 10% FBS at this stage, while GEM-RS-10 condition had 2 fold greater cells than Control. Data also demonstrate that human MSCs cultured in GEM-Novo medium, serum, and 0.5x, 1x or 2x RS-Novo metabolic enhancer maintained their ability to differentiate into adipogenic cells when transferred into a variety of differentiation media. Future work will include evaluation of compatibility with traditional supplements (i.e. specific rProteins and complex supplements such as protein hydrolysates).

P2.16. MAMMAL-FREE CRYOPRESERVATIVE SOLUTION FOR STEM CELLS

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Cryopreservation of the cells is among the important processes in cell therapy and regenerative medicine. Conventionally, mammal-derived components are added into the solution, and FBS supplemented with DMSO is extensively used as cryopreservative solution. In spite of its effectiveness, FBS has several disadvantages, including risk of infection by viruses and abnormal prions. To overcome these disadvantages, various serum-free cryopreservative solutions have been developed. However, current serum-free cryopreservative solutions are inferior to the conventional serum-supplemented ones. Therefore, serum- and mammal-free cryopreservative solutions should be improved. For this purpose, we have been focusing on fructan, a homopolysaccharide containing D-fructose residues obtained from rakkyo (Japanese shallot).
In the previous ESACT meeting, we reported that fructan could protect mammalian cells from toxicity due to DMSO. Therefore, fructan is expected as an alternative to FBS in cryopreservative solutions. The novel cryopreservative solution containing fructan was developed and compared with the conventional FBS supplemented with DMSO. The viability of the cells cryopreserved in fructan solution were similar to that preserved in conventional FBS solution, indicating that the fructan solution could be used as an alternative to the FBS cryopreservative solution.

P2.17. MATURATION LYMPHOCYTE ISOLATE FROM BONE MARROW STEM CELL CANDIDATE RESISTANCE FOR HIV INFECTION

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Acquired immune deficiency syndrome (AIDS) is caused by Human Immunodeficiency Virus (HIV). At the beginning of infection, gp120 virus interacts with CD4 receptor at the surface of the target cell. The interaction between gp120 and CD4 leads to the occurrence of the binding of specific chemokine receptor CXCR4 and CCR5, which are also present on the membrane of the target cell. Therefore, CCR5 and CXCR4 also determine the fate of the target cell. It is the performance of CCR5 and CXCR4, guided by controlling gene that determines susceptibility or resistance to HIV infection. Coding gene CCR5 may mutate to become protective or resistant against HIV infection. In homozygote individuals, it tends to be resistant against infection, while in heterozygote individuals it tends to be susceptible to HIV infection. The aim of this study were to produce candidate TCD4 lymphocyte that is resistant against HIV infection by using gene therapy deletion 32 CCR5 to use for HIV/AIDS treatment, with the methods sample collection, mononucleated cell collection, lymphocyte culture, CD4 identification and maturation using RT PCR and sequencing. This study was performed in several steps, such as mononucleated cell isolation, followed with cell culture, lymphocyte purification, lymphocyte and CD4 expression identification and maturation by RT PCR and sequencing. Conclusion of this study was maturation Lymphocyte T CD4 had achieved after several steps such as mononucleated cell isolation, followed with cell culture, lymphocyte purification and after seven passages
Keywords: maturation lymphocyte, bone marrow stem cell, candidate resistance, HIV.

P2.18. MICROENCAPSULATION TECHNOLOGY: A POWERFUL TOOL TO INTEGRATE EXPANSION AND CRYOPRESERVATION OF PLURIPOTENT HUMAN EMBRYONIC STEM CELLS

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Human embryonic stem cells (hESCs) are an attractive source for cell replacement therapies and *in vitro* toxicology studies due to their inherent self-renewal ability and pluripotency. However, the establishment of effective protocols for large-scale expansion, storage and distribution of hESCs is imperative for the development of high quality therapeutic products and functional screening tools. In this study, cell microencapsulation in alginate was used to develop an integrated bioprocess for expansion and cryopreservation of pluripotent hESC. Different three-dimensional (3D) culture strategies were evaluated and compared: microencapsulation of hESC as single cells, cell aggregates and cells immobilized on microcarriers. Aiming to establish a scalable bioprocess, hESC-microcapsules were cultured in stirred tank bioreactors.
Our results show that, the combination of cell microencapsulation and microcarrier technology resulted in a highly efficient protocol for the production and storage of pluripotent hESCs. This strategy ensured high expansion ratios (approximately 20-fold increase in cell concentration) and high cell recovery yields after cryopreservation. When compared to non-encapsulated cells, an improvement up to 3-fold in cell survival post-thawing was obtained without compromising hESC characteristics.
Microencapsulation also improved the culture of hESC aggregates by protecting cells from the hydrodynamic shear stress and through aggregate size control, assuring the maintenance of cell pluripotency for up to two weeks.
This work demonstrates, for the first time, that cell microencapsulation in alginate is a powerful tool to integrate expansion and cryopreservation of pluripotent hESCs. The 3D culture strategy developed herein represents a significant breakthrough towards the translation of hESCs to clinical and industrial applications.

P2.19. PLATELETPHERESIS: AN ALTERNATIVE SOURCE OF PERIPHERAL BLOOD MONONUCLEAR CELLS TO OBTAIN FUNCTIONAL MONOCYTE-DERIVED DENDRITIC CELLS SUITABLE FOR CO-CULTURE SYSTEMS.

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In the gastrointestinal tract, dendritic cells (DC) are responsible of linking both innate and adaptive immune responses by sampling bacteria from intestinal lumen, either pathogens or commensal bacteria. The intimate interplay between intestinal epithelial cells and DC helps to maintain gut immune homeostasis. In recent years, there is an increasing interest in the use of Lactic Acid Bacteria (LAB) as probiotics. Among the benefits that LAB may exert on health, the modulation of the immune system is a main property. Considering that effects are strain dependent it is important to have in vitro systems to screen large numbers of LAB to identify candidates. As DC and intestinal epithelial cells are key regulators of intestinal homeostasis, in vitro co-culture of both cell types turn out to be a useful tool for that purpose. Classically, human DC are obtained from peripheral blood mononuclear cells (PBMC) derived from buffy coats (BC). During the last years, the use of aphaeresis has been increasing in blood banks to collect platelets, erythrocytes and plasma. As a consequence, the availability of BC is limited and alternative sources for the obtainment of cells for research purposes is needed. Dietz et al (2006) have described the obtainment of PBMC from the cells retained in the leukoreduction system chambers after plateletpheresis (PP) and differentiated DC using a standard 7-day protocol.
In this study we compared the capacity of PBMC derived from PP or BC to differentiate into DC using a 48-hour protocol, which reflects the kinetics of DC differentiation from monocytes under physiological condition. The functionality of the obtained DC was analyzed in a co-culture system using intestinal epithelial HT-29 cells under a pro-inflammatory stimulus with LPS. Surface markers CD86, HLA-DR and pro-inflammatory cytokines (IL-6 and IL-8) production were measured by flow cytometry. DC derived from PP were functionally similar than those derived from BC. In order to test other stimuli, two bacteria commonly used in the food industry -*Streptococcus thermophilus* and *Lactobacillus delbrueckii* were evaluated on the DC response. Significant differences in cytokine secretion were detected, especially IL-10, suggesting that the system was sensible to different tested bacteria.
In conclusion, we have generated functional DC employing a short differentiation protocol using PP as an alternative source of PBMC. Since the initial volume of PP is ten times more concentrated than BC, the use of PP minimizes biological residues generation and reagent consumption. In addition, the monocyte-derived DC were suitable for co-culture systems to study immunomodulatory properties of LAB.
Reference: Dietz, A.B., et al., *A novel source of viable peripheral blood mononuclear cells from leukoreduction system chambers.* Transfusion, 2006. 46(12): 2083-9.

P2.20. AUTOMATION OF HUMAN EMBRYONIC STEM CELL CULTURE

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Human embryonic stem cells (hESCs) have huge potential because they can give rise to any cell type in the body. This is in contrast with adult stem cells which can only differentiate into a limited range of cell types and have a limited number of generations in in vitro culture. The imprecise nature of manual culture and the resulting limitations on economical scale-up of hESC production have opened a potential market for automation technologies to be introduced into human embryonic stem cell laboratories.Automation of manual processes will increase standardisation, reduce uncertainty by reducing variability, and ultimately reduce cost. Automation, as applied to hESC culture is still in the early stages and, as such, is still being utilised with processes which have been optimised for manual culture. Culture of hESCs presents a unique challenge to automation, whereby, the interpretation of an experienced operator should be replaced with more easily measurable and controllable parameters. We aim to optimise the hESC culture process for use with a large-scale automation platform, enabling large quantities of pluripotent stem cells to be manufactured in a reproducible and robust manner.

Disclosures: No relevant conflicts of interest to declare.

P2.21. RECOMBINANT HUMAN GROWTH FACTORS AS AN ALTERNATIVE THERAPEUTICAL APPROACH TO THE WOUND HEALING PROCESS

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Wound healing is a complex process involving three phases: blood clotting and inflammation, proliferation (new tissue formation) and tissue remodeling. By manipulating the growth factors composition, it is possible to accelerate or modify this process. The use of exogenous growth factors has a beneficial effect in the treatment of wound repair. During inflammation, neutrophils and macrophages are recruited to produce two essential growth factors, TGF-β1 and G-CSF. PDGF and TGF-β1 play critical roles in recruiting fibroblasts (proliferation phase). VEGF is essential for angiogenesis. During remodeling, epidermal proliferation is mediated by GM-CSF and TGF-β3. GM-CSF increases neovascularization and granulation tissue formation. While TGF-β1 has a direct involvement in cutaneous scarring, the factor TGF-β3 antagonizes its effect, avoiding an excessive scarring. In order to insure proper glycosylation and conformational folding and prevent immunogenicity, we elected a mammalian cell expression system, to produce several growth factor involved in wound healing process: G-CSF, GM-CSF, PDGF-B, FGF, TGF-β1, TGF-β3, VEGF-B, VEGF-C. cDNAs were amplified from a Human Full-length cDNA Bank and sub-cloned into pGEM®-T-Easy vector. *E. coli* transformants were screened by colony PCR. Upon DNA sequencing, the inserts were transferred to the mammalian expression vector. Mammalian cells were transfected with CSFs constructs, by co-transfection with a G418^r vector for clone selection. CSFs were successfully expressed in two different mammalian cell lineages, as confirmed by qRT-PCR and Western blot, and showed in vitro biological activity. Characterization of mammalian cell clones to the other growth factors is underway. This initiative to produce recombinant growth factors in mammalian cells (biopharmaceuticals) is likely to be useful to better understand the wound healing process and to provide patients with an alternative therapeutical treatment.
Support: FAPESP, CNPq, FINEP

P2.22. SELECTIVE REMOVAL OF UNDIFFERENTIATED HUMAN EMBRYONIC STEM CELLS USING SUPERPARAMAGNETIC PARTICLE AND A CYTOTOXIC ANTIBODY

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Despite the enormous potential of embryonic stem cells (ESC) for regenerative medicine, one of the most pertinent concerns using differentiated cells from ESC is the presence of residual undifferentiated ESC because they carry the risk of teratoma formation. It is expected that a single dose of about 10^7 - 10^9 cells are required for the treatment of a patient. Presuming a sufficiently large safety margin with a probability of less than 10^{-1} undifferentiated cells per dose, a clearance factor of 10^8 - 10^{10} has to be ensured respectively. Using current available cell-affinity separation methods i.e. magnetic particle separation, we found that the removal of undifferentiated stem cells to 10^{-1} is hard to achieve.
In this study, a panel of monoclonal antibodies (IgMs and IgGs), specific for cell surface antigens on undifferentiated hESC, were evaluated and screened for their selectivity to remove undifferentiated hESC. By combining a single step magnetic particle separation followed by treatment with a cytotoxic antibody[1] (mAb 84) that kills undifferentiated hESC, we show that the necessary purity can be achieved. We evaluated several parameters of this method to optimize the efficiency for cell separation. The applicability and robustness of this method is shown in a case study using pools of undifferentiated hESC and human fibroblast cells (hF) at different ratios (50 - 5% hESC). After the single step magnetic particle separation, purities of 98 - 99.9% were achieved with the IgM mAb. This was validated by flow cytometry analysis using antibodies specific for undifferentiated hESC as well as qRT-PCR analysis of undifferentiated hESC related genes. Furthermore, when the unbound cells after single step magnetic particle separation with IgM was injected into SCID mice 11 out of 12 mice did not show teratoma formation after 28 weeks post-injection. While magnetic particle separation is frequently used for selective capture of cells from heterogeneous cell pools[2], our two-steps method can be applied for specific and complete removal of undifferentiated hESC[3].

References:

[1] Choo A.B.H. et al. (2008) Selection Against Undifferentiated Human Embryonic Stem Cells. Stem Cells 26 (6): 1454 - 1463
[2] Schriebl, K. et al. (2010) Stem cell separation: A bottleneck in stem cell therapy. Biotechnology Journal 5: 50-61
[3] Schriebl, K. et al. (2010) Selective removal of undifferentiated human embryonic stem cells (manuscript in preparation).

P2.23. STUDY OF EXPANSION OF PORCINE BONE MARROW MESENCHYMAL STEM CELLS ON MICROCARRIERS USING VARIOUS OPERATING CONDITIONS

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Bone marrow mesenchymal stem cells (BM-MSCs) represent promising source for tissue engineering and cell therapy, due to their multipotency, immunoregulation and self-renewal properties. The expansion phase of these cells prior to differentiation and/or injection to the patient remains a critical step. BM-MSCs are classically expanded in small scale culture systems, with low control of culture conditions. However, tissue engineering and cell therapy require very large quantities of cells that cannot be easily achieved using processes in static flasks. Microcarriers, classically used for industrial large scale culture of continuous cell lines, could be advantageously applied to stem cells expansion such as BM-MSCs. In the present work, the influence of some operating parameters on porcine BM-MSCs growth kinetics and morphology was characterized in a microcarrier culture system, in order to improve cell expansion while maintaining their multipotency.
Prior to the expansion phase, the adequate seeding phase conditions were determined (cell to bead ratio, agitation mode, medium composition,…). Then, kinetic studies of porcine BM-MSCs cultures were performed at different agitation rates (0, 25, 75 rpm) by using Cytodex 1 microcarriers in spinner flasks, with 50% medium renewal every 2 days. Results indicated that under agitated conditions, BM-MSCs cell population reached a maximum cell concentration (1.5 x 10^5 cell/mL; x 5 multiplication factor) before to decline whatever the agitation rate used. However, cultures without agitation reached a stationary phase at the same maximum cell concentration and no decrease of cell population was observed during 300 h. It was pointed out that in the early stage of the culture, cells under agitated conditions formed small aggregates at the surface of microcarriers. Once those aggregates reached a critical size, they left from the microcarriers and remained in suspension, where no more growth could be observed. As this aggregation behavior was not observed with the culture at 0 rpm, it could be assumed that agitation was responsible for cell aggregation. Nevertheless, to allow homogeneous and controlled stirred cultures, we demonstrated that addition of fresh microcarriers enabled to prolong cell growth without cell aggregation until 2.8 x 10^5 cell/mL (x 9 multiplication factor). Following expansion on microcarriers, BM-MSCs were harvested by trypsination and differentiated in adipocytes, chondrocytes and osteocytes, indicating that they preserved their multipotency ability.
BM-MSCs culture on microcarriers in stirred systems allowed the expansion of the cells while maintaining their multipotency. By adding fresh microcarriers, aggregation could be prevented and the expansion phase extended. This culture process is expected to be transferred to a larger controlled culture system, in order to fulfill the need of high quantities of multipotent BM-MSCs.

P2.24. STRATEGIES IN UMBILICAL CORD-DERIVED MESENCHYMAL STEM CELLS EXPANSION: INFLUENCE OF OXYGEN, CULTURE MEDIUM AND CELL SEPARATION

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Mesenchymal Stem Cells (MSCs) from different sources attract tremendous interest in cell-based therapies for their ability to differentiate into different cell lineages. MSC are already used in clinical trials as cell suspensions. Due to its easy availability and high frequency of MSCs, the human umbilical cord (UC) presents a promising and non-controversial source for these cells. A large number of cells is required which may still limit implant preparation in clinical applications. Another important question is the ability of the cells to survive after implantation, when oxygen and nutrition supply is limited due to the lack of vascularisation.
In this work we studied the influence of hypoxia as well as different glucose concentrations on the expansion, metabolic activity and differentiation capacity of the UC-MSCs *in vitro*. Additionally, cell separation by centrifugal elutriation was performed and proliferative activity of the different cell populations was investigated.

P2.25. AFFINITY MATURATION THROUGH PHAGE DISPLAY OF A STEM CELL SPECIFIC ANTIBODY

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Antibodies are a useful class of proteins that have high specificity and affinity for a binding companion. They are however, not necessarily optimized for their desired functionality such as cytotoxicity, binding affinity or specificity. Protein engineering techniques involving the generation of a library followed by a high-throughput screen such as phage display can tune the antibodies towards better binding functionality. An improved antibody will be useful for therapies involving the use of stem cells.
We will report on improving the affinity of a stem cell specific antibody and the validation of the improved variants through secondary assays such as surface plasmon reasonance. As an initial step, the scFv was confirmed to functionally display on the surface of phage using western blot and flow cytometry analysis. The library was then created by incorporating degenerate codons at complementary determining regions on the antibodies. The library was then biopanned for up to 4 rounds against podocaylxin and the isolated clones were analyzed further by secondary binding assays.

P2.26. TOWARDS HUMAN CENTRAL NERVOUS SYSTEM *IN VITRO* MODELS FOR PRECLINICAL RESEARCH: STRATEGIES FOR 3D NEURAL CULTURE AND GENE DELIVERY

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The development of new drugs for human Central Nervous System (CNS) diseases has traditionally relied on 2D *in vitro* cell models and genetically engineered animal models. However, those models often diverge considerably from that of human phenotype (anatomical, developmental and biochemical differences) contributing to a high attrition rate – only 8% of CNS drugs entering clinical trials end up being approved. Human 3D *in vitro* models are useful complementary tools towards more accurate evaluation of drug candidates in pre-clinical stages, as they present an intermediate degree of complexity in terms of cell-cell and cell-matrix interactions, between the traditional 2D monolayer culture conditions and the complex brain and can be a better starting point for the analysis of the *in vivo* context.
We have been developing 3D *in vitro* models of the CNS, using human midbrain-derived neural stem cells (hNSC) as a scalable supply of neural-subtype cells. We have focused on the implementation of long-term culture systems for neural differentiated phenotypes as neurospheres, following a systematic technological approach based on stirred-tank bioreactors. Control of chemical and physical environmental parameters allowed for the differentiation of hNSC into neurons, astrocytes and oligodendrocytes in well-defined, reproducible ratios. Detailed cell characterization of the differentiated neurospheres was performed along culture time using spinning disk confocal microscopy, flow cytometry, qRT-PCR and Western Blot.
To develop the full potential of this human CNS model system it is of paramount importance to establish reliable and robust methodologies for gene transfer and manipulation of gene expression. To address this issue we have used canine adenovirus type 2 (CAV-2) viral vectors, that present high cloning capacity, long-term transgene expression and low immunogenicity and have been shown to preferentially transduce neurons. A CAV-2 vector carrying a GFP reporter gene was used for optimization of conditions for transduction of differentiated neurospheres and investigation of vector entry and transport, as well as assessment of transduction impact on cell survival and neurosphere composition.
The model system developed in this work constitutes a practical and versatile new *in vitro* approach to study human CNS, and is expected to increase the relevance of *in vitro* preclinical research of human CNS disorders. Furthermore, this culture strategy can be adaptable to induced NSC or NSC derived from induced pluripotent stem cells, broadening the applicability of these models even further.

P2.27. STEM CELLS: FROM BASIC THEORETICAL ASSUMPTIONS AND MATHEMATICAL CONCEPTS TO THE COMPUTATIONAL MODELS. APPLICATIONS FOR CANCER RESEARCH AND THERAPY OF HUMAN RARE DISEASES

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Stem cells, could be discussed by Totipotent cells (i. g. placents); Pluripotent stem cells; Embryonic stem (ES) cells; In Vitro Fertilization (IVF); Embryonic Carcinoma (EC); Multipotent Stem Cells, [1]. Differentiation of ES cells and regeneration using adult stem cells are of the more important properties of these cells, [2]. The need of understanding of mechanical and structural testing and theory of living cells has been appeared about a century ago. Engineering mechanics techniques- high resolution of AFM imaging, blood, blood vessels and nerves, oscillations in the living organisms and cell-wall oscillations in plants and movement of cilia, have been used. The aim of the work, presented could be formulated as follows: to analyze some theoretical concepts, mechanisms and models of mathematical modeling of stem cells. Concepts for mathematical modeling of hematopoietic tumor stem cells stochastic dynamics has been analyzed as well in the work. A novel computational model, based on the thesis, [3], for numerical study of adult stem cells has been developed too. The model, gives an opportunity on the basis of author's numerical algorithms and FORTAN programs to be presented different curves, reflecting effects of the model's parameters. In conclusion has been pointed out some future problems of stem cells for various biopharm applications (neural stem cells (NSC), future directions for human ES Cell Culture optimization; stem cells used as vehicle for gene therapy.

[1]. Katya Marinova Simeonova, ESACT2009, 7-10 June, 2009, Citywest hotel, Dublin, Ireland, www.esact2009.org poster presentation P242, page 362, BURSARY, to be published in Springer book
[2]. Katya M. Simeonova, Ganka M. Milanova, Light-Activated Tissue Regeneration and Therapy III, July 11-15, 2010, Sheraton Hotel and Conference Center, Burlington, Vermont, USA, oral presentation (full financial support)
[3]. Surbhi Mittal, Thesis, December 2005

P2.28 A NOVEL PEPTIDE TO ENHANCE RECOMBINANT BMP-2 PRODUCTION IN MAMMALIAN CELL CULTURES

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Background: Due to their osteoinductive properties, recombinant human BMPs (rhBMPs) have been used successfully for bone regeneration and replacement. However, rhBMPs are produced at very low yields, affecting their affordability as biologics. BMP-2 is synthesized as a precursor, proBMP-2, which undergoes enzymatic cleavage by proprotein convertases (PCs) to form the mature BMP-2. Furin is an enzyme of the PC family that can cleave BMP-2.
Objectives of Study: To determine the effect of the polyarginine furin inhibitor, IND-1, on rhBMP-2 production in CHO and HEK cells over-expressing rhBMP-2. In addition, the mechanism by which IND-1 influences the rhBMP-2 production was investigated.
Methods: Two stable cell lines expressing the *hBMP2* gene, CHO-BMP2 and HEK-BMP2, were cultured in the presence of IND-1 in short (24 h, multi-well) and long-term (two-month, perfusion flasks) cultures. The rhBMP-2 produced was characterized by Western blot and its activity assessed using the C2C12 cell-based assay. The amount of proBMP-2 and mature BMP-2 produced was quantified by ELISA. The expression of the *BMP2* and *FURIN* genes in the HEK-BMP2 cells treated with or without IND-1 was measured by real-time RT-PCR. IND-1 uptake was estimated by measuring fluorescence of cell lysates following incubation with FITC labeled IND-1. The PC activity in CHO-BMP2 cells following incubation with IND-1 was measured using the Boc-RVRR-AMC substrate. PC activity of cell lysates incubated directly with IND-1 was also measured. Furin-specific siRNA was used to knock down the furin expression in CHO-BMP2 cells and its effect on the rhBMP-2 production was determined.
Results: Stably transfected CHO-BMP2 cells secreted 36 kDa rhBMP-2 dimers that were biologically active. In 24 h CHO or HEK cultures, IND-1 treated cells had significantly greater amounts of proBMP-2 ($\geq$ 10-fold, $P < 0.001$) and mature BMP-2 ($\geq$ 3-fold, $P < 0.001$) in their conditioned medium. In long-term CHO-BMP2 culture, IND-1 continued to increase yields of BMP-2 and proBMP-2 protein without affecting cell growth or viability. IND-1 treatment had no effect on the expression of *BMP2* and *FURIN* mRNA levels, indicating IND-1's effect on rhBMP-2 yield is post-transcriptional. While IND-1 was taken up by the cells and inhibited PC activity in the cell lysates, IND-1 treated cells showed no changes in their PC activity at doses 50 times higher than required to affect BMP-2 yields. Knockdown of furin at both the mRNA ($\geq$ 80%) and the protein level ($\geq$ 70%), did not affect rhBMP-2 yields. These results suggest that furin inhibition is unlikely to be the mechanism by which IND-1 enhances rhBMP-2 yields.
Conclusions: The addition of IND-1 to the cell culture medium significantly enhanced the yields of both pro- and mature BMP-2 in CHO and HEK cell lines. These increases were sustainable over an extended time period with regular IND-1 treatments. However, the enhanced rhBMP-2 yield is unlikely due to the well-established role of polyarginines as furin inhibitors.

P2.29. APPLICATIONS OF NANOPARTICLES IN MOLECULAR AND CELLULAR BIOLOGY AND CLINICAL MEDICINE

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Nanoscale materials (nanotubes, nanoparticles, nanocomposites) possess extraordinary physico-mechanical properties, electronic, optical, chemical, electrical and so on properties. Moreover these nanomaterials, have very small nanosizes and chirality structure. Both these facts, mentioned above give an opportunity for application of nanomaterials in many fields of technique, engineering, electronics, optics, optoelectronics, transistors, and others [1]. Recently has been discovered an very important application of nanoscale materials (especially nanoparticles) in molecular and cellular biology and medicine. Information about experimental and theoretical works, "imaging protein interactions", "microfluidic devices", "cell finger printing", "cancer research", "plasma protein protection" development of Zebrafish cancer models and so on has been given in [2]. The aim of the work, presented could be formulated as follows: to analyze the synthesis methods for example: a two phase method using biocompatible block polymer; Deposition -precipitation (DP) process; growth of gold shell; Catalytic Oxidation of Ethanol on Gold Electrode in Alkaline Media and others. Following Drude method (model), [3] for describing of the optical properties as a free electron model state could be defined some important characteristics of these properties. Nanotechnology based on gold nanoparicles for In Vivo and In Vitro Diagnostics of cancer have been discussed as well in [4]. Some new computational models, reflecting the optical properties of nanoshells, depending of different model's parameters will be developed in the work too. In conclusion has been pointed out that the paper could be used as a very actual and useful tool for medical and biomedical scientists, physicians, molecular biology specialists, and in nanomedicine too. All these basic research, discussed in the work, could be a very successful step from molecular to cellular biology to the clinic applications.

Key words: nanoparticles, nanoshells, optical properties, nanotechnology, computational models

[1]. Katya Marinova Simeonova, Ganka Marinova Milanova, ISCOM2007, Book of Abstracts, poster 123, page 136, Grant ISCOM2007, 24-29 Sept 2007, Peniscola, Spain
[2]. Katya Marinova Simeonova, ESF-EMBO Symposium, Hotel Eden Roc, Sant Feliu de Guixols (Costa Brava), Spain, 3-8 November 2007, www.esf.org/conferences/07227, Poster presentation (Grant from ESF-EMBO)
[3]. B. I. Drude, J. Optic. Soc. Amer. A., 1994, 11, p.1491
[3]. NCI Alliance for Nanotechnology in Cancer, nanUtah, 2007, Oct. 26, 2007, Piotr Grodzinski, PhD, Director, NCI Nanotech. All., NCI

P2.30. PERFUSION CULTURE OF MACROTISSUE ASSEMBLED BY MICROTISSUE

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Microfabrication-reassembling technology, also called "Bottom-up approach" or "Modular tissue engineering", is an appealing strategy to build complex 3D viable tissues *in vitro* by the assembly of microtissue. The microtissues were produced by first seeding human fibroblasts on microcarriers and then cultivating them in a spinner botter for 16 days. The microtissues were then loaded into a cylindrical chamber and let to maturate and assemble into a 3D macrotissue (1.7 cm in hight and 2 cm in diameter) by perfusion culture for 2 weeks. Results of SEM, viability and histology assays showed the microtissues fabricated by spinner culture had high viability, and the macrotissue had a strong elasticity of compression and a loose core, but viable cells and ECM of the macrotissue mainly distributed in the outer surface regions, which was caused by the mass transfer limitation newly formed in the reassembly process. It need further efforts to investigate and optimize the reassembly process for the construction of complex 3D viable tissues.

P2.31. HUMAN HAIR FOLLICLE EQUIVALENTS *IN VITRO* FOR TRANSPLANTATION AND CHIP-BASED SUBSTANCE TESTING

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The ability to create an organoid, the smallest functional unit of an organ, *in vitro* across many human tissues and organs is the key to both efficient transplant generation and predictive preclinical testing regimes. The hair follicle is an organoid that has been much studied based on its ability to grow quickly and to regenerate after trauma. Replacing hair lost due to pattern baldness or more severe alopecia, including that induced by chemotherapy, remains a significant unmet medical need. By carefully analyzing and recapitulating the growth and differentiation mechanisms of hair follicle formation, we recreated human hair follicles in tissue culture that were capable of producing a hair shaft and revealed a striking similarity to their *in vivo* counterparts. Extensive molecular and electron microscopy analysis were used to track the assembly of follicular keratinocytes, melanocytes and fibroblasts into the final hair shaft producing micro-follicle architecture. The hair follicle generation process was optimized in terms of efficiency, reproducibility and compliance with regulatory requirements for later transplantation. In addition, we developed a procedure to integrate the de novo created human micro-follicles into our existing chip-based human skin equivalents for substance testing. This would allow the evaluation of the role of hair follicles in dermal substance transport mechanisms for cosmetic products. Finally, we describe the challenges and opportunities we are facing for first-in-man transplantation trials.

P2.32. CLINICAL-GRADE GENERATION OF ACTIVE NK CELLS FROM CORD BLOOD HEMATOPOIETIC PROGENITOR CELLS FOR IMMUNOTHERAPY USING A CLOSED-SYSTEM CULTURE PROCESS

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Natural killer (NK) cell-based adoptive immunotherapy is a promising treatment approach for many cancers. However, development of protocols that provide large numbers of functional NK cells produced under GMP conditions are required to facilitate clinical studies. In this study, we translated our cytokine-based culture protocol for ex vivo expansion of NK cells from umbilical cord blood (UCB) hematopoietic stem cells into a fully closed, large-scale, cell culture bioprocess. We optimized enrichment of CD34+ cells from cryopreserved UCB units using the CliniMACS system followed by efficient expansion for 14 days in gas-permeable cell culture bags. Thereafter, expanded CD34+ UCB cells could be reproducibly amplified and differentiated into CD56+CD3- NK cell products using bioreactors with a mean expansion of more than 2,000 fold and a purity of >90%. Moreover, expansion in the bioreactor yielded a clinically relevant dose of NK cells (mean: 2-4x10E9 NK cells), which display high expression of activating NK receptors and cytolytic activity against K562. Finally, we established a versatile closed washing procedure resulting in optimal reduction of medium, serum and cytokines used in the cell culture process without changes in phenotype and cytotoxic activity. These results demonstrate that large numbers of UCB stem cell-derived NK cell products for adoptive immunotherapy can be produced in closed, large-scale bioreactors. The production process has been accepted by the Dutch national authorities and facilitate a phase I clinical trial in elderly AML patients in Nijmegen.

P2.33. XPANSION MULTI-PLATE BIOREACTOR: THE SCALABLE SOLUTION FOR ADHERENT STEM CELL EXPANSION

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Adherent stem cell expansion processes use traditional polystyrene T-flasks or multitray stacks. Such culture methods are not suitable for large-scale production since they involve large numbers of T-flasks or stacked trays, large incubation rooms and multiple manual operations. Capital and operating costs for large-scale production are also prohibitive. To date, implementation of other accepted approaches for large scale adherent cell production such as microcarriers in bioreactors is impeded by adherent stem cell sensitivity to shear stress. Stem cell adherence and detachment are also much more complicated to manage in bioreactors than traditional cell lines.
In response to the lack of suitable large-scale expansion and recovery systems for adherent stem cells, Artelis has developed a new 2D bioreactor, Xpansion, which is composed of multiple, hydrophilised polystyrene plates stacked in a closed bioreactor which enables controlled media flow for dissolved oxygen and pH control. Due to its large surface area of up to 125 000 cm^2 and multi-plate design, Xpansion enables production of large amounts of cells in a process easily adapted from traditional T-flask or stacked tray methods. A scale-down of the bioreactor, Xpansion One, has been developed to allow bench top testing of the system.
Computational fluid dynamic simulations and validations made on 3D models and Xpansion prototypes have demonstrated that media circulation through all layers is evenly-distributed. It also demonstrated that linear speed is constant between the plates and most of the plate area. Only the plate extremities are subjected to slightly lower or faster speeds. This characterization also showed that, at the average operating linear speed of 2mm/sec, 90% of plate surfaces were subject to shear stress of less than 6 mPa. Culture results obtained with the Xpansion One versus traditional T-flasks demonstrated the same cell growth trends, cell morphology and cell density at harvest.

P2.34. OSTEOGENIC DIFFERENTIATION OF ADIPOSE MESENCHYMAL STEM CELLS WITH BMP-2 EMBEDDED MICROSPHERES IN A ROTATING BED REACTOR

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The aim of this work was to study the effect of BMP-2 on the osteogenic differentiation of human mesenchymal stem cells from adipose tissue. The cells were cultivated in a rotating bed bioreactor system (ZRP® system, Zellwerk GmbH) on the aluminium oxide based macroporous ceramic Sponceram® for four weeks. The ceramic discs were loaded with PLGA microspheres releasing BMP-2.
During the cultivation glucose and lactate concentrations were measured. After the experiments histological stainings were performed, (DAPI, von Kossa and alizarine red). Furthermore the mRNA of the cells was isolated and RT-PCR was performed to investigate the expression of different bone markers. Furthermore the concentration of alkaline phosphatase was measured in medium samples (Sigma fast™, Sigma).
The glucose consumption increased during the cultivation indicating a continuous cell growth. The DAPI staining of the ceramics showed a homogenous spreading of the cells on the ceramic and the histological staining showed matrix calcification. Furthermore the expression of typical bone markers was shown. In summary osteogenic differentiation of MSC on Sponceram, caused by BMP-2 released from PLGA microspheres, was demonstrated.

P2.35. AN INTEGRATED APPROACH TO ELIMINATE RESIDUAL PLURIPOTENT STEM CELLS – IMPROVING THE SAFETY OF STEM CELL THERAPY

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Pluripotent stem cells (PSC) such as human embryonic stem cells (hESC) and induced pluripotent stem (iPS) cells have enormous potential for regenerative medicine because of its ability to proliferate indefinitely and differentiate in vitro to any cell type in the body. However, one of the most pertinent concerns using differentiated cells from PSC is the presence of residual undifferentiated cells, which carry the risk of teratoma formation.

Over the last few years, our group has developed several strategies to eliminate residual PSC based on a panel of monoclonal antibodies (mAbs) to novel cell surface markers on hESC. These include cytotoxic antibodies, cytotoxic peptides and cell-cell separation. mAb 84, is an example of a novel cytotoxic IgM, which selectively binds and kills hESC and iPS cells but not differentiated cells. The killing occurs within 30 min of incubation in vitro in a dose-dependent and complement-independent manner by pore formation. Furthermore, in animal models, teratoma formation by hESC and iPS cells was eliminated following treatment with mAb 84 (>24 weeks). Based on the antigen target of another antibody, mAb 375, we have identified a cytotoxic peptide which internalizes and kills PSC via apoptosis within 48 h of incubation. Peptide-treated cells exhibited hallmarks of apoptosis, such as elevated caspase-3 activity and phosphatidylserine externalization. Lastly, we have developed a single step magnetic particle cell-cell separation strategy based on mAb 85, a non-cytotoxic mAb that has a high selectivity and affinity for undifferentiated hESC. The robustness of this method was demonstrated using pools of hESC and fibroblast cells mixed at different ratios (50 – 5% hESC). After the separation step, purities of 98 – 99.9% were achieved.

In conclusion, combining these different strategies together can offer an integrated approach to systematically eliminate residual undifferentiated cells from differentiated cell products. This would evidently improve the safety of the procedure and alleviate concerns over the use of PSC as the starting cell population for cell therapy applications.

P2.36. CARBON MONOXIDE PREVENTS NEURONAL APOPTOSIS BY MODULATION OF ASTROCYTIC METABOLISM: PURINERGIC SIGNALLING

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Carbon monoxide (CO) can be classified as a promising therapeutic molecule. In low doses, this endogenous product, can play a beneficial role in several cerebral models, including primary culture of neurons and astrocytes, as well as isolated non-synaptic mitochondria. CO generates small amounts of reactive oxygen species (ROS), which act as signalling intermediaries and induce a preconditioning state. Herein, a model of primary co-culture of neurons and astrocytes (2D) is used to explorer the role of CO in cell-to-cell communication. Neuronal survival was assessed after being co-cultured with astrocytes pre-treated or not with CO. Neuronal cell death was triggered by *tert*-butylhydroperoxide (*t*-BHP), an oxidative stress inducer. CO-pretreated astrocytes reduced neuronal cell death in the co-culture systems. Since there is no physical contact between neurons and astrocytes, the existence of extracellular factors involved in this cell to cell interaction is suggested. Additionally, when media was conditioned with CO-treated astrocytes, neuronal survival was higher. Therefore, CO seems to induce the release of some neuroprotective factor from astrocytes.

The classical energetic molecule ATP and its degradation product, adenosine, are the candidate molecules for being neuroprotective. The variation on ATP/adenosine content in the media due to CO presence was assessed by HPLC. Neuronal survival was determined under several conditions. Addition of adenosine or alpha,beta-methyleneadenosine 5'-triphosphate lithium salt (ATP resistant to degradation) into the neuronal media allowed to clarify the players involved in this cell-to-cell communication. Furthermore, several chemical inhibitors of purinergic receptors (both P1 and P2), namely PPADS, suramin, MRS 2179 or SCH 58261, partially reverted CO protection in co-cultures. Still, the expression of purinergic receptors with or without CO-treatment was analysed by western blot and RT-Q-PCR.

Overall, one can conclude that CO neuroprotective role is not limited to a direct action into a single cell type. This gasotransmitter has the ability to operate in a complex system, as the brain, in order to increase astrocytic protection against neuronal cell death. This innovative approach takes advantage of endogenous molecules and natural cellular organisation to achieve cell survival. Based on this knowledge, CO-based therapies for the treatment of several diseases remain an opened window to be explored.

P2.37. LARGE SCALE EXPANSION AND TRI-LINAGE DIFFERENTIATION OF HUMAN MESENCHYMAL STROMAL CELLS

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Multi-potent human mesenchymal stromal cells (hMSC) are a potential source for autologous cells for tissue repair, either differentiated into cells targeted for a specific tissue or as un-differentiated hMSC. Whether the requirements are for clinical or research use, obtaining a substantial number of cells can constitute a bottle neck for the investigator. We here present a protocol enabling the clinician or researcher to rapidly expand a population of hMSCs utilizing the potential of Thermo Fischer Scientific HyClone AdvanceSTEM media, developed specifically for the optimal expansion and maintenance of undifferentiated hMSCs and Nunc brand cell factories. Several different seeding densities from 350 – 4000 cells/cm^2 was investigated in flask format and a relatively low seeding density of 350 cells/cm^2 was chosen for use in the cell factory hMSC expansion protocol. In the cell factories the hMSC population was rapidly expanded more than 800 fold in 12 days. The easy to use protocol provides the investigator with several decision points regarding growth format, cell density and culture period while maintaining the multi-potency of the hMSCs. Subsequent to expansion the differentiation of the expanded hMSCs into adipocytes and osteoblast lineages was demonstrated in cell factories and chondrocytes differentiation in polypropylene tubes. The differentiation was documented using commercial kits.

P2.38. COVALENTLY GRAFTED CELL CULTURE SURFACES FOR STEM CELL DIFFERENTIATION AND TISSUE ENGINEERING

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Thermo Scientific Nunc HydroCell surface enables cultivation of cells that are sensitive to unwanted activation and differentiation arising from cell adhesion. The problem with using traditional cultureware for suspension cell culture is that the heterogeneity nature of the culture leads to partial attachment of these cells to culture surface often facilitated by serum in the media. The Nunc HydroCell dishes minimizes adhesion of large protein molecules in serum and on cell surfaces, resulting in better quality of suspension cell culture and higher efficiency for the induced differentiation. Stem cells derived from exfoliating human deciduous teeth have the ability to differentiate into neuronal and glial cells in vitro. Dental pulp may, therefore, be a potential source of cells in cell replacement therapies for various neurological disorders. This study demonstrates that adult rat dental pulp cells can be propagated as neurospheres in cultureware with Nunc HydroCell surface.
Thermo Scientific Nunc UpCell surface is a novel temperature-responsive cell culture surface that allows collection of cells by controlling the external temperature of the culture, therefore completely abolishing the usage of cell damaging proteolytic enzyme such as trypsin. The proprietary technology involves covalent grafting of a thermo-responsive polymer, PIPAAm, onto the cell culture surface. The polymer changes its property across the lower critical solution temperature (LCST) of 32°C. At 37°C, the surface allows adherent cells to attach, and at room temperature (20-25°C), the surface facilitates spontaneous cell detachment. This presentation demonstrates that the temperature reduction cell detachment process preserves CD140a surface molecules on mesenchymal stem cells from human bone marrow and human preadipocytes, and improves their recovery and viability. Moreover, when MDCK cells are cultured on UpCell to 100% confluency, they can be collected as "cell-sheet" with the establishment of tight cell-to-cell junctions and the intact extracellular matrix naturally deposited by the cells as demonstrated by transmission electron microscopy.

P2.39. INNOVATIVE ANIMAL COMPONENT-FREE SURFACE FOR THE CULTIVATION OF HUMAN EMBRYONIC STEM CELLS

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The concerns over contaminants from animal components and batch-to-batch variability of the coating matrices for human stem cells culture have significantly hindered the usage of this type of surface for translational and clinical applications. The Thermo Scientific Nunclon Vita is an energy-treated polystyrene surface free of animal components. It enables culture of human stem cells without matrix or feeder layers. Human embryonic stem (ES) cells are grown directly on the surface in conditioned media containing ROCK-inhibitor, and can be sustained for more than ten passages without signs of differentiation. During this presentation, we will present data that demonstrate karyotypic normality, pluripotent status, and induced differentiation to embryoid body formation of H1 and H9 human ES cells cultured on Nunclon Vita surface.

P2.40. DEVELOPMENT OF A SERUM-FREE HUMAN BONE MARROW MESENCHYMAL STEM CELL EXPANSION SYSTEM SUPPORTS SUPERIOR GROWTH PERFORMANCE AND RETENTION OF MULTIPOTENCY AND IMMUNOPHENOTYPE

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Fulfilling the clinical promise of mesenchymal stem cells (MSCs) in cell therapy and tissue engineering will require solutions to reliably expand and manufacture these primary cells. MSC expansion has traditionally employed serum supplementation of growth media, but serum presents challenges due to its lot-to-lot variation, chemically undefined nature, and potential for transmission of infectious agents.

A novel serum-free MSC expansion medium, BD MOSAIC™ , yields proliferation in culture significantly exceeding that of cells in conventional serum-containing medium through multiple passages. Cells expanded in this serum-free medium maintained their multipotency in *in vitro* adipogenic, chondrogenic and osteogenic differentiation protocols as measured by lipid accumulation, glycosaminoglycan accumulation, and alkaline phosphatase induction, respectively. Serum free-expanded cells also maintained the consensus MSC immunophenotype (positive for CD73, CD90, CD105; negative for CD14, CD34, CD79a, HLA-DR). The immunomodulatory ability of serum free-expanded cells was likewise equivalent to that of serum-expanded cells in an *in vitro* T-cell proliferation assay. This chemically defined medium, developed through the application of bioinformatic and screening principles, may thus assist in the progress of MSCs from the laboratory bench to the clinic.

P3.01. ASSESSING THE TRANSLATION STATE OF HIGH PRODUCING CHO CELLS

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During the past decades, bioprocessing has become increasingly important since it can provide complex biopharmaceutical proteins for the treatment of human diseases. Continuous enhancement of the bioprocesses is required to increase cost efficiency and meet the growing demand for these proteins of interest, especially monoclonal antibodies.Translation is one of the critical steps in the biosynthesis of the protein of interest, as it determines the effective protein level in the cells and requires high amount of energy (ATP) to proceed. The mTOR pathway is used by the cells to sense environmental stress signals and to regulate their translational machinery accordingly. Interestingly, there has been several studies (Santoro et al. 2009; Underhill et al. 2003) focused on enhancing the translation activity of Chinese Hamster Ovary (CHO) cells with no proper assessment of the translation state.
In this study, we introduced a new approach to assess the translation state of high producer CHO-mAb cells. The polysome profiling technology was used to compare batch and fed-batch cultures. For both cultures, the overall translation activity started decreasing when entering the stationary growth phase. However, the extent of decrease of translation activity was less for the fed-batch, which resulted in the maintenance of a constantly higher global translation state as compared to the batch culture. In addition, the involvement of the mTOR pathway in dictating the overall translation state was confirmed by probing phosphorylated 4E-BP levels. The specific translation efficiencies for the heavy chain (HC) and light chain (LC) of the monoclonal antibody were also monitored. Results indicate an overall loss in efficiency in the case of the batch culture that seems to correlate with the trends in specific productivities. Based on the findings, it is suggested that polysome profiling can be used as an efficient and reliable approach to investigate the translation in CHO cells.
References:
Santoro R, Lienemann P, Fussenegger M. 2009. Epigenetic engineering of ribosomal RNA genes enhances protein production. PLoS One 4(8):e6653.
Underhill M, Coley C, Birch J, Findlay A, Kallmeier R, Proud C, James D. 2003. Engineering mRNA translation initiation to enhance transient gene expression in chinese hamster ovary cells. Biotechnol Prog 19(1):121-9.

P3.02. DISSECTION OF THE ROLES OF THE mTOR PATHWAY IN GROWTH AND PRODUCTIVITY OF INDUSTRIALLY-RELEVANT CHO CELLS

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Understanding the molecular mechanisms that govern productivity and growth of recombinant CHO cells is essential to devise informed approaches to increase commercial viability and availability of biopharmaceuticals. This work has focused on the changes in the intracellular mammalian target of Rapamycin complex 1 (mTORC1) signalling pathway in response to the environmental conditions of culture. The study of a recombinant CHO cell line in the serum-free suspension batch culture indicated a gradual decrease in the activity of mTORC1, as defined by the decreased extent of site-specific phosphorylation in western blotting analysis of two widely ascribed downstream target proteins (ribosomal protein S6 kinase 1 (S6K1) and 4E-BP1, an inhibitor of translation initiation). The decline in the activity of mTORC1 paralleled decreases in growth rate, recombinant protein specific productivity and global protein translation and an increase in the percentage of cells in the G1/G0 phase of the cell cycle (determined by flow cytometry). To further clarify the role of the mTOR pathway in cell growth and protein production, cells in batch culture were treated with Rapamycin, a specific inhibitor of mTORC1 in several cell types. Treatment with Rapamycin stalled the growth of the CHO cell line, but only briefly, and this effect was transient as cells reverted to the predicted growth rate. Under the condition of Rapamycin challenge, recombinant protein specific productivity at the beginning of batch culture, the longevity of batch culture, and the final antibody titre were higher than control. The Rapamycin addition produced discriminating effects on downstream signalling targets, abolishing S6K1 phosphorylation but no effect on 4E-BP1 phosphorylation. Since Rapamycin did not affect the phosphorylation of 4E-BP1 and the rate of global translation, we conclude that inactivation of 4E-BP1 plays a more significant role in the maintenance of recombinant protein synthesis. Analysis of the downstream targets of mTORC1 suggests that S6K1 is important for cell growth in CHO cells; however, alternative pathways may counter-regulate the effect of inactivated S6K1 with long-term or continuous treatment.

P3.03. TRANSCRIPTIONAL PROFILING OF HIGH-PRODUCTIVITY CHO HOST CELL LINES

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Chinese hamster ovary (CHO) cells are the most prevalent cells in mammalian cell culture technology and a lot of effort has been put into the engineering and improvement of this important cell line. Most approaches aim for the generation of high growth rates and more efficient protein production and secretion. By repeated cycles of transient transfection and cell sorting, we have previously established clonal host cell lines derived from CHO-K1 and CHO-S (Pichler et al. 2011) with a stable high producing phenotype that resulted in an increase in the specific transient protein production rate (qP) of up to 5-fold (CHO-S) and up to 3-fold (CHO-K1) compared to the respective parents.
In order to gain a deeper understanding of the molecular mechanisms causing this effect, transcriptome analysis of messenger RNA (mRNA) and microRNA (miRNA) of the parental strains were compared to those of the clonal cell lines by cross-species profiling. Microarray profiling of mRNA is a well established method for the analysis of transcriptomic expression data, however miRNA profiling is a rather novel, yet promising tool for more detailed cell line characterization. At present, over 1000 human miRNAs are known and they exhibit a high degree of sequence homology across several species (see Poster Hackl et al. on the sequence of CHO miRNAs). Moreover, their involvement in the regulation of fundamental cellular processes such as apoptosis or proliferation makes them interesting targets for cell line development.
In the present study, we compared expression profiles of optimised CHO-S subclones (CHO-S-D2&4F11) and CHO-K1 subclones (CHO-K1-1D9&4F10) to their respective parents. Surprisingly, fewer mRNAs as well as miRNAs were differentially regulated in CHO-S- D2&4F11 (~120 mRNAs, ~10 miRNAs), while in CHO-K1-1D9&4F10 ~250 mRNAs, ~30 miRNAs were differentially regulated. The alterations in mRNA expression monitored in the subclones were mostly found both in transfected and untransfected cell lines, thus these changes were permanent and not induced by transient production. The expression level of many genes that were differentially regulated in CHO-K1-1D9&4F10 approached the levels shown in the CHO-S parent, indicating an already optimised gene expression pattern in CHO-S. This explains the lower number of regulated genes in CHO-S-D2&4F11. MiRNA profiling revealed consistent up-regulation of the miRNA cluster (miR23-24-27) in all subclones. Members of this cluster have been reported to be involved in glutamine and glucose metabolism as well as cell cycle control and proliferation.
Further data analysis will focus on the correlation of differentially expressed mRNA and miRNA expression with an emphasis on the identification of novel target genes and miRNAs for more detailed functional characterization and potentially for engineering.
Pichler J. et al. Biotechnol Bioeng. 2011 108(2):386-94

P3.04. DETERMINATION OF INTRACELLULAR PRODUCTIVITY, CELL CONCENTRATION AND VIABILITY OF RECOMBINANT CHO DG44 CELLS BY THE FLOW CYTOMETER MACSQUANT®

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The MACSQuant® Analyzer is a benchtop cell analyzer with a broad range of applications such as total cell counting and multicolor flow analysis. In this work the MACSQuant® analyzer was used for determination of cell density, viability, and for intracellular staining of recombinant IgG4 heavy and light chains. Transfected cell pools could be monitored due to their ability of expressing the recombinant antibody by this method.
Cell concentrations were determined by flow analysis and viabilities were determined by propidium iodide (PI) staining. This approach was compared to other methods of automated cell counting (e.g. Cedex). Furthermore, a combination of PI and Annexin V-FITC staining allowed the detection of apoptotic cells. Apoptotic cells are stained positively for Annexin V-FITC that binds to phosphotidylserine (PS), but are negative for staining with PI. Dead cells are stained positive for Annexin V-FITC and for PI, whereas viable cells are negative for both Annexin V-FITC and PI.
Intracellular staining of IgG-Fc- and κ-chains allowed the monitoring of recombinant antibody producing cells. During amplification of the target gene in CHO DG44 cells by increasing MTX concentrations it was shown that one transfected cell pool was stable (about 98% productive cells), whereas the rate of productive cells of another pool dropped from 30% to 1%. For recombinant CHO DG44 single cell clones a correlation between cell specific production rates and the dot blot pattern could be observed. This method also was successfully used to monitor the genetic stability of recombinant CHO DG44 cells over a long period of time.
To combine the determination of intracellular staining of the product with cell concentration and viability, the described method is a powerful tool to characterize recombinant CHO cells during cell line development up to stable cell banks.

P3.05. ANALYSIS OF GLYCOLYTIC FLUX AS A RAPID SCREEN TO IDENTIFY LOW LACTATE PRODUCING CHO CELL LINES WITH DESIRABLE MONOCLONAL ANTIBODY YIELD AND GLYCAN PROFILE

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There is growing interest in the development of defined culture media for the production of therapeutics from established animal cell lines. Cultured mammalian cells are major vehicles for producing therapeutic glycoprotein, and energy metabolism in those cells profoundly affects process productivity and product quality. Regulation of glucose feed rate promotes a higher efficiency of glucose and nitrogen source utilization, with lower production of metabolic byproducts such as lactate. In mammalian cell lines currently selected for the production of recombinant antibody, approximately 80% of the metabolized glucose is converted into lactic acid. Therefore, it is important to assess the suitability of the cell line for the manufacturing process through examining how nutrient feeding affects the productivity and product quality. The negative effect of lactate on growth, productivity and glycan profile and the ability of CHO cells to consume endogenous lactate in the presence of different carbon and nitrogen sources open up the possibility of defining different culture strategies to allow growth under conditions of glucose depletion.
In this study, we utilized an XF96 analyzer to measure glycolytic fluxes based on the secretion of extracellular acid from lactate production. This enabled a rapid screen for lactic acid production from cells exposed to alternate energy and carbon sources, such as galactose or fructose. The screen identified a subset of batch and fed-batch cultures, using different carbon sources, which drastically reduced lactate production. Specific metrics of the study included cell growth, product yield, and glycan profile. This screening approach based on metabolic glytolytic flux analysis, can be used to predict the metabolic behavior of both batch and fed-batch systems as a function of the extra-cellular nutrient/metabolite concentrations. We believe this approach can be used to optimize monoclonal antibody (MAb) production and quality in the future.

P3.06. SITE-DIRECTED MUTAGENESIS OF HUMAN COAGULATION FACTOR VIII FOR IMPROVED SECRETION: EXPERIMENTAL AND *IN SILICO* APPROACHES

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Factor VIII (FVIII) is a blood coagulation factor involved in the blood coagulation system. Its deficiency causes hemophilia A, the most prevalent type of hemophilia. The protein suffers several essential post-translational modifications, and therefore recombinant FVIII used in replacement therapy is produced in mammalian cell cultures. Even when cloned in the same expression vector as other proteins, human FVIII (hFVIII) is secreted in a much lower concentration. One reason for this is the high-affinity interaction with intracellular chaperones. Hydrophobic amino acids in a 110-amino acid region have been already characterized as binding sites for ERGIC-53 (also known as BiP), a chaperone that assists FVIII folding in the endoplasmic reticulum. In the present work, the hydrophobic residues Phe270, Phe277, Leu300 and Leu335 were changed to neutral or charged amino acids in a B-domain deleted FVIII, in order to analyze the effects of those mutations on FVIII secretion. After stable transfection of CHO cells, mRNA concentration was measured in the different stable populations and was shown not to be significantly affected by the single residue modification. All the muteins were biologically active, with Leu300Ser and Leu335Ser being 1.4 and 2.7 times more active than the wild-type control. Mutants Leu335Ser and Phe277Ser were only secreted during the stationary growth phase, while the other proteins were secreted from the beginning of the exponential growth phase. Higher hFVIII productivity was obtained for the Phe270Ser, Phe277Ser and Leu300Thr mutants (1.6, 1.8 and 1.3 fold, respectively, when compared with the wild type BDD-FVIII).
Using homology molecular modelling, tridimensional structures for the hamster BiP chaperone and the BDD-hFVIII were proposed. Docking studies confirmed that the Asn253-Asp357 region in hFVIII was involved in the interaction with BiP. Only the Leu335 residue was shown to directly interact with BiP, but this seemed not to be crucial, since experimental mutations in residues at positions 270, 277 and 300 modified the FVIII-BiP interaction strength, indicating that charge and/or size modifications affect the local tridimensional structure and, consequently, the interaction intensity. Other interacting hydrophobic residues were also identified as possible targets for further site-directed mutagenesis.
In summary, in the present work several FVIII mutant proteins were obtained, all of them conserving the pro-coagulant activity. Hence, due to the increased secretion in the exponential growth phase, the FVIII molecules with Phe270Ser and Leu300Thr mutations may represent promising therapeutic hFVIII molecules for more economic production processes.

P3.07. ENHANCING RECOMBINANT MRNA TRANSLATION VIA MICRORNA INTERACTIONS WITH THE 3'-UTR FOR IMPROVED RECOMBINANT PROTEIN YIELD

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The production of many complex recombinant proteins is achieved industrially using mammalian expression systems. Despite improvements to the levels of recombinant protein these systems can express over the last few decades, the potential to engineer mammalian cells for further enhanced recombinant protein production still remains. Alternatively, a better understanding of the cellular constraints upon recombinant protein production would allow for the design of novel approaches to screen for high producing cell lines. It has been suggested that mRNA translation is a key cellular process that could limit the qP obtainable from these systems and also be central to the control of other processes such as cell growth. A recently discovered mechanism by which mRNA translation is controlled is via the expression of microRNAs (miRNAs). miRNAs are endogenous single stranded non-coding pieces of RNA of around 22 nt in length that can either stall mRNA translation or activate cleavage of target mRNAs. Chinese hamster ovary (CHO) cells are one of the most widely used host cell lines for producing biopharmaceuticals. We used CHO cell lines engineered to express a model monoclonal antibody to investigate the role of miRNAs in controlling cell growth and volumetric productivity. Ultimately this work aims to provide informed strategies to manipulate the mRNA in general, and the control of mRNA translation, in order to increase recombinant protein expression levels from mammalian cells.

P3.08. QUANTIFICATION OF INTRACELLULAR NUCLEOTIDE SUGARS AND FORMULATION OF A MATHEMATICAL MODEL FOR PREDICTION OF THEIR METABOLISM

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The US FDA and the European Medicines Agency have recently proposed to apply the Quality by Design (QbD) paradigm to the manufacture of biopharmaceuticals. Its implementation requires the use of all available knowledge of a given product, including the parameters that affect its quality, for the design, optimization and control of the manufacturing process. The goal is to ensure that quality is built into the product at every stage of the manufacturing process. Most licensed monoclonal antibodies (mAbs) are based on the immunoglobulin G isotype and contain a consensus N-linked glycosylation site on the C□2 domains of their heavy chains. Studies have found that the oligosaccharides attached to this site dramatically influence the efficacy of mAbs as therapeutics either by reducing their serum half-life or by directly affecting the mechanisms they trigger *in vivo*, thus defining glycosylation as a critical quality attribute of mAbs under the QbD scope. It has been recently proposed that detailed mathematical models will play a critical role in the design, control and optimization of biopharmaceutical manufacturing processes under the QbD scope. To our knowledge, there are currently no mathematical models that relate mAb glycosylation with cell culture conditions. Several reports have shown that glycosylation is directly affected by the intracellular availability of nucleotide sugar donors (NSDs), which are the co-substrates for the glycosylation reactions that occur in the Golgi apparatus. During culture, cells synthesize all the relevant NSDs from glucose through the nucleotide sugar metabolic pathway. In an effort to relate process conditions with mAb glycosylation, we have generated a dynamic mathematical model for this metabolic pathway. The kinetic model was subsequently reduced based on the methodology described by Nolan and Lee. In order to estimate the unknown parameters of the reduced model, the intracellular concentration of glucose and eight NSDs was monitored through the course of a batch culture of CHO cells. The cells were harvested and their metabolism quenched prior to sample preparation for analysis. The intracellular glucose concentration was determined using a fluorescence-based assay kit and the intracellular NSD concentrations were determined with an ion-pairing reverse-phase liquid chromatographic technique. Finally, a series of fed-batch cell culture experiments were performed in order to validate the reduced model and assess confidence in the estimated parameters. The obtained time-course concentrations of NSDs follow the same trends that have been reported previously for four of the eight NSDs. However, implementation of the metabolism quenching method reduces uncertainty in the measurements associated to metabolite degradation and leakage during sample preparation, thus yielding more robust results than previous studies. Regarding the modeling component of this work, our results show that the model has the ability to reproduce the intracellular concentration of all eight NSDs and the intracellular glucose concentration as a function of extracellular glucose availability and biomass balances. The model has also shown good predictive ability of intracellular NSD concentrations under fed batch operation. Our mathematical model for NSD metabolism, when coupled to a model for Golgi N-linked glycosylation, generates a direct link between extracellular glucose concentration, which is a readily measurable process variable, and protein glycosylation. Such a combined model has great potential for the design, control and optimization of manufacturing processes that produce mAbs with *built in* glycosylation-associated quality as proposed under the QbD paradigm.

P3.09. INCREASING PRODUCTIVITY OF HYBRIDOMA CELL LINES BY CELL SORTING BY SIDE SCATTERING LIGHT

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Due to the fact that the side scattering light of cells is caused by inner membranes we suggest that cells with a high side scatter contain a large amount of mitochondria and a large endoplasmatic reticulum. In a simple approach we separate cells with a high side scatter to build sub populations with higher productivity via fluorescence activated cell sorting (FACS). We obtain cells with a strong energy metabolism caused by a high amount of mitochondria and a high protein productivity caused by a large endoplasmatic reticulum. The advantage of this technique is that no staining dye or complex procedure is needed to reach the goal of increasing the productivity of a cell line.
Subsequent flow cytometric analysis show constant increased sides scatter in the following populations. This could be caused by a higher content of diverse cell organelles. Further studies to compare the content of mitochondria and endoplasmatic reticulum will be performed.

P3.10. HUMANIZATION STRATEGIES FOR AN ANTI-IDIOTYPIC ANTIBODY MIMICKING HIV-1 GP41

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Anti-idiotypic antibodies could represent an alternative vaccination approach in human therapy. The anti-idiotypic antibody Ab2/3H6 was generated in mouse and is directed against the human monoclonal antibody 2F5, which broadly and potently neutralizes primary HIV-1
isolates. Ab2/3H6 is able to mimic the antigen recognition site of 2F5 making it a putative candidate for HIV-1 vaccine purposes. In order to reduce immunogenicity of therapeutic proteins, humanization methods have been developed. The mouse variable regions of Ab2/3H6 were subjected to three different humanization approaches, namely resurfacing, complementarity determining region (CDR)-grafting and superhumanization. Four different humanized Ab2/3H6 variants were characterized for their binding affinity to 2F5 in comparison to the chimeric Ab2/3H6. The resurfaced and the 'conservative' CDR-grafted variants showed similar binding properties to 2F5 when compared to the chimeric version, while the 'aggressive' CDR-grafted antibody showed reduced affinity and the superhumanized type lost its binding ability. In this study, we developed humanized Ab2/3H6 variants that retained the same affinity as the parental antibody, and are therefore of potential interest for future clinical trails.

P3.11. IS CELL OVERPOPULATION A RISK FOR MY PRODUCTIVITY?

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Increased pressure on costs and time-to-market are main trends of the current biopharmaceutical industry landscape. For marketed products, the main focus is to reduce cost of goods by improving process performance. The upstream process for one of the human recombinant proteins manufactured at Merck Serono S.A. was investigated in order to decrease the process variability and to improve its productivity. Indeed, the total production of the human recombinant protein in the upstream process has shown a high level of variability throughout all manufacturing campaigns.
The production of the human recombinant protein is performed using transfected CHO cells cultured on macroporous microcarriers. A first bioreactor of 75 LT containing microcarriers is inoculated with cells to promote cell growth. Then, when a defined cell concentration is reached, the cells are trypsinised from the microcarriers and the whole content of the bioreactor is transferred to a 300 LT production bioreactor containing new macroporous microcarriers. The production phase is performed in perfusion mode in a serum-free medium. In order to understand cell distribution and viability on microcarriers, samples were taken after trypsinisation of the 75 LT growth bioreactor and analyzed by fluorescent microscopy. The study showed that the macrocarriers are heterogeneously colonized by the cells, also with variable cell viability on each individual macrocarrier. This observation led to the hypothesis that trypsin activity used for the transfer from the 75 LT growth bioreactor to the production bioreactor could be optimized in order to increase cell distribution homogeneity and as a consequence, cell viability on microcarriers.
Historically, while the duration of trypsinisation of the cells from the microcarriers in the 75 LT bioreactor was fixed at 20 minutes, the trypsin activity was not defined, ranging from 1 to 2.2 arbitrary units. Kinetic experiments with various activities of trypsin could demonstrate a direct relationship between the quantity of cells detached from the microcarriers and the level of trypsin activity, until reaching a plateau. The optimal trypsin activity was defined at the start of the plateau, corresponding to an activity of 2.9 arbitrary units.
The results obtained in this study led to implement a fixed trypsin activity of 2.9 arbitrary units in production. This process change was concomitant with a 1.5 fold average increase in cumulative and specific productivity per run during the 2010-2011 manufacturing campaign, probably by a better distribution and subsequently better overall cell viability on microcarriers in the production bioreactor.

P3.12. IN-DEPTH ANALYSIS OF HIGH PRODUCING HEK293 CELLS USING A SYSTEMS BIOLOGY APPROACH

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Mammalian cells are the organisms of choice for the production of recombinant proteins requiring post-translational modifications such as antibodies, certain hormones (e.g., EPO, TSH, FSH) and cytokines. Compared to bacteria and yeast however, mammalian cell culture is slow, expensive and productivities are low. A lot of effort has gone into improving certain aspects of mammalian cells associated with higher product titers. These include genetic engineering strategies to delay apoptosis, increase the rate of proliferation, the capacity of the protein processing machinery and the efficiency of the metabolism to reduce the production of growth inhibiting by-products like lactate and ammonia. However, the outcome of these manipulations has been mixed, with few studies able to generate an improved phenotype.
One of the main reasons for this lack of success is that the engineering strategies were based on our current understanding of mammalian cells or rather on our assumptions of what is required for a particular phenotype. While our understanding of certain aspects of mammalian cells (e.g., apoptosis) may be quite detailed, our knowledge about the interplay of the individual components to create a certain function is still very limited. To enable engineering of a superior host cell we first need a better understanding of the cellular interactions.
Such knowledge can only be acquired by employing a systems biology approach combining datasets from all functional levels e.g., transcriptomics, proteomics and metabolomics. With this in mind, we have collected complete 'omics datasets of a high producing Hek293 cell line and its parental cell line. Combined analysis of these datasets has the potential to identify important features for high productivity. This approach can be applied to other cell lines e.g., CHO cells as soon as complete sequence information is available.

P3.13. 3D6 AND 4B3 – RECOMBINANT EXPRESSION OF TWO ANTI-GP41 ANTIBODIES AS DIMERIC AND SECRETORY IGA

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Each year, the human immunodeficiency virus (HIV), potentially leading to the acquired immunodeficiency syndrome (AIDS), newly infects several million individuals on a global scale. The majority of these infections are sexually transmitted. There, during sexual intercourse, mucosal membranes of the rectal or genital tract represent the major port of virus entry. Although current therapies can reduce disease progression in infected individuals, no cure is yet available or within reach in near future. As a consequence increased attention is now being paid to develop drugs that could prevent virus acquisition.
The monoclonal antibodies (mAb) 3D6 and 4B3 have originally been isolated as IgG1 isotype from seroconverted HIV-1 patients where they selectively bind to the principal immunodominant domain of gp41. However, the primary antibody class to elicit mucosal immunity is IgA. In vivo, dimeric IgA (dIgA) reaches the luminal side of mucosal tissues via the polymeric immunoglobulin receptor (pIgR) expressed basolaterally on epithelial cells lining the mucosa. Upon binding of dIgA to pIgR, the complex is transcytosed to the luminal side. After cleavage of pIgR, its extracellular portion, termed secretory component (SC), remains attached to the antibody to form secretory IgA (sIgA).
The aim of this project is to establish mammalian cell lines for the recombinant production of 3D6 and 4B3 as dimeric as well as secretory IgA. While dIgA is expressed by a single cell line, sIgA will be produced by an in vitro association of dIgA with SC. Both dIgA and sIgA variants will be characterized and the contribution of the heavily glycosylated SC on IgA stability will be investigated.

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P3.14. RNA INTERFERENCE OF COFILIN IMPROVES RECOMBINANT PROTEIN PRODUCTIVITY IN ADHERENT CHINESE HAMSTER OVARY CELLS

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Chinese hamster ovary (CHO) cells are the most commonly used mammalian cell line for production of biopharmaceutical proteins that require proper folding and glycosylation for full activity. Gene silencing using RNA interference (RNAi) has been recently applied in CHO cells to improve cellular productivity by altering gene expression, apoptosis, and metabolism and to improve antibody efficacy by altering glycosylation. Previous characterization of a gene-amplified CHO cell line identified several proteins with altered expression. Cofilin, a key regulatory protein of actin cytoskeletal dynamics in mammalian cells, was down-regulated in this high-producing cell line. Here, RNAi is used to decrease cofilin levels as a genetic approach to cell line engineering. CHO cells expressing the model protein human secreted alkaline phosphatase (SEAP) and the therapeutic protein tissue plasminogen activator (tPA) were treated with short interfering RNA (siRNA) and short hairpin RNA (shRNA) to reduce cofilin expression. Transient reduction of cofilin expression by siRNA enhanced specific productivity in these cell lines by up to 80%. CHO cell lines expressing cofilin-specific shRNA vectors showed up to a 65% increase in specific productivity. These results suggest that alteration of the actin cytoskeleton using RNAi technology is a new approach to enhance recombinant protein productivity in CHO cells.

P3.15. EFFECT OF PRODUCTIVITY ENHANCERS ON GROWTH AND PRODUCTIVITY OF A PANEL OF 148 GS-CHO CELL LINES

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The deliberate inhibition of cell growth to increase specific production rate (Q_P) (controlled proliferation) is a technique often employed when trying to increase product titres. Three methods commonly used for controlled proliferation are (i) use of chemical agents, (ii) hypothermic conditions and (iii) genetic manipulation. Typically, a cell line has already been selected as a manufacturing cell line prior to assessing such methods. Consequently, the likelihood of successfully implementing such strategies to increase Q_P is unpredictable: resulting in the frequently heard comment that results are 'cell line specific'. But what if we were to look at these methods with many cell lines, at a much earlier stage of development?: 'To what extent does the response to such methods vary in a large panel of cell lines all producing the same antibody?'; 'Would their use in an earlier stage of cell line development enable the selection of a 'better' manufacturing cell line?'

In this study, the effect of two chemical agents, sodium butyrate (NaBu) and sodium acetate (NaAc), on a panel of 148 previously characterised (Porter *et al*, 2010, Biotechnol Prog 26: 1455-1464) GS-CHO cell lines were investigated. Three fed-batch shake-flask cultures were initiated for each cell line. The first was a control culture, in the second the culture medium was supplemented with 1 mM NaBu, and in the third the culture medium was supplemented with 7.5 mM NaAc.

Analysis of the entire data set, for each condition, reveals that the distribution of the time integral of viable cell concentration (IVC) values for both the NaBu and NaAc conditions are lower than that of the control (mean ± 1 standard deviation [SD] = 585 ± 258 x 10^6 cells/mL.h, 1640 ± 1219 x 10^6 cells/mL.h and 1984 ± 435 x 10^6 cells/mL.h respectively). For Q_P, the distribution of values for the NaBu condition are higher that that of the control (mean ± 1 SD = 0.88 ± 0.64 pch and 0.36 ± 0.31 pch respectively). The NaAc condition shows no improvement (mean ± 1 SD = 0.45 ± 0.35 pch). For product concentration, the distribution of values for the NaBu condition are lower than that of the control (mean ± 1 SD = 487 ± 383 mg/L and 692 ± 586 mg/L respectively). Little difference is observed between the control and the NaAc condition (mean ± 1 SD = 681 ± 545 mg/L). Data were analysed by one-way ANOVA and Tukey's multiple comparison test at a 5% significance test. The analysis reveals that there is a significant difference between the control and NaBu conditions for IVC, Qp and product concentration. There is also a significant difference between the control and NaAc conditions for IVC but not for Qp and product concentration.

The response to the productivity enhancers varied, as could be predicted from comments that any increases in Q_P, when using productivity enhancers, are 'cell line specific'. The results also suggest that there is no advantage in using NaBu or NaAc, at the concentrations tested, during early cell line selection stages. These conditions did not enable the identification of a cell line capable of achieving a higher product concentration than any of those in the control condition.

P3.16. INFLUENCE OF GLUTAMINE ON TRANSIENT AND STABLE RECOMBINANT PROTEIN PRODUCTION IN CHO AND HEK-293 CELLS

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Glutamine is an essential component in culture media for most mammalian cell lines, and it is often used as an alternative to glucose as an energy source. Here we show that glutamine can be reduced or eliminated from culture medium for growth-arrested cells that have been transiently transfected and for bioprocesses with stable cell lines. We have observed that glutamine reduction results in an improvement of transient recombinant IgG yields by up to 50% in both CHO and HEK-293E cells in 7 day bioprocesses. We studied the metabolic profile of transiently transfected CHO-DG44 and HEK-293E cells under these conditions. Lower ammonia accumulation in the cell culture medium was observed when the glutamine concentration was reduced. For bioprocesses involving stable CHO-DG44 cell lines or pools of recombinant cells expressing a recombinant monoclonal antibody, elimination of glutamine resulted in increases in antibody titers of up to 80% under mild hypothermia at 31 °C . Our data demonstrate that under growth-arrested conditions, reduction or complete removal of the glutamine in the medium is an effective and economically useful approach to improve protein production in both transiently and stably transfected mammalian cells.

P3.17. BACTERIAL ARTIFICIAL CHROMOSOMES IMPROVE RECOMBINANT PROTEIN PRODUCTION IN MAMMALIAN CELL LINES

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Stable recombinant protein production in mammalian cells is a main topic in biotechnology. Expression vectors are a critical step in the generation of stable cell lines; consequently, a considerable effort has been invested in the development of expression systems. An ideal expression vector should fulfil three prerequisites:

1) Expression levels should not be influenced by the genomic integration site.
2) Expression should be maintained over time.
3) Expression should be copy number dependent.

Bacterial Artificial Chromosomes (BACs) are vectors derived from the *E. coli* F factor with a cloning capacity of up to 400Kb. Due to the large cloning capacity, BACs are able to accommodate a whole mammalian locus, and have been widely used in the mouse transgenic field as expression vectors because BACs fulfil the prerequisites of an ideal expression vector. Indeed, in the mouse transgenic field it has been shown that expression from BACs is not affected by the genomic integration site, BACs confer copy-number dependent expression and expression is stable over time. Therefore, BACs should be useful as expression vectors in the field of recombinant protein production in mammalian cells lines.
As a proof of principle, we have explored the applicability of BACs as expression vectors in HEK293 cells[1] using the constant region of the human IgG1 (Fc) as a protein of interest. We have compared Fc expression levels in HEK293 stable cell lines using a conventional expression vector and a BAC-based expression vector containing the Rosa26 locus (a locus that is considered "open chromatin" and highly transcribed). Stable cell lines generated with the BAC-based expression vector showed a 10 times increased in Fc expression levels compared to cell lines generated with a conventional vector. Expression from the BAC-based vector was copy number dependent and stable for more than 30 cell passages. Thus, BAC-based expression vectors should be an ideal tool for stable recombinant protein production in mammalian cells.

Blaas L, Musteanu M, Eferl R, Bauer A, Casanova E (2009) *Bacterial artificial chromosomes improve recombinant protein production in mammalian cells.* BMC Biotechnol. 2009, **9**:3

P3.18. MINING BIOPROCESS DATA FOR DISCOVERY OF KEY PARAMETERS INFLUENCING HIGH PRODUCTIVITY AND QUALITY

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Majority of biopharmaceuticals and vaccines are currently produced in modern manufacturing facilities equipped with highly automated process monitoring and data archiving systems. Yet fluctuations in process productivity and product quality invariably occur across different runs even in the same facility. It is thus critical to understand the root of such deviations and identify key parameters that can be intervened for enhanced process performance and consistency. On-line, off-line, and raw materials data obtained from 51 runs were investigated for discovery of prominent patterns in the deviation of final product titer and glycosylation patterns. A support vector regression approach was used to construct multivariate models to predict final process performance using process data accumulated at various stages. In addition, the impact of different parameters was determined as a correlation to final product yield, or glycosylation profiles. The analysis reveals that final process performance can be predicted with high confidence in an early stage, and a marked improvement in predictability occurs during a transition period in the mid stage. An interestingly strong correlation between product titer and product quality was also observed. Furthermore, several parameters with significant impact on process performance were identified, including stirrer speed, viable cell density, and lactic acid concentrations. This approach represents an important step towards understanding process characteristics for enhanced process robustness, and thus contributes to the advance of bio-manufacturing.

P3.19. SELECTION OF HIGH PRODUCTIVITY CELL LINES PRODUCING NOVEL HUMANISED AND CHIMERIC ANTIBODIES FOR CANCER THERAPIES

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The Clinical Program of the Ludwig Institute for Cancer Research (LICR) aims to translate basic laboratory discoveries into early phase clinical trials in cancer patients. The LICR Antibody Program has developed six recombinant humanized antibodies into human trials, and has a pipeline of novel antibodies in progress. Through the combined efforts of the LICR laboratories and GMP production facilities, LICR sponsored clinical trials are now currently being conducted in sites in the US, Europe, Australia and Japan.

A key component of the LICR approach to selective tumour targeting with antibodies is the identification of highly specific antigen targets, and generation of humanised antibodies to these targets. The development of high producing cell lines is crucial in the development of each antibody project. We have implemented a strategy to generate high-yield and high-quality antibody producing cells, incorporating a combination of high producing expression systems, and the use of Biosensor technology with conventional methods of cell line development to detect promising transfected cell lines in the early stages of the process. This approach has resulted in higher productivity in shorter time compared to yields obtained with existing strategies.

P3.20. PREDICTING PRODUCTIVITY IN CHINESE HAMSTER OVARY PRODUCTION CELLS

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Improving the rate of recombinant protein production in Chinese hamster ovary (CHO) cells is an important consideration in controlling the cost of biopharmaceuticals. However, the molecular factors and complex pathways underpinning productivity in CHO are poorly understood. Here we present the first predictive model of productivity in CHO bioprocess culture based on gene expression profiles. We utilised a supervised regression algorithm, partial least squares (PLS), to produce a predictive model of cell-specific productivity (Qp) derived from transcriptomic data on stationary phase, temperature-shifted CHO production cell line samples. The final model was capable of accurately predicting Qp to within 4.44 pg/cell/day root mean squared error in cross model validation ($RMSE^{CMV}$) with a $Q^{2,CMV}$ of 0.72. To incorporate additional validation of the model, we utilised 10 unseen samples with a similar range of Qp values as an independent testing set, generating a $RMSE^{prediction}$ of 3.11 pg protein/cell/day. Several of the genes constituting the model are linked with biological processes relevant to protein metabolism and some have been identified to be differentially expressed in previous CHO and NS0 bioprocess profiling studies specifically examining productivity. Additionally, three of the selected genes have been demonstrated to functionally impact productivity in bioprocess culture. Additional preliminary siRNA functional screening results demonstrating that some of the selected genes exhibit a functional impact on productivity in CHO will be presented.

P3.21 ENHANCED PROTEIN SYNTHESIS AND SECRETION USING A RATIONAL SIGNAL-PEPTIDE LIBRARY APPROACH AS A TAILORED TOOL

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There is a steadily increasing demand for producing higher yields of biopharmaceuticals as recombinant proteins in mammalian cells. UniTargetingResearch AS is engaged in the development and commercialisation of tools to optimise protein synthesis and secretion by ensuring that the mRNA encoding the protein of interest is efficiently directed to the endoplasmic reticulum. This is heavily dependent on the presence of specific genetic targeting elements, namely a selected signal sequence (SS) in combination with appropriate 5´ and 3´ untranslated regions. Here our focus is on the SS, which is translated into the signal peptide (SP).
We have shown in earlier studies that SPs vary greatly concerning their ability to promote high-level protein production. Further, we have since tested a large series of SP mutants and again observed unexpectedly great differences in performance (0 to >300%). Based on a comparison of the success levels of individual SPs and their amino acid (AA) composition, we now use bioinformatics to make predictions with respect to which AAs in specific positions have a major impact on protein synthesis/secretion.
We have developed a tool, UTR®Tailortech, that provides us with the opportunity of generating rational libraries of selected SSs randomised at chosen positions and using criteria optimal for CHO cells. This considerably minimises the size of the "haystack" in which the "needles" have to be detected, while simultaneously enriching the library for best performers. The tool also comprises the concept of generating libraries that are re-usable. The pre-made, high-quality libraries can in a seamless manner be linked with any protein-coding region contained in any expression vector. When combined with high-throughput screening technology, a tailored SP for any specific protein can readily be defined.
The results from proof-of-concept studies have demonstrated impressively the tremendous potential of UTR®Tailortech. It is currently incorporated in an extensive trial conducted by Novartis Pharma AG in order to further optimise the company's proprietary mammalian expression system.

P3.22. *ATF4* OVER-EXPRESSION INCREASED IGG$_1$ PRODUCTIVITY IN CHINESE HAMSTER OVARY CELLS

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[2] *Tanabe Mitsubishi Pharma, Osaka, Japan* [3] *Insitute of Technology and Science, University of Tokushima, Japan*
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The endoplasmic reticulum (ER) is the major organelle of synthesis of protein and forms a membranous network throughout the cell. According to Shimizu and Hendershot[1], about one third of the total proteins produced are synthesized in the ER. The ER lumen possesses a unique environment for high quality control for protein folding and assembly. It contains high concentrations of molecular chaperones, folding enzymes, and ATP, which aid in proper maturation of proteins[2]. However, if the amount of proteins to be folded exceeds the capacity of the folding machineries, unfolded proteins will accumulate in the ER and Unfolded protein response (UPR) will start. UPR aims at regaining the homeostasis inside the ER by attenuating the protein translation, activating the folding machineries, degradation of the unfolded proteins, and finally apoptosis. Activating transcription factor 4 (ATF4) is a central UPR pathways which is involved in folding and processing of secretory proteins. Our previous research showed that *ATF4* over-expression is efficient for increasing the productivity of antithrombin-III[3,4]. In this study, we investigated if this approach is product-specific or not.
The gene encoding *ATF4* was cloned from CHO-K1 inserted into the multiple cloning site of pcDNA3.1 vector with hygromycin cassette as a selection marker. The *ATF4* expression vector was then transfected into CHO DP-12-SF cell line[5] producing humanized anti IL-8 Immunoglobulin-1 (IgG$_1$). Twenty six single cell clones were then established using the limiting dilution method. To examine if pcDNA3.1/Hygro(+)-ATF4 was integrated into the CHO chromosome, PCR analysis were performed using the primers designed for non-coding region of the expression vector. Only 5 clones were confirmed with the insert at 1.2 Kb; CHO DP-12-ATF4-3, -9, -10, -12, and-16. RT-PCR revealed that only 3 clones, CHO DP-12-ATF4-10, -12, and -16, showed positive *ATF4* expression. After 144 hours of cultivation, only clones with confirmed *ATF4* expression showed significant increase in specific IgG production rate ranging from 1.8 to 2.5 times the parental CHO DP-12-SF cell. Clone DP-12-ATF4-16 that showed the highest specific productivity with about no change in the specific growth rate was subjected to further analysis for quantification of mRNA of heavy and light chains to determine if over-expression affects the transcription of mRNA or not. The result was found in agreement with our previous research that over-expression of *ATF4* did not significantly change the level of the product mRNA[4]. It suggested that *ATF4* over-expression may improve the translation and the secretion without affecting the transcription.

1) Shimizu Y and Hendershot LM (2007) Adv Exp Med Biol 594:37-46
2) Gomez E *et al.* (2008) Biochem J 410: 485-493
3) Ohya T *et al.* (2008) Biotechnol Bioeng 100:317-324.
4) Omasa T *et al.* (2008) J Biosci Bioeng 106:568-573.
5) Kim W-D *et al.* (2010) Appl Microbiol Biotechnol 85:535-542

P3.23.EXPRESSION SATURATION IN AN INDUSTRIAL CHO SETTING: VECTOR COPY NUMBER IS NO LONGER A BOTTLENECK

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For the last quarter of a century, expression saturation in CHO has been achieved with aid of integrated vector amplification techniques designed to flood the nucleus with transcripts. The presentation will first review these methods from a historical context before outlining how the need for such amplification is significantly reduced with modern industrial methodology.

P3.24.INFLUENCE OF CELL SPECIFIC PRODUCTIVITY ON PRODUCT QUALITY

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The quality profile or micro heterogeneity of a protein has been shown to have a significant impact on its physical, chemical and biological properties both *in vitro* and *in vivo.* For macromolecules such as monoclonal antibodies, this micro-heterogeneity is important as it has an effect on the safety and efficacy of the protein. Micro-heterogeneity is evaluated in terms of post translational modifications such as glycosylation, charge variants, aggregates and fragments profile. The biggest challenge in process development is to find a balance between increasing productivity while maintaining product quality. To control product quality during process development, attempts are being made by altering process parameters, nutrient feeds etc. It is common to correlate lower cell-specific productivity with longer residence time for the protein in the cell organelle like ER and Golgi. This longer residence time is believed to facilitate the enzyme infrastructure available in the cell to act on the protein for example by producing a fully glycosylated structure essentially altering the micro-heterogeneity of the protein.
Results from our Experiments suggest that product quality profile may not always depend on specific productivity as normally believed. It is possible to produce a protein with desired product quality profile with high specific productivity. Our study reveals that different clones with the same productivity can have different product quality profiles alternatively the same clone with different specific productivity can be manipulated to produce the same desired product quality by altering the cell culture parameters or addition of supplements. This observation also influences the acknowledged methodology for selecting clones with higher productivity while still maintaining their product quality profile. The results supporting the points made above will be presented in the poster.

P3.25 STUDY OF A RECOMBINANT CHO CELL LINE PRODUCING A MONOCLONAL ANTIBODY BY ATF OR TFF EXTERNAL FILTER PERFUSION IN A WAVE BIOREACTOR™

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A recent upstream trend in the biopharmaceutical industry is that the perfusion mode of operation tends to gain interest as relative to batch and fed-batch modes. Major advantages of perfusion are high cell numbers and high total productivity in a relatively small size bioreactor. Moreover, perfusion is optimal when the product of interest is unstable or if the cell line product yield is low. On the other hand, disadvantages are for example technical challenges originating from non-robust cell separation devices as well as sterility concerns from the more complex set-up needed.
In the present work, the use of a WAVE Bioreactor system 20/50 in perfusion mode with disposable hollow fiber filters as external cell separating devices was investigated. A comparison between ATF and TFF filtration was performed using a recombinant CHO cell line producing an IgG as a model system. Kinetics (growth, death, consumption of glucose, glutamine and production of lactate, ammonia and monoclonal antibody) were determined during the perfusion cultures and differences between the ATF and TFF filtration modes were studied. Anti-foam addition was performed to counter-act excessive bubble formation resulting from the wave motion. With both filtration devices, a stable system with high CHO cell density was reached and maintained for a prolonged time by performing regularly bleeds, daily if required. In summary, proof-of-concept experiments for perfusion cultivation of CHO cells in a WAVE Bioreactor system with external hollow fiber filters as cell separating devices have been successfully performed. The use of a disposable bioreactor equipped with a disposable separation device offers a solution alleviating technical and sterility challenges occurring in perfusion processes.
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P3.26.EFFECT OF IRON SOURCES ON THE GLYCOSYLATION MACROHETEROGENEITY OF HUMAN RECOMBINANT IFN-γ PRODUCED BY CHO CELLS DURING BATCH PROCESSES

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In the biopharmaceutical industry, the control of glycosylation to satisfy the quality consistency of recombinant proteins produced during a process has become an important issue. The glycosylation pattern of recombinant proteins could be influenced by different factors including the cell line used, environmental factors such as oxygenation, temperature, shear stresses, extracellular pH... and the availability of nutrients.
In this work, the impact of different iron sources on the CHO cell growth, as well as on the production and the glycosylation of a human recombinant IFN-γ were investigated. Iron is an important nutrient and is reported to support essential functions in cells. Thus, cutures were performed in Erlenmeyer flasks and in two different media, namely RPMI and BDM. Whereas RPMI is a classical medium containing serum, BDM is a chemically defined medium without any proteins or serum addition. Iron citrate is present in the BDM medium but completely missing in RPMI serum medium. As previously reported, the BDM medium is able to support a better CHO cell growth and an higher IFN-γ production compared to RPMI medium supplemented with serum. In addition, when BDM medium is used, CHO cells are capable to maintain a high percentage of doubly-glycosylated glycoforms of recombinant IFN-γ produced during the entire process. Conversely, mono-glycosylated and non-glycosylated IFN-γ forms increased during batch cultures performed with RPMI serum medium. Our results showed that addition of iron citrate to RPMI serum medium improved the cell growth, as well as the IFN-γ production. Furthermore, the glycosylation pattern of IFN-γ remained constant when iron citrate was added in the medium. Addition of ammoniacal ferric citrate, iron citrate complexed to selenium or ferric-EDTA to RPMI serum medium also allowed to maintain a constant macroglycosylation pattern of IFN-γ produced during batch cultures. However, using ferric-EDTA, the percentage of doubly-glycosylated IFN-γ glycoforms was lower than the values obtained when other iron sources as named above are used. Thus, the addition of a biavailable iron source to culture media could improve the physiological cell properties as well as the quality of a recombinant protein expressed in CHO cell cultures.

P3.27. SINGLE USE BIOREACTORS FOR THE CLINICAL PRODUCTION OF MONOCLONAL ANTIBODIES – A STUDY TO ANALYZE THE PERFORMANCE OF A CHO CELL LINE AND THE QUALITY OF THE PRODUCED MONOCLONAL ANTIBODY

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In recent years, the use of disposables in the pharmaceutical industry has increased extensively. Disposables can be used in many areas of biopharmaceutical production. They reduce not only the investment costs, but also require less man power to operate, since time consuming change-over procedures are significantly reduced. Single use bioreactors (SUBs) are very different to traditional bioreactors in terms of their design and the resulting physical characteristics (e.g. power input, mixing time and oxygen transfer coefficient). The aim of this study was to compare the performance of one CHO cell line in a SUB with its performance in a traditional stainless steel bioreactor (SSB). The study was performed on a 250 L scale in a GMP environment. Same media lots were used and the corresponding bioreactors were inoculated from the same preculture campaign. Cell culture performance was characterized by measuring viable cell density, viability, lactate dehydrogenase (LDH) activity and the specific productivity. In addition, product quality was assessed by ion exchange chromatography (IEC), size exclusion chromatography (SEC) and glycan analysis using HPAEC-PAD and LC-MS Pepmap for characterization of microheterogeneity. Furthermore, the process related impurities (DNA and Host cell protein (HCP)) were considered. To achieve valid results, three batches produced in each system were compared.
We find that the cell culture performance was only marginally different between SUB and SSB. Most notably, LDH release was higher in SUB. However, the observed differences did not impact product quality. We conclude that both bioreactor systems can be used equivalently for the supply of material for clinical trials.

P3.28. PRODUCTION AND SIALIC ACID CONTENT OF GLYCOPROTEIN IN THE TEMPERATURE-SHIFT FED-BATCH CULTIVATION OF RECOMBINANT CHINESE HAMSTER OVARY CELLS

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The importance of sialic acid content on glycoprotein was widely reported, and the impact of temperature changes exerted the crucial effects on glycoprotein production and sialic acid content. In this paper, we demonstrated that the sub-physiological temperature enhanced the glycoprotein productivity in recombinant Chinese Hamster Ovary (CHO) cells and prolonged the culture duration, resulted in the higher glycoprotein production. However, there was also an increase in less sialylated glycoforms, led to a loss of 10-20 μg/mg sialic acid to glycoprotein. This correlated with the decreased in sialic acid precursor pool and sialyltransferase activity. An optimal fed-batch strategy was developed based on the addition of galactose and the temperature-up-shift, resulted in the similar glycoprotein production and 66.8% improvement in sialic acid content.

P3.29. IMPACT OF CULTURE CONDITIONS ON PRODUCT QUALITY: ASSESSING CONSISTENCY IN A PERFUSION SYSTEM

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Perfusion systems offer advantages when compared to batch culture methods such as higher cell densities and extended culture time. Moreover, the low residence time in the biorreactor minimizes product degradation. However, perfusion systems require mandatory studies in order to demonstrate product's quality attributes consistency and functional equivalence. These characteristics will help to ensure that the culture course have no impact on the safety and efficacy profile of the biological product. This concept applies not only to structural integrity of the protein but also to process and product-related attributes that are relevant to safety.
The purpose of this work was to perform comparability studies regarding quality attributes for two different glycoproteins produced during different culture periods (initial versus extended perfusion culture in agitated biorreactor). Both proteins have multiple N-glycosylation sites, with numerous glycoforms due to inherent variability in the post translational attachment of carbohydrate residues. To assess comparability between samples several tests were performed including charge distribution profile by isoelectrofocusing and capillary electrophoresis, molecular weight heterogeneity and protein subunits dissociation analysis by SDS-PAGE, detection of degradation products by western blot, presence of aggregates by size exclusion chromatography, oxidation status by reverse phase chromatography, sialic acid analysis and in vivo biological activity. Profile of native and neutral oligosaccharides was also performed by fluorescence labelling and detection of N-glycans.
Results obtained for samples from both culture periods demonstrate that products were statistically indistinguishable from each other for the two glycoproteins included in this work. Further, similar levels of residual process-related substances (host cell proteins and DNA) were observed regardless the culture period, indicating that a similar level of purity is achieved in both conditions.
The demonstration of a high degree of similarity in quality attributes helps to ensure that the production process have no impact on the safety and efficacy of both biological products.

P3.30. IDENTIFICATION OF COPPER AS A KEY COMPONENT IN CHEMICALLY DEFINED MEDIA

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In recent years, considerable efforts have been directed towards the development and implementation of chemically-defined media for industrial cell culture processes. Improvements in product titer can significantly reduce the cost of goods and increase plant capacity when producing human protein therapeutics in mammalian cell cultures. To consistently achieve future titer goals in the range of 4-10 g/L and above, the development and optimization of high-performance cell culture media, together with improved cell lines, is required.
During the development of our early-stage CHO cell culture process platform we identified copper as a key component affecting productivity, cell metabolism and product quality.
Using copper at an appropriate concentration resulted in a significant increase (up to 100%) in product titer and also resulted in more favorable metabolic profiles (lactate consumption). However, an increase in copper also led to a change in charge variant profiles for several molecules. The amount of basic variants was increased in the presence of copper. Strategies to optimize culture performance while minimizing changes in product quality and potential mechanisms responsible for the observed product quality differences will be discussed.

P3.31. CELL LYSIS-MEDIATED MONOCLONAL ANTIBODY REDUCTION - A METHOD FOR RISK ASSESSMENT

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The potential for monoclonal antibody (MAb) disulfide reduction and eventual product loss during harvest operations warrants a lab-scale method to assess the risk associated with cell culture production processes. A cell line's susceptibility to lysis has again become an important characteristic as excessive cell lysis is usually correlated with the level of MAb reduction. A working hypothesis is that as cells lyse they release cellular components including macromolecules (e.g. reducing enzymes) and active proton carriers (e.g. NADPH) into the cell culture fluid (CCF). Released cellular components in turn partner to hydrolyze the interchain disulfide bonds of the MAb. Lysis susceptibility can be screened by subjecting cultures to a flow contraction device (FCD) in which variable degrees of lysis between cell lines are achieved at modeled energy dissipation rates (EDR). The EDRs generated by the FCD are ~10e5 - 10e8 W/m^3 across flow rates of 10 - 100mL/min. These EDRs are sufficient to cause lysis in CHO cells. In the overall method shown here, lysis susceptibility screening is integrated with analysis of MAb reduction in resulting lysates. Part of this method includes screening for the effect of cell-size on lysis where cell-size is modulated through the use of a salt-shock technique just prior to the lysis event. The salt-shock technique is a robust strategy to prevent cell lysis-mediated MAb reduction.

P3.32. SELECTIVE PRODUCTION OF HOMOGENEOUS GLYCOFORMS OF ANTIBODY THERAPEUTICS

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Glycosylation, at asparagine 297, within the IgG-Fc, is essential to the generation of interaction sites for ligands that trigger downstream biologic effector activities, mediated through cellular Fc receptors (FcγR) and the C1q component of complement. Effector mechanisms have been shown to be abrogated or severely compromised for aglycosylated or deglycosylated forms of IgG. X-ray crystallographic analysis provides coherent diffraction for the oligosaccharide and show that it is "sequestered" within the internal space of the C_H2 domains. The site of oligosaccharide attachment is proximal to the N-terminal region of the C_H2 domain, from which point it "runs forward" such that terminal sugar residues are exposed at the C_H2/C_H3 domain interface. Multiple non-covalent interactions between the oligosaccharide and the protein result in a reciprocal influence on conformation. The oligosaccharide released from normal polyclonal IgG-Fc is comprised of a core complex diantennary type heptasaccharide and exhibits heterogeneity due to varied addition of galactose, fucose, sialic acid and bisecting N-acetylglucosamine sugar residues; therefore polyclonal human IgG is comprised of multiple IgG-Fc glycoforms. Analysis of recombinant IgG antibody therapeutics produced in CHO, NSO or Sp2/0 cells show that the IgG-Fc is hypogalactosylated, relative to normal polyclonal IgG-Fc, contains variable levels of fucosylation and sialylation and lacks bisecting N-acetylglucosamine. Studies of homogeneous IgG-Fc glycoforms, generated *in vitro,* demonstrate significant differences in effector functions between different IgG-Fc glycoforms. Thus, the presence or absence of α(1-6) fucose profoundly influences the affinity of IgG-Fc binding to FcγRIII and hence the killing of antibody sensitized cancer cells by NK cells.

The IgG-Fc glycoform profile of an antibody is determined during its passage through the Golgi apparatus and studies of human IgG, in health and disease, demonstrate that the glycoform profile of IgG-Fc, secreted by plasma cells, is subject to local environmental conditions. It has been suggested that the IgG-Fc glycoform profile may be determined by levels of expression of glycosyl transferases; however, studies of human antibodies glycosylated within the IgG-Fab as well as the IgG-Fc demonstrates complete processing of the IgG-Fab oligosaccharide, i.e. galactosylation and sialylation.

It suggests that IgG-Fc oligosaccharide processing is dependent on the conformation of the IgG-Fc as it transits the Golgi; presumably determining access to appropriate transferases. This proposition is confirmed by studies in which we replaced wild type amino acid residues shown to form non-covalent interactions with sugar residues with alanine. We demonstrate replacement of single amino acid residues that result in increases in IgG-Fc galactosylation and sialylation, from essentially zero to 70 - 80 %. Since these studies were in antibody expressing CHO cells the sialic acid was added in an α(2-3) linkage; subsequently we transfected the cell line with the α(2-6) sialyl transferase gene, resulting that secretion of quantitatively equal amounts of the α(2-3) and α(2-6) glycoforms. These findings may be exploited for the generation of fully sialylated antibody therapeutics since these glycoforms have been reported to express anti-inflammatory properties.

P3.33. ANTIBODY DISULFIDE BOND REDUCTION IN CHO CELL CULTURE PROCESSES

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In the biopharmaceutical industry, therapeutic monoclonal antibodies are primarily produced in mammalian cell culture systems. After initiating a production bioreactor, various process additions and parameter manipulations are performed to maximize growth and antibody production and yield suitable product quality. At the end of the production culture, the antibody product is typically separated from the cells using centrifugation and/or filtration. During the scale-up of a monoclonal antibody production process, we observed significant reduction of the antibody's disulfide bonds during the harvest operations. This antibody reduction event was catastrophic as the product failed to meet the drug substance specifications and the bulk product was lost. Subsequent laboratory studies demonstrated that cells subjected to mechanical shear during the harvest operations released cellular components that resulted in this antibody reduction phenomenon. Several methods to prevent this antibody reduction event were developed using a small-scale model. Some of these methods were implemented at manufacturing-scale and shown to successfully prevent the reduction of the antibody's disulfide bonds. The results of the small-scale reduction prevention studies and the translation into manufacturing processes will be discussed here as well as some of our understandings into the molecular mechanism of antibody disulfide bond reduction by endogenous intracellular enzymes.

P3.34. QUANTITATIVE AND QUALITATIVE ANALYSIS OF ERYTHROPOIETIN-FC PRODUCTION IN A HIGH DENSITY AND GLUTAMINE-FREE ADAPTED CHO CELL LINE

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An increasing demand necessitates improvements both in yield and product quality of recombinant protein therapeutics. The yield is directly dependent on the integral of viable producer cells (IVCD) and cell specific productivity, whereas quality as the result of post-translational modifications is determined by the state of the cellular machinery and bye-products released into the culture. Thus efforts are underway to establish improved host or production cell lines and optimized processes that achieve higher IVCD, reduced nutrient consumption & waste accumulation and increased specific as well as overall productivity, while maintaining adequate product quality.
In this work, an erythropoietin (EPO-Fc_{parent}) producing CHO cell line was adapted to grow to high density in glutamine-free chemically-defined medium (EPO-Fc_{-Gln}). Glutamine contributes to ammonium accumulation which is known to alter protein glycosylation, thus leading to compromised product quality. The adapted cells were then compared with their parents for growth behavior, nutrient consumption, waste accumulation and recombinant protein production and quality during repeated batch cultures in parallel bioreactors. Although EPO-Fc_{-Gln} cells have an increased lag phase, both cell lines achieved comparable total biomass and IVCD. Despite the fact that EPO-Fc_{-Gln} were deprived of a major energy source, glucose consumption and lactate production were comparable. However, the EPO-Fc_{-Gln} culture produced only half the amount of ammonium. Specific productivity was decreased 18 percent in the EPO-Fc_{-Gln} culture.
In addition to these basic data, the concentration of intracellular nucleotides and nucleotide sugars, the status of oxidative stress of cells, and the glycosylation quality of the product were analysed. Significant differences were observed in oxidative stress: the EPO-Fc_{-Gln} cells had a higher intracellular concentration of both reactive oxygen species (ROS) and superoxide (SO), but also of glutathione, which helps cells to neutralize oxidative stress. At the same time these cells also had a higher concentration of GSSG, which is responsible for disulfide bond formation and has been reported to help achieve high productivities. Several intracellular nucleotide sugar concentrations were also increased in the EPO-Fc_{-Gln} cells, notably that of CMP-NANA, the precursor for terminal sialic acid, an important marker for protein quality. Analysis of protein quality revealed something that we will put in next week, when we have the data.
Overall, the methods used for this study reveal interesting correlations between cell behavior and the analysed metabolites which can be used both for cell line and process optimization.

P3.35. HOST CELL LIMITATION OF POST-TRANSLATIONAL MODIFICATIONS: BALANCING CELLULAR PRODUCTIVITY AND PRODUCT QUALITY

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This presentation will report on the challenge of host cell limitation for a post-translational modification (PTM) encountered while expressing two recombinant therapeutic proteins. The two proteins are blood clotting factors, referred to here as Factor □ and Factor □; these are complex proteins (vitamin K dependent serine proteases) requiring several PTMs. Product quality, as measured by bioactivity (*in vitro* clotting activity), is paramount for these proteins to be effective therapeutics. Two sets of cell lines, one expressing Factor □ and the other Factor □, were generated and subject to extensive screening in order to identify production cell lines. A proteolytic cleavage enzyme was co-expressed with each factor as it enhances cleavage of the propeptide, which converts the proteins into active forms. For expression of Factor □, transfections with multiple vectors resulted in cell lines of a wide range of specific cellular productivity, Qp. A general inverse relationship between the expression levels of Factor □ and that of the proteolytic enzyme was apparent; it suggested the existence of a cellular energetics limitation for the combined levels of the two recombinant proteins. Productivity and product quality analyses showed that the specific activity of the expressed Factor □ clearly decreased with increasing Qp of Factor □. The decline in the specific activity with increasing Qp was attributable to the limitation of at least one PTM, □ carboxylation, which is crucial for bioactivity. Western blots using anti-Factor □ identified un-□ carboxylated Factor □ species by i) a shift in gel mobility and ii) absence of binding by an anti-Gla antibody. While cell lines of low Qp produced no detectable un-□ carboxylated Factor □ (an undesirable species) those of high Qp produced un-□ carboxylated species in a Qp dependent manner. For expression of the Factor □, three independent rounds of cell line developments were undertaken. The lead cell lines from each round differed significantly in their Qp levels. The Qp-to-specific activity inverse relationship was also evident among the three Factor □□cell lines; the declining specific activity in this case was also attributable to un-□ carboxylated product species. Thus limitation of the cellular machinery for □ carboxylation poses a major challenge to recombinant expression of vitamin K dependent clotting factors in active form. Striking the appropriate balance between cellular productivity and product quality was the key to cell line selection for these proteins. Various approaches pursued to address this limitation will be discussed.

P3.36. CONTROLLING SIALYLATION OF RECOMBINANT GLYCOPROTEIN IN CELL CULTURE PROCESS

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Biological functions of many recombinant glycoproteins are known to be affected by its sialic acid levels. Therefore, it is not only desirable to maximize the level of sialic acid, but also important to control its level within a consistent range to minimize the lot-to-lot variability of the final drug product.
Herein, we report the effects of various media additives and the culture temperature, on the sialic acid level of a recombinant glycoprotein. Cell culture was treated with various individual precursors and co-factors involved in sialylation pathway in a screening study. Selected components from this screening study were used in a dosing study to determine the dose-response relationship between these components and the average level of sialic acid. Bolus addition of galactose, N-acetyl mannosamine (ManNAc), manganese and dexamethasone were seen to increase sialic acid levels significantly. Temperature of cell culture is also known to affect glycosylation in mammalian cells. In the case of our glycoprotein, lower temperatures during the production resulted in higher level of sialic acid.
Hence, we have demonstrated that sialic acid level in our glycoprotein can be altered through addition of certain media additives and process parameter optimization. The information obtained from these studies may be useful for implementing an effective sialylation control strategy for manufacturing of recombinant glycoprotein

P3.37. INCREASING ANTIBODY YIELD AND MODULATING FINAL PRODUCT QUALITY USING FREEDOM™ CHO PRODUCTION PLATFORMS

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Over the last decade, antibody titers in mammalian cell culture systems in excess of 3 g/L have been achieved through the use of novel medium and nutrient feed designs. However, despite these advances, it is still a challenge to consistently and rapidly create a stable mammalian cell line and develop a cell culture process capable of supporting both high antibody yield and acceptable antibody glycosylation within limited efforts. We used CHO DG44 and CHO-S™ cells stably expressing a variety of IgGs to investigate media effects on pool selection, single cell cloning, antibody productivity and glycan profile of antibody produced in the derived clones. We compared several selection, screening and cloning media and processes (batch vs. fed batch) to determine the most efficient and effective strategy for identifying high producing clones up to 2-3 g/L while keeping the workload manageable to 1-2 operators. Our results show that i) cell growth, protein titer and glycosylation are all affected by both the basal medium and nutrient feed strategy and ii) a single culture medium (CD FortiCHO™ Medium) can support all phases of the cell line development workflow; therefore, avoiding adaptation (and potential genetic selection) of the cells to different media.

P3.38. IMPACT ON PRODUCT QUALITY OF HIGH PRODUCTIVE GS-CHO CELL LINES

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The Clinical Program of the Ludwig Institute for Cancer Research (LICR) aims to translate basic laboratory discoveries into early phase clinical trials in cancer patients. A key component of the LICR approach has been to focus on the identification of antibodies selectively targeting antigens preferentially expressed in tumour tissue, and the molecular engineering of chimeric or humanised antibodies to these targets. The development of robust and high producing cell lines is crucial in the development of each antibody construct.
The Cell Biology group is located within the Biological Development Facility of LICR Melbourne-Austin Branch and is responsible for the production of monoclonal antibodies from hybridomas or from industrially relevant mammalian cell lines (CHO, NS0, etc). The Cell Line Development activities are driven by the powerful combination of industrial relevant cell lines transfected with cancer-relevant DNA targets using the Glutamine-Synthetase system (GS) from Lonza. During the course of the year 2010 the group generated 20 new GS-CHO cell lines. Here we propose to analyse two case-studies of high-productive cell lines in terms of product quality and biological activity of the protein produced with these cell lines.

P3.39. DISULPHIDE BOND REDUCTION OF A THERAPEUTIC MONOCLONAL ANTIBODY DURING CELL CULTURE MANUFACTURING OPERATIONS

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Disulphide bonding is critical to maintaining IgG tertiary and quaternary structure for therapeutic monoclonal antibodies (MAb). Both inter- and intra-chain disulphide bonds are formed intracellularly in the expression host prior to secretion and purification during MAb production processes. We describe identification during cell culture manufacturing operations, and remediation through process optimisation, of disulphide bond reduction of a therapeutic IgG1 monoclonal antibody (MAb) during late stage commercial development. The MAb was co-developed with MacroGenics, Rockville, MD.
The original Phase III MAb production process incorporated cell settling following harvest of the 16 day, fed batch, production bioreactor. Following settling, the bioreactor material was clarified through a filtration sequence (depth filter + membrane filter) and then purified through downstream processing. LC-MS and CE-SDS analyses detected significant reduction of IgG1 inter-chain disulphide bonds in the eluted mainstream from the Affinity Capture chromatography unit operation (which immediately followed harvest and clarification), whereas bonding was intact prior to harvesting the production bioreactor. LC-MS peptide map analysis of the reduced IgG1 identified disulphide scrambling of the IgG1 molecule which may have occurred during subsequent re-oxidation of the disulphide bonds.
Investigations into the production bioreactor and harvest and clarification unit operations identified low dissolved oxygen and high free thiol levels correlated with antibody reduction. Lab scale and full scale (pilot plant) investigations delineated critical process controls required to maintain disulphide bonds in the oxidised state. These primarily involved timing of harvest and clarification, and additional controls to maintain minimum levels of dissolved oxygen during the latter stages of the production bioreactor and during primary recovery. Over 15 batches have since been produced at manufacturing scale with no observed impact to disulphide bonding (as assessed by CE-SDS and LC-MS peptide mapping). The relationship between disulphide reduction and dissolved oxygen and free thiol levels is not first order, and additional lab scale investigations have indicated the involvement of a catalytic component.
In summary, gross disulphide bond reduction was identified during late stage development of an IgG1 monoclonal antibody being commercialised for a therapeutic indication. Disulphide bond reduction had a second, or higher, order link to low dissolved oxygen levels in process intermediates, and the involvement of a catalytic factor is also indicated. Implementation of an appropriate control strategy (and associated process analytics) informed by process development has ensured no recurrence of this issue.

P3.40. MANIPULATION OF ANTIBODY GLYCOFORMS IN A HIGH-YIELD GS-CHO PROCESS TO MEET COMPARABILITY REQUIREMENTS

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For clinical re-supply, an antibody production process was moved in house from a CMO. Although the master cell bank was transferred, the remainder of the cell culture process had to be redeveloped. Application and optimization of our platform process for titer led to a product with a similar set of Fc oligosaccharide structures, although there was a reduction in overall galactosylation from 18% to 3%. In this poster, we demonstrate that the combined addition of uridine, manganese, and galactose (UMG) can be used for fine-tuning antibody galactosylation with minimal impact on other glycoforms, other product quality attributes, or cell culture performance. The impact was reproducible from 2L to 1000L scale and appears to be a generally useful approach since similar results were observed for a second GS-CHO cell line expressing a different antibody. The level of galactosylation could be controlled in the first cell line from 3% to 23% by varying the concentration of these additives and in the second cell line from 5% to 29%. This approach enabled design of a process that started with 3% galactosylation to meet the comparability target of 18% galactosylation by adding the appropriate amount of these components to the culture medium.

P3.41. SWITCH BETWEEN CHO CELL TYPES TO IMPROVE EXPRESSION YIELDS FOR A HUMAN RECOMBINANT ANTIBODY

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Production yields of recombinant proteins as human biotherapeutics are determined by the productivity of the cell line to be used for successful clinical and commercial supply. Various expression systems have to be screened to ensure high productivity at high quality for a given protein.
A human recombinant IgG antibody was produced in the Chinese hamster ovary-derived (CHO) K1 and dihydrofolate reductase (DHFR)-deficient DG44 cell lines. Stable clones were generated using a fluorescence halo picking technique integrated into various subcloning rounds. In the case of CHO K1, a strategy of sequential transfections was used to improve expression levels. The traditional gene co-amplification approach using the DHFR-inhibitor methotrexate (MTX) was followed for the DHFR-deficient DG44 cell lineage.
Performances of clones derived from both cell types were compared in lab-scale fermentation and micro-scale bioreactor runs, and showed a four-fold higher yield for the DG44-derived clones. This was a sum of slower growth rates enabling longer production phases in batch/fed-batch cultures, and higher cell-specific productivities. A rapid growth phase followed by an immediate loss of productivity and viability was observed in K1-derived clones. Gene co-amplification of DG44 leading to a more than five-fold productivity increase of selected clones did not impact the clone development timelines negatively in comparison to K1, with less cloning rounds required. Long-term stability also in the absence of MTX was confirmed for producer clones, and product features of purified materials from both cell types were compared. Quantitative PCR was used to study parameters influencing antibody gene expression at genome and mRNA levels.
In conclusion, the DG44 cell line was superior to K1 in terms of yields. The timelines to generate clones suitable for production were similar for both cell types.

P3.42. EXPRESSION OF RECOMBINANT HUMAN COAGULATION FACTORS VII (RFVII) AND IX (RFIX) IN VARIOUS CELL TYPES, GLYCOSYLATION ANALYSIS, AND PHARMACOKINETIC COMPARISON

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Clearance mechanisms for rFVII (or the active enzyme rFVIIa) and rFIX are influenced by post-translational modifications, especially N-glycosylation. This should be considered when choosing a recombinant expression system in view of the varying ability of frequently used cell lines to perform modifications similar to human proteins. Differences in the pharmacokinetic properties of recombinant FVIIa versus plasma-derived (pd)FVII or desialylated rFVIIa are known for human FVII(a). Asialo rFVIIa clears quickest, whereas pd FVII, having a higher degree of sialylation, is cleared to a lesser extent[1]. In the case of FIX, the degrees of serine phosphorylation and tyrosine sulfation in the activation peptide have been postulated to influence pharmacokinetic behavior, especially *in vivo* recovery[2].
We chose CHO, BHK and HEK293 cells for expression to compare post-translational protein modifications of rFVII. Protein activities of rFVII determined by antigen and activity assays were similar for each cell type. Glycosylation analysis revealed differences between N-glycans made by HEK293 cells to those in rFVII from CHO and BHK cells and pdFVII. A lower degree of sialylation, a high content for N-acetylhexosamines, and absence of tri- or tetra-antennary structures were found for HEK293-rFVII compared with the other materials. We used HEK293 cell lines to generate highly phosphorylated and sulfated rFIX for *in vivo* studies. rFIX from the same clone and production run was purified using two different down-stream processes: the first to enrich high phosphorylated and sulfated protein, the second to purify total rFIX at high yield. These HEK293-derived rFIX isoforms were compared with CHO-rFIX and pdFIX in a pharmacokinetic study in FIX knock-out mice. *In vivo* recovery and the area under the curve were statistically significantly higher for high-phosphorylated and sulfated rFIX than for total rFIX derived from HEK293 cells. However, both parameters were lower for both HEK293-rFIX preparations than for CHO-rFIX, and, in agreement with literature, lower for CHO-rFIX than for pdFIX. Mean residence times and terminal half lives were similar for all.
In conclusion, these data showed that HEK293 cells were not adequate for rFIX or rFVII production due to improper N-glycosylation compared with the material from other cell lines, or from human plasma. Consequently, an advantage of this human cell line for the production of "more-human-like" biotherapeutics could not be observed.

[1] Appa, R. S. et al., 2010. Investigating clearance mechanisms for recombinant activated factor VII in a perfused liver model. *Thromb Haemost* 104(2), 243.
[2] Ewenstein, B. M. et al., 2002. Pharmacokinetic analysis of plasma-derived and recombinant F IX concentrates in previously treated patients with moderate or severe hemophilia B. *Transfusion* 42, 190-7.

P3.43. CELL CULTURE TEMPERATURE AFFECTS ANTIBODY AGGREGATE FORMATION IN CHO CELLS

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For manufacturing of therapeutic antibodies (Ab), low levels of Ab aggregates are desirable to minimize the potential risk of immunogenicity to patients. During production of an Ab with a stably transfected CHO cell line, we observed atypical variability from 1-20% aggregates formed during cell culture that negatively impacted Ab purification.
Analytical characterization using size exclusion chromatography and capillary electrophoresis revealed aggregates were mediated by hydrophobic interactions. Analysis of 30 cell cultures at different scales, temperatures and cell ages showed a significant inverse correlation of aggregate levels with culture temperature. In particular, Ab aggregates increased 5-15 fold when production cultures were carried out at 33°C as compared to 37°C. We also examined heavy chain (HC) and light chain (LC) transcripts and found mRNA levels increased 2-4 fold at 33°C as compared to 37°C. ER chaperone expression and ER size also increased at 33 versus 37°C (25-75%) but to a lesser extent than LC and HC mRNA. Finally, we observed a 2-5 fold increase in Ab aggregate formation at 33°C versus 37°C in three CHO cell lines expressing different Abs.
Overall, our studies showed that cell culture temperature could be used to modulate aggregate level during production of monoclonal Abs and that enhanced HC and LC transcript levels combined with limited ER processing capacity may increase Ab aggregation.

P3.44. IDENTIFICATION AND VERIFICATION OF SCALE RELATED FACTORS THAT INFLUENCE GLYCOSYLATION OF A COMPLEX CHO-DERIVED PROTEIN THROUGH MULTIVARIATE DATA ANALYSIS AND MANIPULATION OF ENVIRONMENTAL CONDITIONS WITHIN THE BIOREACTOR

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Protein glycosylation is a complex process, which is finely orchestrated in mammalian cells, requiring the interaction of a large number of intra- and extracellular factors. A complete understanding of the factors influencing glycosylation is crucial in order to predict, and consequently manage protein glycosylation during commercial scale manufacturing.
In this study we describe methods to identify and experimentally verify some of the cell culture and environmental factors that influence the glycosylation profile of a complex CHO-derived glycoprotein, and how these factors can be influenced by scale.
To identify potential root cause(s) for the subtle variations in sialylated N-linked glycan profiles occasionally noted between small and large scale bioreactors a two step approach was applied: (i) data driven analysis using Multivariate Data Analysis (MVDA) and (ii) the generation of experimental data to verify the MVDA models. MVDA also served as a means to improve both process knowledge and the ability to predict the effects of scale on the final product. Using this approach, it was found that out of all the variables analysed, ammonium ion levels were the primary influencing factor for the subtle variation in glycosylation profiles noted across the scales. This finding is consistent with the observation that the ammonium ion profile for this process is different across the scales which further demonstrates that while qualified small-scale systems mimic large scale conditions, minor differences are usually acknowledged due to the complexity of the scale-down process.
Bench scale experiments were executed to replicate those large scale environmental conditions that were predicted by MVDA models to influence the glycosylation profiles across scales, with a particular emphasis on the ammonium ion levels. Through the artificial manipulation of the cell culture environment at bench scale, it was possible to eliminate the subtle variations in sialylated N-linked glycans and therefore ensure consistent glycosylation profiles across all scales.
This study confirms the utility of MVDA to identify factors influencing the glycoform profiles of complex CHO-derived recombinant glycoproteins and importantly also provides experimental data to verify the output from MVDA.

P3.45. CONTROLLING THE ANTIBODY QUALITY OF ANTIBODY MIXTURES

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The synergistic effects of combinations of antibodies reported for both cancer treatment and versus complex antigens in infectious diseases demonstrate that antibody mixtures represent a promising new class of therapeutics.
Symphogen has developed an expression platform, Sympress™, for consistent production of mixtures of antibodies. With Sympress, it is possible, with a given number of antibodies in the mixture, to generate an optimal Ab composition and a robust production process showing high batch-to-batch consistency, gram-scale titers and low production costs.
Simultaneously with the development of Sympress, a characterization strategy for release of these antibody products has been designed and developed. The antibody composition is evaluated by orthogonal methods, such as ion exchange, size exclusion, mass spectroscopy, by evaluation of antibody mRNA levels by T-RFLP and also by potency assays. This has gained regulatory acceptance of this new drug class.
Optimization of the manufacturing process is important in any biologics CMC development program, and key parameters such as titer are typically improved during clinical development of a given product. For antibody mixtures this represented a dual challenge in that not only the product quality but also the relative antibody composition has to be maintained, We here show several examples of how to control the antibody quality from the stage of clone selection to the stage of bioprocess screening in scale-down models and bioreactors. In one example, bioprocess optimization increased antibody titers but also increased the heterogeneity of each individual antibody in the mixture. However, by adjusting the pH settings and by regulating key nutrients in the process it was possible to maintain product quality simultaneous with a doubling of the antibody titer.
The combination of high-throughput analytical tools and process development with several antibodies has allowed us to systematically develop and refine the Sympress platform technology so it delivers robust high-quality antibody mixtures.

P3.46. EVALUATION OF IMPACT OF CELL CULTURE PROCESS CONDITIONS ON CONSISTENCY OF TRISULFIDE VS NATIVE DISULFIDES LINKAGES IN AN IGG1 MOLECULE

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Molecular heterogeneity was detected in a recombinant IgG1 mAb and attributed to the presence of a protein trisulfide moiety. The predominant site of trisulfide modification was the bond between the heavy and light chains. Trisulfide bond formation has been recently observed in immunoglobulins (IgGs) produced in culture by mammalian cells. This molecular heterogeneity has no observable effect on antibody function based on in-house studies at Biogen Idec. Nevertheless, to minimize the heterogeneity of therapeutic monoclonal antibody (mAb) preparations, an understanding of impact of cell culture process conditions on trisulfide vs disulfide linkage formation is desirable.
Trisulfide levels in samples from 2000-L bioreactors using conditions developed at the 200 L scale showed consistency. To investigate variables that might impact trisulfide formation, a number of cell culture parameters such as cell density and feed strategies were varied in bench bioreactor studies. Trisulfide analysis of the samples from these runs revealed that the trisulfide content in the LC5-HC5 linkage varied considerably from <1% to 39%. Changes in culture conditions that seemed relatively minor and did not significantly affect growth and culture productivity resulted in large differences in trisulfide levels. In particular, culture duration and feeding strategy were important variables and product with reproducible trisulfide levels can be obtained by tightly controlling cell culture conditions. Therefore, trisulfide/disulfide heterogeneity may be eliminated from IgG1 molecules via control of cell culture process conditions.

P4.01. SCREENING OF ANTIBODY EXPRESSING CLONES WITH AN AUTOMATED SHAKEN DEEP-WELL SYSTEM

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In cell line development, one of the main goals is to identify cell lines with the highest antibody production levels, and this process typically requires large numbers of clones to be screened. One strategy for screening a large number of clones in a small footprint is to use miniaturized systems such as shaken deep-well plates. To integrate such systems into cell line development efficiently, automation of plate and liquid handling must be developed and incorporated. In this talk, we will present a fed-batch shaken deep-well plate culturing platform with an automated liquid handling system where cell counting and protein titer measurement tools have been integrated. We will also demonstrate that our system can screen up to 5 times more clones than can be done with standard manual methods and is capable of identifying cell lines with fed batch antibody production levels greater than 6g/L.

P4.02. USE OF MICRO BIOREACTOR SYSTEMS TO STREAMLINE CELL LINE EVALUATION AND UPSTREAM PROCESS DEVELOPMENT FOR MONOCLONAL ANTIBODY PRODUCTION

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Early process development for monoclonal antibody production has traditionally been undertaken in shake flasks and stirred tank bioreactors. More recently a number of scaled down systems have become available which allow increased experimental throughput and can facilitate shortened timelines. These range in complexity from simple microtitre plates through mid range systems such as the TAP AMBR system or the Pall Micro24 bioreactor to fully automated systems such as the SimCell.
This work describes the potential integration of the Duetz Microflask system (Applikon) and the Micro-24 bioreactor system (Pall) into cell line evaluation and early process development programmes. Preliminary cell line selection using the Duetz Microflask system is discussed elsewhere and this work describes the further selection of cell lines in fed batch cultures using both systems. Fermentation parameters including titre and response to feeding during the fermentation were used to assess cell line performance and the data is compared to that obtained in conventional systems.
This work demonstrates the potential to use these systems for combined cell line selection and early process development at a small scale which can result in reduced timelines and increased throughput. The relative advantages and limitations compared to conventional systems are also discussed.

P4.03. CELL LINE SELECTION USING THE DUETZ MICROFLASK SYSTEM

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The identification of a small number of monoclonal antibody (mAb) producing candidate cell lines from large numbers of clones generated post transfection is one of the potential bottlenecks of cell line development. Clone numbers are reduced significantly during initial medium exchange and static scale up stages but significant numbers can still progress to evaluation in shaking cultures. This is often carried out in shake flasks where the number of clones that can be evaluated may be restricted due to resource limitations.
This work describes the use of the Duetz Microflask system to evaluate cell lines in shaking culture prior to scale up to production shake flasks. The reduced scale and potential for automation of this microtitre plate based system can lead to increased throughput and reduced timelines to the selection of clones for final evaluation in 'production equivalent' conditions.
The performance of multiple clones producing the same monoclonal antibody molecule and of sets of clones producing different mAb molecules was assessed in 24 well microtitre plates sealed with Duetz Sandwich Cover Lids and compared to that achieved in shake flasks.
The data presented will demonstrate that 24 well plates fitted with Duetz Sandwich Cover Lids can be used as an intermediate cell line screening stage to significantly reduce the numbers of clones required for final cell line evaluation in production conditions and show the potential for this system to become an integral part of a streamlined cell line development process.

P4.04. CULTURE, BACULOVIRUS INFECTION AND TRANSIENT TRANSFECTION OF INSECT SF-9 CELLS IN TUBESPIN® BIOREACTOR 50 TUBES

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Spodoptera frugiperda Sf-9 insect cells are widely used for recombinant protein production. Generally, the cells are grown in suspension in either spinner flasks or Erlenmeyer flasks. For gene delivery, infection with a recombinant baculovirus vector or transfection with high-cost liposome-based reagents are commonly used. Here we present TubeSpin® bioreactor 50 tubes (TubeSpins) as a simple, low cost, and highly efficient culture system for the growth and transfection of Sf-9 cells. Sf-9 cells had a considerably better growth behavior in TubeSpins than in spinner flasks. Cells were inoculated at 1 x 10^6 cells/mL and reached maximal cell densities of 16 x 10^6 cells/mL in TubeSpins and 6 x 10^6 cells/mL in spinner flasks. The cell viability in these batch cultures remained higher than 90 % for 10 days in TubeSpins but only for 4 days in spinner flasks. Infection with a recombinant baculovirus coding for green fluorescent protein (GFP) was performed with Sf-9 cells grown in TubeSpins, spinner flasks or Erlenmeyer flasks. All cultures resulted in similar GFP-specific fluorescence levels. Sf-9 cells in TubeSpins were transfected with a plasmid encoding the GFP gene under the control of the baculovirus IE-1 promoter using polyethylenimine (PEI) as the DNA delivery vector. Transfection efficiencies of up to 58 % were observed. In separate transfections with a plasmid encoding the tumor necrosis factor receptor-Fc fusion protein (TNFR:Fc), volumetric productivity of 28 mg/L was achieved in 3-day cultures. This study highlights the use of TubeSpins for the cultivation and PEI-mediated transfection of Sf-9 cells in suspension. Efforts for further scale-up in orbitally shaken bioreactors are underway.

P4.05. RESPIRATORY QUOTIENT (RQ) ESTIMATION FOR MAMMALIAN CELLS IN HIGH-DENSITY PERFUSION CULTURE

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We present robust methods for online estimation of oxygen uptake and carbon dioxide production rates (OUR and CPR, respectively) during perfusion cultivation of mammalian cells. Perfusion system gas and liquid phase mass balance expressions for O_2 and CO_2 were used to estimate OUR, CPR and thus RQ for CHO cells in perfusion culture over 12 steady states with varying dissolved oxygen (DO), pH, and temperature set points. Under standard conditions (DO = 50%, pH = 6.8, T = 37.5°C), OUR and CPR ranges were 5.14 – 5.77 and 5.31 – 6.36, respectively, resulting in RQ values of 0.98 – 1.14. Changes to DO had a slight reducing effect on respiration rates with OUR and CPR values of 4.64 and 5.47 pmol/cell-d, respectively, at DO = 20% and 4.57 and 5.12 pmol/cell-d at DO = 100%. Respiration rates were lower at low pH with OUR and CPR values of 4.07 and 4.15 pmol/cell-d at pH = 6.6 and 4.98 and 5.35 pmol/cell-d at pH = 7. Temperature also impacted respiration rates with respective OUR and CPR values of 3.97 and 4.02 pmol/cell-d at 30.5°C and 5.53 and 6.24 pmol/cell-d at 37.5°C. Despite these changes in OUR and CPR values, the RQ values in this study ranged from 0.98 – 1.23 suggesting that RQ was close to unity. The mass balance-based OUR and CPR expressions developed in this study allow robust real-time estimation of respiration rates which, both independently, and in conjunction with metabolic flux analysis, provide additional quantitative information on cell physiology, both during bioprocess development and commercial biotherapeutic manufacturing.

P4.06. APPLICATIONS OF THE SIMCELL ROBOT TECHNOLOGY FOR PROCESS DEVELOPMENT

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The 'SimCell' robot has been used to screen cell lines and fermenter parameters at the microlitre scale. The robot allows us to cultivate cell lines at the 650ul scale and to control parameters such as pH, cell density and DOT at this scale of operation. Data will be presented where clones have been evaluated in a fed-batch operation (two feeds) and where parameters such as pH setpoint and medium composition have been optimised prior to lab scale fermentation. Data will also be presented to compare the SimCell scale data to lab scale data.

P4.07. EVALUATION OF MAMMALIAN TRANSGENIC CLONES THROUGH THE USE OF A SMALL-SCALE SYSTEM THAT PREDICTS BIOREACTOR PERFORMANCE

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The ability to predict the behavior of transgenic clones in a bioreactor by using a small-scale model allows for an efficient means of evaluating a large number of cell lines. We have developed a model system using orbiting spin tubes that predicts bioreactor performance with high accuracy. The model system allows for assays to run for as long as two weeks with intermittent feeding and intermittent sampling for phenotypic measurements. One person can manage the evaluation of a large number of candidate cell lines simultaneously, and the results of the evaluation translate well to cell line performance at larger scale. In this poster we present a correlation between assay results from the small-scale spin tube model and performance of those same cell lines in bioreactors. The data represent both antibody and non-antibody projects.

P4.08. DEVELOPMENT OF AN AUTOMATED, MULTIWELL PLATE BASED SCREENING SYSTEM FOR SUSPENSION CELL CULTURE

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Rationale:
The drive to develop new processes faster and more efficient requires a streamlined workflow.
Resource intensive approaches like the use of shake flasks limit the accessible design space for the development of highly productive processes or the characterization of established processes. Process automation provides the appropriate tools to address the following key points:

Increasing experimental throughput	⇨ enable full factorial design of experiments
Increasing process information	⇨ improve process understanding
Automate repetitive manual work	⇨ gain efficiency, focus on high value tasks

Technology:
We development an automated, multiwell plate based screening system for cell culture processes.
The system is setup to be generic and can utilize multiwell plates of different configurations as bioreactors (6 to 48 wells per plate). The currently most frequently used configuration is the 6-well plate.
The screening system is based on off-the-shelf commercial laboratory automation equipment. It is fully automated and handles plate transport, feeding and seeding of cells, daily sampling and preparation of metabolite assays.
The integration of all required analytical instrumentation to perform these metabolic assays into the system enables a hands-off operation and renders the system independent from the analytical capabilities available in development thus preventing a potential bottleneck in sample processing.

Application:
The system enabled an increase in throughput of approximately 10 -fold compared to classical shake flask-based approaches. The comparability of the overall process performance as well as product quality obtained in the screening system to shake flask as well as bioreactors up to 1000L scale was successfully shown.

P4.09. HYDRODYNAMIC STRESS IN ORBITALLY SHAKEN BIOREACTORS

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Orbitally shaken bioreactors of scales from 50 mL to 2'000 L have been developed for the cultivation of suspension-adapted mammalian cells, but the hydrodynamics of these bioreactors is not well understood. Here we study the hydrodynamics of orbitally shaken bioreactors to allow predictions of suitable conditions (e.g. shear stress level, oxygen transfer efficiency) for mammalian cell cultivation. First, the hydrodynamics were studied experimentally in a 30-L cylindrical bioreactor. The velocity fields were measured with Laser Doppler Velocimetry (LDV) and used to estimate the local shear stress within the vessel at various operating conditions. The free surface was captured with a high-speed camera to establish similarity laws to allow comparisons of the flow at different scales. The fluid motion and the free surface were simulated with Computational Fluid Dynamics (CFD). These results were compared to the experimental data and were also used to model the effects of several parameters (vessel diameter, working volume, shaking diameter and agitation rate) on the hydrodynamics of orbitally shaken bioreactors, allowing the computation of both the mass transfer coefficient (k_La) (oxygen transfer efficiency) and the shear stress level for a given shaking regime. To define thresholds of shear stress for mammalian cells experimentally, CHO cells were cultivated in cylindrical vessels of various scales at different agitation rates, shaking diameters, and working volumes. Cell damage was evaluated by measuring cell debris accumulation with flow cytometry and by quantifying lactate dehydrogenase levels in the medium. These results will help determine key parameters for establishing mammalian cell cultivation conditions with CHO or other cell lines and will facilitate the scale-up of orbitally shaken bioreactors.

P4.10. ORBSHAKE BIOREACTORS FOR SUSPENSION CULTURES OF ANIMAL CELLS – AN ENGINEERING PERSPECTIVE

Kühner, M. [1], Tissot, S. [2], Cesana C. [1], Anderlei T. [1], Zhang X. [2], Burki C. [3], de Jesus M. [3], Stettler M. [2], Muller N. [2], Bertini O. [3], Broccard G.[2], Monteil, D.T. [2], Baldi, L. [2], Hacker, D.L. [2], and Wurm, F.M. [2, 3]

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Orbital shaking for the cultivation of animal cells has been developed from similar systems previously used for microbial cultures. The mechanical and electrical engineering of incubator shakers was modified to allow humidification in combination with a controlled CO_2 supply, and a robust electrical drive and mechanical platform for the containers of different volumes and weights was implemented. Based on this experience, cylindrical orbitally shaken (OrbShake) bioreactors (OSRs) with nominal volumes of 250 L (now available on the market), 2000 L, and 3500 L (both as prototypes) have been developed, using disposable bags for containing the cell culture. At these scales, mammalian cell culture is usually performed in stirred-tank bioreactors (STRs). We are investigating the suitability of large-scale OSRs as an alternative for mammalian cell culture. Cells were cultivated in OSRs with nominal volumes from 30 to 3500 L (diameter to height ratio of 1:1.2 – 1:1.5). Cell cultures in OSRs at working volumes up to 1000 L were comparable in cell density and recombinant protein production to those in 3-L STRs. The cultures at working volumes up to 100 L were performed either with or without probes for pH and dissolved oxygen (DO). To better understand large-scale OSR performance, a number of key engineering parameters, such as the mass transfer coefficient of oxygen (k_La), the volumetric power input (P_V), and the mixing time were evaluated under conditions suitable for mammalian cell culture. In STRs, the k_La typically decreases as the scale increases. For OSRs, in contrast, we observed k_La values around 10 h^{-1} at all scales of operation. In large-scale OSRs, the P_V was 0.2 kW/m^3, as determined by a thermodynamic method, and the mixing times were 30 s or less. Our results show that similar values of k_La, P_V and mixing time are achievable at both small- and large-scales in OSRs. These results suggest that OSRs are a viable alternative to STRs for animal cell culture, even at large volumetric scales. A concise overview of the various steps of development of orbital shaking and OrbShake bioreactors, from an engineering perspective, will be presented.

P4.11. DEVELOPMENT & OPTIMIZATION OF PRODUCTION PROCESSES SUPPORTING MANUFACTURING OF BIOLOGICAL PRODUCTS ON THE DUCK EB66® CELL LINE

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Over the last ten years, Vivalis established, developed and optimized the proprietary cell line EB66®. This cell line was initially derived from duck embryonic stem cells as an alternative to chicken embryonic fibroblasts and eggs used for the manufacturing of human and veterinary vaccines. EB66® cells display attractive characteristics such as an efficient upscaling to 250L, a very short population doubling time of ~ 15 hours, very high cell densities (over 30 millions cells/mL), and a unique metabolic profile resulting on low ammonium and lactate accumulation with low glutamine consumption, even at high cell densities. Those cells, adapted to serum free media (SFM) in the early-stage development, demonstrated a high susceptibility to a wide spectrum of human and animal viruses and revealed attractive properties for the production of Monoclonal antibodies (mAb) with enhanced antibody-dependent cell cytotoxicity (ADCC) activity linked to their natural low level of fucosylation. The EB66 cell substrate constitutes therefore a very attractive platform for the industrial manufacturing of vaccines and antibodies and is already well endorsed by the industry as illustrated by its licensing to 29 Biotech and Pharma companies worldwide. In addition, fully characterized GMP MCB and WCB are available, a Biological Master File (BMF) has been filed with the US FDA and a first Investigational New Drug (IND) filing has been approved in 2010 in the USA for a human vaccine candidate produced on EB66 cells.
In order to further optimize the current efficient processes based on SFM, investigations were conducted with the aim to develop a new generation of media based on chemically defined (CD) formulations. Preliminary data, based on metabolic requirement of the EB66® cells, demonstrate that CD media could efficiently replace SFM during product manufacturing. Main results of this study will be presented.

P4.12. INFLUENCE OF OPERATING PARAMETERS OF A SETTLING-BASED PERFUSION PROCESS ON EXPANSION OF VERO CELLS ATTACHED ON MICROCARRIERS

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The growing demand for biologicals produced by animal cells motivates the development of more efficient and reliable culture production processes. In the particular case of the industrial production of viral vaccines by Vero cells, the cell propagation phases are mainly devoted to reach high cell density in less time while maintaining a good cell physiological state. Perfusion bioreactors, based on continuous medium renewal and cell retention, can meet these objectives. So, the aims of this work were to evaluate the performances of adherent Vero cell cultures performed inside a perfused bioreactor using a gravitational settler as cell retention device.
Among the various technologies for cell retention, gravitational settler is a promising device for large-scale perfusion culture process. This simple device takes advantage of the difference between the cell settling rate and the medium harvesting flow rate. Moreover, in the particular case of cells attached on micro-particles, it is more suitable than in the case of single suspended cells. So, in a first time, the design of an efficient gravitational settler has been evaluated by applying chemical engineering methods to the specific case of cells attached on Cytodex-1 microcarriers. Our study addressed several aspects such as the ratio between cell and settler diameters, the flow around the carriers, the terminal velocity fall, the potential group effect and the culture medium flow rate.
Then, Vero cell cultures have been performed in a 2 L perfused bioreactor to evaluate the influence of two major operating parameters, the microcarrier concentration and the seeding cell density, by using a D-optimal experimental design. The other parameters, such as serum-free medium composition, medium flow rate, pH, pO_2 or temperature were maintained at the same values for all experiments. The kinetics of cell growth, nutrient consumption, and metabolite production were followed all over the cultures and kinetics parameters were calculated. The cell viability and apoptosis were analyzed by Crystal violet method and with a Guava cytometer. Moreover, in order to better control the process, the culture permittivity was on-line monitored by using the Fogale cell sensor and correlated to the viable cell density.
Both theoretical analysis and experimental results will be presented and the experimental design will be discussed based on key performance criteria in order to propose optimized operating conditions. Based on this study, the scale-up of this perfusion mode to the industrial-scale could be then considered.

P4.13. DETERMINATION OF SCALE PARAMETERS IN SHAKEN BIOREACTORS

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Shaken bioreactors a frequently used as small scale systems for cell cultivation. Starting from 50ml bioreactors (spin tubes), Erlenmeyer flasks up to 2L roller bottles all different types of bioreactors are placed on a shaker for cultivation.
But what are the differences between the bioreactors?
To compare the bioreactors the scale up parameter aeration rate and oxygen transfer rate were determined of all different vessel types. The shown data will support the user to find the most suitable small scale system for cultivation and to scale up his process into bigger vessels.

P4.14. MICROBIOREACTORS: WHY SMALL IS THE NEW BIG FOR MAMMALIAN AND MICROBIAL CELL LINE OPTIMIZATION AND PROCESS DEVELOPMENT

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The ability to efficiently and successfully develop cell lines and processes that will meet the demands of a commercial launch early in development is a key factor for reducing timelines and resource demands for biopharmaceutical organizations; ultimately benefitting the patient. In the past cell lines and processes were initially developed in uncontrolled environments that poorly modeled bioreactors (commercial method of production) and not until late in development were cells placed in controlled bioreactors. The decision to screen uncontrolled in the past was based often on resource limitations (bioreactors, number of clones, cost) not on whether it was the best way to screen or develop commercial ready products. New tools and methods are available today to screen and develop processes under controlled conditions earlier than ever before and with a smaller footprint to allow delivery of a robust cell line and process earlier and also generate data for Quality by Design (QbD) initiatives. One of those tools is the Micro-24 MicroBioreactor, a 24 well system with individual pH, Dissolved Oxygen, and temperature control which allows users to maximize the likelihood of obtaining a “winning” cell line prior to commercialization. Data will be presented showing the Micro-24 MicroBioreactor utilized successfully in cell line selection activities (ranking clones) and process optimization (parameter optimization) activities and its scalability to larger bioreactors, demonstrating the advantages of a controlled “high-throughput” bioreactor system that allows rapid, very early stage process development which can contribute to shorter development timelines and lower development costs.

P4.15. IMPACT OF CELL PRODUCTION SCALE-UP ON GLYCOSYLATION OF A RECEPTOR FC FUSION PROTEIN

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The potential therapeutic value of many proteins—including enzymes, cell-surface receptors, cytokines and peptides—can be realized by fusing these proteins to the Fc region of human immunoglobulin G. Of the 31 mAb products approved as human therapeutics in the USA to date, 4 are Fc fusion proteins, and many more are in clinical testing. Notwithstanding their structural similarity to mAbs, these glycoprotein constructs can present unexpected production challenges. At Genentech, we sought to manufacture a fusion protein consisting of the extracellular domain of the p55 TNF receptor with human IgG1 Fc (TNFR-IgG) as part of a clinical development program. The receptor domain of the fusion protein contains 3 N-linked, complex oligosaccharides, in addition to the 1 N-linked, complex oligosaccharide in the Fc region of IgG. Initial clinical production was performed using a 1000 L cell culture process, and testing in human subjects showed an AUC_{1week} of 93 µg/h per mL for TNFR-IgG. As the clinical program progressed, the production process was improved to meet productivity targets. As a result, a 20-60% decrease in AUC_{1week} was noted for test material generated from this more highly productive process. Process changes had been made to increase specific productivity and enhance cell mass, resulting in a 3-fold increase in titer. An analytical investigation of 8 manufacturing batches of bulk drug substance was undertaken to identify structural changes in TNFR-IgG that might be correlated with the change in pharmacokinetics of the molecule. As part of that investigation, three terminal sugars were measured---sialic acid, galactose and N-acetylglucosamine. While terminal galactose was relatively unchanged across the 8 production batches, the amount of terminal N-acetylglucosamine varied. Indeed, we observed an inverse correlation between terminal N-acetylglucosamine and AUC_{1week}. Thus, process changes associated with cell production resulted in a change in pharmacokinetics of the TNFR-IgG fusion protein that turned out to be significant enough to require an adjustment in clinical dosing. After this was realized, additional changes to the process were implemented reducing specific productivity and restoring the glycan composition and, with that, AUC. This final process was successfully implemented at 12,000 L production scale.

P4.16. CRITERIA FOR BIOREACTOR COMPARISON AND OPERATION STANDARDISATION DURING PROCESS DEVELOPMENT FOR ANIMAL CELL CULTURE

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[3] *Bielefeld University, Germany*
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Development of bioprocesses for animal cells has to deal with different bioreactor types and scales. Bioreactors might be intended for seed train and production, research, process development, validation or transfer purposes. During these activities, not only the problem of up- and downscaling might lead to failure of reproducibility, but also the use of different bioreactor geometries and operation conditions. In such cases, the criteria for bioreactor design and process transfer should be re-evaluated in order to avoid an erroneous transfer of cultivation parameters.
With main focus on laboratory scale bioreactors, criteria such as power input, mixing time, stirrer speed, etc. for bioreactor design and process transfer during the cultivation of a novel human cell line for production purposes (AGE1.HN, ProBioGen AG, Germany) have been systematically compared. A common optimum value for exponential growth rate as a function of mixing time has been found in bioreactors having significant differences in their inner geometries. The obtained results have been employed for process standardisation and transfer between research institutions.

This work is a part of SysLogics: Systems biology of cell culture for biologics, a project founded by the German Ministry for Education and Research (BMBF).

P4.17. INTRODUCTION OF A NEW BIOPROCESS SCHEME TO INITIATE CHO CELL CULTURE MANUFACTURING CAMPAIGNS

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Agility to schedule and execute cell culture manufacturing campaigns quickly in a multi-product facility will play a key role in meeting the growing demand for therapeutic proteins. In an effort to shorten campaign timelines, maximize plant flexibility and resource utilization, we investigated initiation of cell culture manufacturing campaigns using CHO cells cryopreserved in large volume bags in place of the conventional seed train process. This approach involves cultivating cells in a perfusion bioreactor, and cryopreserving cells in multiple disposable bags. Each run for a campaign would then come from a thaw of one or more bags directly into the inoculum train, followed by fed-batch production.
This presentation will review the development and optimization of individual steps of this new bioprocess scheme (from cell scale-up to freezing in bags) prior to its roll out in GMP manufacturing. Prior to GMP implementation, extensive development data was also gathered to ensure that the quality of the drug manufactured using this new bioprocess scheme is comparable to material manufactured using the conventional seed train cell source and is acceptable for use.

P4.18. CELL LINE AND UPSTREAM PROCESS DEVELOPMENT FOR A NOVEL HSA FUSION PROTEIN

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At HGS, we have developed a platform process to generate manufacturing cell lines for monoclonal antibodies using the GS-CHO expression system. In this presentation, we will describe our efforts in creating a manufacturing cell line for a novel HSA fusion protein using the platform process. In addition, effects of various factors including cell lines, basal and feed media, and culture times, on productivity and product quality will be described. By choosing the right cell line combined with the right media, we were able to develop a high titer cell culture process with desired product quality.

P4.19. APPLICATION OF HYDROCYCLONES FOR CONTINUOUS CULTIVATION OF SP-2/0 CELLS IN PERFUSION BIOREACTORS. EFFECT OF HYDROCYCLONE OPERATING PRESSURE

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Recently, hydrocyclones have been extensively evaluated for their application in the separation of mammalian cells in perfusion bioreactors. The high centrifugal force derived within hydrocyclones is the key mechanism for cell separation inside such small-sized simple devices. This is usually accompanied by a very short residence time of the cells inside the separating equipment, usually not more than 0.2 s. Moreover, hydrocyclones have other specific characteristics, which highly recommend their application for the production of pharmaceutical products in perfusion cultivation bioreactors. They are characterized by their high performance, robustness, lack of movable parts, ease of *in situ* sterilization and suitability for cleaning-in-place processes.
In the present work, the application of hydrocyclone for cell separation in continuous cultivation of SP-2/0 cells in perfusion bioreactors has been investigated. The effect of operating pressure of the hydrocyclone on cell viability as well as separation efficiency was studied. Recombinant mouse lymphoid cell line SP-2/0, cultured on serum-free ZKT-1 medium, was used.
The obtained results showed that cells were able to grow with high viability after the operation of hydrocyclone at both tested pressure values (0.85 and 1.30 bar). Maximal cell concentrations of about 8.2×10^6 and 6.0×10^6 mL^{-1} were achieved at an operating pressure of 0.85 and 1.30 bar, respectively. This corresponds to a 5- and 3.5-fold increase than the batch cultivation, respectively. During the whole course of perfusion, cell viability ranged from 92 to 98 at both tested pressure drops. Concerning separation efficiency, increasing the hydrocyclone operating pressure from 0.85 to 1.30 bar resulted in an increase in the average total separation efficiency from 89 to 95%., respectively. Additionally, results also showed that hydrocyclone preferably separates more viable cells in the underflow, and hence, back to the bioreactor system. Consequently, more dead cells are separated in the overflow, thus leaving the bioreactor system. This will lead, finally, to the improvement of the system viability and product quality. From the aforementioned results, it can be concluded that hydrocyclones can be successfully applied for cell retention in mammalian cell perfusion bioreactors.

P4.20. COST-EFFICIENT DEVICE ENHANCES DATA QUALITY DERIVED FROM SHAKE FLASK CULTIVATIONS

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Shake flasks are a common small scale format for cultivation of mammalian suspension cell cultures. Incubators providing a suitable environment by carbon dioxide (CO_2) supply and humidity control are used to ensure sufficient oxygen transfer and homogenization of cell suspension by appropriate mixing. In order to match scale-up parameters power inputs are adjustable either by adaptation of the shaking frequency, eccentricity of the shaking movement or working volumes in shake flasks.
However, removal of shake flasks from the incubator for feeding or sampling procedures during development of fed batch cultivations may lead to a sudden loss of CO_2 in the headspace of the shake flasks altering pH of the cell suspension. Moreover, temperature drops, cell settling, local pH gradients by addition of feed solutions, and oxygen limitations may occur. After removal of shake flasks from the incubator requiring opening the door only a few seconds, recovery of the CO_2 content in the incubator may take up to ten minutes. This effect is even more significant if shake flasks are removed and put back into the incubator consecutively.
To avoid the necessity of removing the shake flasks from the incubator for feeding and sampling, a prototype of an autoclavable shake flask adapter was constructed which enables addition and removal of liquids via three ports. The adapter provides an internal screw thread for the shake flask itself and an external screw thread for the vented cap. A modification of the shake flask itself is not necessary. In contrast to attached sterile filters, oxygen transfer into the shake flask was shown not to be limited by this device.
Continuous feeding, e.g., is very easy to perform via continuous pumps or syringe based dosing systems. Moreover, monitoring and control of e.g. dissolved oxygen and pH is possible using in-line analytics from Presens®, and incubator settings for CO_2 and frequency as actuating variables. For scale-up studies, comparable and representative results can be gathered using this device since main disturbance variables can be minimized or knocked out.

P4.21. LIMITATION OR DENATURATION? A GENTLE WAY TO SUPPLY ANIMAL CELL CULTURES WITH CONCENTRATED FEED SOLUTIONS

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A widely used format for the production of therapeutic proteins or biomass is the fed batch fermentation. Cell densities of mammalian cell cultures nowadays often exceed 100x10^5/mL making it difficult to provide sufficient amounts of required substrates due to bad solubility and/or stability of certain substances or substance classes.
Therefore a common approach is to use feed solutions with extreme (mostly basic), non- physiological pH-values that allow dissolving or stabilizing required amounts of certain substances. Especially continuous feeding strategies require stable feed solutions, for shelf life must exceed at least the durability of feeding periods.
To avoid effects generated by feed solutions with high or low pH values, a feed mixing device was developed, enabling continuous mixing of at least two solutions just before addition into the cultivation vessel. In consequence, feed components can be dissolved individually at pH ranges ideal for good solubility and/or good stability of these components, independent of the pH of the cultivation medium, i.e. different from the physiological pH value. Four fed-batch cultivations were performed in 2L-bioreactors with a continuous feeding strategy (Figure 1).

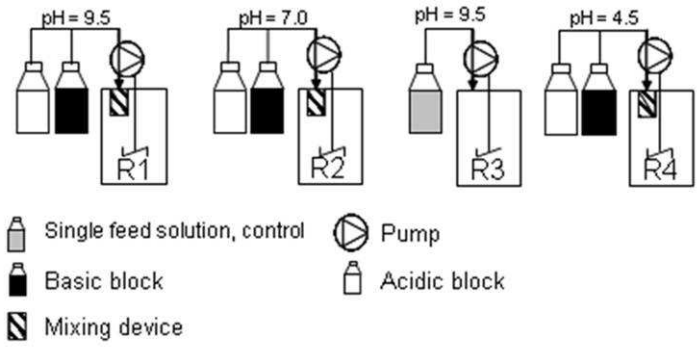

Figure 1: experimental setup for fed-batch cultivations using feed mixing device. Feed rate and component addition compared to the control was identical.

A high-concentrated, internal standard feed solution with a pH value around 9.5 was divided into two blocks with acidic and basic pH, respectively. Components with a good solubility in acidic environment were dissolved in the acidic block; all other components were dissolved in the basic block. To keep volume flow (feed rate) comparable to the standard, component concentration in each block was doubled. Continuous mixing of both blocks with an identical volume flow using the feed mixing device provides exactly the same component mass flow compared to the original basic feed solution. Appropriate pH adjustment of acidic and/or basic blocks leads to different overall pH of the mixed solution. Mixed feed solutions with overall pH values of 4.5, 7.0 and 9.5, respectively, were compared to the single feed solution as control.
It could be shown that acid and base addition by pH-control was directly affected by the pH of the mixed feed solution. Overall accumulation of metabolic parameters like ammonia and lactate was altered. Agalactosyl-species [Gal(0)] of created IgG-antibody was decreased significantly in favor of the monogalactosyl-species [Gal(1)] by the acidic feed compared to the control.

P4.22. HIGH YIELDING AND SCALABLE CHO TRANSIENT SYSTEM: EFFICIENT PRODUCTION OF PRECLINICAL GRADE RECOMBINANT PROTEIN

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An efficient, rapid and productive expression strategy is required to meet the increasing requirements for research grade material during early drug development. The development, optimisation and implementation of a scalable high yielding proprietary CHO transient expression system would be presented. The system has been successfully scaled up to 250L and is capable of expressing several hundred mg/L of recombinant protein. This CHO transient system can now provide gram amounts of preclinical grade material.

P4.23. EVALUATION OF ABER-INSTRUMENTS AND FOGALE-NANOTECH CAPACITANCE PROBES IN LONG-TERM HIGH-DENSITY CELL CULTURES

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The reliable monitoring and estimation of viable cell densities is one of the critical tasks in mammalian cell culture, in fed-batch cultures to control feeding strategies or in high-density long-term perfusion cultures to maintain steady-state conditions . Currently the O2 flow rate is often used as a substitute for cell density for example to control the purge setting to ensure constant cell densities during perfusion processes. Due to changes in cellular metabolism or physical changes to the reactor system (e.g. increase of backpressure in membrane aerated reactors or change of total gas-flow rates) purge settings based on O2 flow have to be adjusted during the course of a reactor run. Therefore, systems that can monitor cell densities on-line are desirable.
Capacitance probes have been used in the biotechnology field for more than two decades, primarily in microbial or yeast fermentation. The advantage of capacitance probes over other on-line sensors is its fairly simple and straightforward measurement principle. Here two currently commercially available capacitance probes were evaluated in high cell density bioreactor cultures; the Aber biomass monitor 230 (Aber Instruments Ltd, Aberystwyth, UK) and the Fogale Biomass System (Fogale Nanotech, Nimes, France).
Initial off-line measurements were performed to evaluate these probes regarding measurement limits and physical and/or chemical factors that could influence the accuracy of the capacitance readings. Subsequently the two probes were evaluated in three different perfusion processes using recombinant BHK cell lines with and without cleaning pulses.
The off-line beaker experiments demonstrated, that both capacitance probes were able to reliably measure cell densities in the range of about 0.1 • 106 cells/mL to at least 40 • 106 cells/mL. Only viable cells are measured and no significant influence of cellular debris was found. The reactor experiments showed that fouling occurred after approximately 40 – 50 days in almost very culture if no or only infrequent cleaning pulses were applied. Nevertheless an automated rigorous cleaning regime with a cleaning pulse of every 8 hours provided reliable capacitance reading at least during two long-term cultures. Overall the results show that both capacitance probes can be used in high cell density cultures of mammalian cells although fouling occurred in almost every culture unless a rigorous cleaning regime is applied from the start of each campaign.

P4.24. IMPROVING VOLUMETRIC PRODUCTIVITY OF A STABLE HUMAN CAP CELL LINE BY OPTIMIZATION OF BIOPROCESS STRATEGY

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Since product quantity and quality are important issues for recombinant proteins, a human cell derived expression technology offers significant advantages in quality, serum half- life and safety. High volumetric productivity with first-class quality is the ultimate ambition during process development.
CEVEC's proprietary expression system based on human amniocytes offers significant advantages for the production of complex human proteins and antibodies. The key benefits are the stable and high expression of recombinant proteins with human type posttranslational modifications, the robust growth behavior with competitive high cell densities and the easy handling in serum free suspension. CAP cells meet all regulatory guidelines, they are of non-tumor origin and from ethically accepted source.
In order to test the performance of CAP cells for the production of very complex proteins but also for antibodies, stable C1-Inhibitor and IgG-expressing CAP cells were developed. C1-Inhibitor is a serine protease inhibitor (serpin) and one of the most heavily glycosylated plasma proteins bearing numerous complex N- and O-glycans, whereas IgGs contain a single N-glycan.
Subsequent to single cell cloning, a fed batch process for stable protein expressing CAP cell lines was developed. Based on the results of multiple small scale experiments in shake flasks, the optimal medium and feeding supplements were chosen. Several parallel cultivations led to the optimized physical parameters, process conditions and specific feeding schedules.
By testing different commercial peptones in combination with other supplements, we achieved optimized cell densities and viabilities and improved cell specific productivities.
The optimized fed batch procedure yields high product concentrations with improved volumetric productivity and is applicable for the C1-Inhibitor-expressing and IgG-expressing cell lines

P4.25. DEVELOPMENT AND FINE-TUNING OF A SCALE DOWN MODEL FOR PROCESS CHARACTERIZATION STUDIES OF A MONOCLONAL ANTIBODY UPSTREAM PRODUCTION PROCESS

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It has always been an objective of process development and more recently it has also become a regulatory expectation to build robustness into and demonstrate proper control of a manufacturing process, thus ensuring that the biological product meets consistently its quality attributes and specifications. This is achieved mainly through systematic process development and understanding. Once a process is locked and ahead of consistency runs at the intended commercial scale, process characterization studies (PCS) further contribute to the demonstration of process robustness and the justification of process control ranges. These studies characterize the relationship between process parameters and process performance as well as product quality attributes. For practical reasons PCS are performed in a scale down model of the manufacturing process studied. Therefore, it is essential to establish a scale down model that is representative of the commercial scale.
Here we describe a systematic approach to develop a 2 L scale down model of a cell culture process for recombinant protein production. The cell culture process modeled is a 12,000 L scale Sp 2/0 cell line-based fed batch process producing a monoclonal antibody. In a first step, the scale down model was defined such that volume-dependent parameters were scaled down linearly and volume-independent ones were kept at the same set-point as in the large scale bioreactors. Process performance in this initial scale down model was then compared to commercial scale production in terms of cell growth, metabolism, product accumulation and product quality. This initial scale down model simulated the production process well with regard to viabilities, maximum cell densities achieved, viability, product accumulation and metabolite profiles.
However, a slight delay of the cell density peak was observed in the small scale model. Fine-tuning of the model focused on troubleshooting this growth difference. Experiments showed that dissolved CO_2 levels impact this cell line's growth profile. By adjusting the aeration strategy to match dissolved CO_2 profiles in small and large scale we have been able to match the cell growth profiles of the two systems.

P4.26. PROCESS DEVELOPMENT OF ATROSAB, AN ANTI TNFR1 MONOCLONAL ANTIBODY: IN THREE STEPS FROM RESEARCH TO GMP

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The humanized monoclonal antibody ATROSAB is targeted against the TNF receptor 1(TNFR1) specifically. TNF is a central mediator of inflammation and key target for intervention in inflammatory diseases such as rheumatoid arthritis, psoriasis and Crohn's Diseases. Notably, blockade of the second TNF receptor, TNFR2, has been associated with increased sensitivity to viral infections or increased susceptibility to demyelinating disorders and lymphomas. In this context, a selective inhibition of TNF-induced TNFR1 but not TNFR2 signaling activity holds great promises to overcome undesired effects observed with less specific TNF antagonists currently used in clinic.
The recently humanized antibody was used to establish a rCHO-K1cell line under serum-free, chemically defined conditions. The process development was based on two sets of shake flask experiments, three 10L bioreactor runs and finally 3 GMP production runs in 300 L scale. The scale up strategy was based on mixing time as a function of the bioreactor geometry, tip-speed, Reynolds number, and the power input of the systems.
The data obtained from all three culture systems were basically identical based on product quality attributes as well as final product concentration, indicating for suitable scale-down models in early process development. The product quality was characterized employing SDS-PAGE, IEF, HP-SEC, a potency assay and glycosylation pattern with HPAEC-PAD. All samples were found to be within defined specifications. The product concentration could be increased by more than 7 fold from basal medium screening to the final production process.

P4.27. BATCH CULTURE PERFORMANCE OPTIMIZATION WITH FED-BATCH CULTURE COMPONENTS

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Concentrated feed supplements are an integral part of fed-batch culture strategy. In these studies, supplements normally used as feeds in fed-batch strategies were instead used to fortify basal media for batch culture. Six commercially available cell culture supplements were investigated at concentrations ranging from 5-30% (v/v) in various culture media. Cell types examined included CHO, NS0 hybridoma, SP2/0 hybridoma, HEK293, and SF9 insect cells. Product levels were monitored for the two hybridoma cell lines. In batch culture of supplement-fortified basal media, peak viable cell densities were increased by up to 300% of control, with similar gains seen in product titre. Titration of supplement concentration within the basal medium afforded the best performance gains, as individual cell lines vary widely in response. As expected, performance improvements were dependent on both the initial culture medium and the cell lines used. In general, the approximate magnitude of performance increase correlated to the richness / leanness of the culture medium, with leaner media having the most room for improvement.

P4.28. AVOIDING PITFALLS IN COMMERCIAL BIOLOGICS MANUFACTURING PROCESS ADAPTATIONS AND IMPROVEMENTS

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In modern biotechnology, the production of recombinant proteins and antibodies is of increasing importance. Since the early 80s and 90s when the first commercial biotech production processes were developed, a large number of products have been launched for the treatment of diseases. Especially in the early nineties biotech products derived from mammalian cells shaped the biopharmaceutical industry and made life-saving contributions to patients' lives by targeted therapies. Due to the increasing demand patterns as well as new product launches, significant (bio) process development has been conducted, creating a large knowledge base and fostering implementation of advanced technologies in new manufacturing processes. In order to deliver high quality products and to satisfy increasing demands of existing and new product portfolios, process development and optimizations have been augmented with an increase in global biotech manufacturing capacity over the last decade. However, due to mergers and acquisitions most companies have grown manufacturing asset bases leading to several differences in facility design and unit operation platforms. Therefore, in most technology transfers, manufacturing processes have to be adapted to the existing equipment ("facility fit"). In addition, new technologies driven out of research and development (know why translated to know how) have created value in existing production processes. In many cases, process modifications are necessary for supply chain and financial risk mitigation. These areas are meant to enhance manufacturing processes and supply chain reliability without compromising safety or the right of patients to quality products – The major question remains: *How to verify comparable product quality and quantity?*
This presentation will review a case study from Genentech where modifications to commercial processes were performed in order to achieve the following:

- Facility fit
- Continuous improvement of technology standards
- Business/ supply chain risk mitigation.

Special consideration to the implementation of HTST (High temperature short time) as an additional viral barrier for cell culture media and additives (next to downstream processing measures) is of particular interest due to recent reports from industry. The case study will also review the necessary process adaptations for facility fit of a non-platform process, as well as examples for implementing continuous improvement measures (e.g. disposable applications).

P4.29. FED-BATCH AND PERFUSION CULTURE PROCESSES: OPERATIONAL, ECONOMIC AND ENVIRONMENTAL FEASIBILITY UNDER UNCERTAINTY

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Historically the use of continuous processes has been hampered by system complexity, logistical problems and validation issues. However some of the latest advances in continuous technologies claim to overcome some of these obstacles with the promise of more cost-effective processing compared to traditional batch processes. This presentation will evaluate the current and future potential of batch and continuous cell culture technologies via a case study based on the commercial manufacture of monoclonal antibodies. The case study compares fed-batch culture to two perfusion technologies: spin-filter perfusion and an emerging perfusion technology utilising alternating tangential flow (ATF) perfusion reactors. The operational, economic and environmental feasibility of these technologies is evaluated using a prototype dynamic decision-support tool built at UCL encompassing process economics, discrete-event simulation and uncertainty analysis so as to enable a holistic assessment. The technologies are compared across a range of scales and titres so as to visualise how the ranking of the technologies changes in different industry scenarios. The impact of uncertainty and failure rates on the feasibility of the technologies are explored using Monte Carlo simulation, allowing their robustness to be evaluated. The presentation also looks to the future of antibody manufacture, in particular the impact of the trend towards multiple staggered single-use bioreactors in place of stainless steel bioreactors and the resulting effect on the overall process rankings.

P4.30. K_LA AS A PREDICTOR FOR SUCCESSFUL PROBE-INDEPENDENT MAMMALIAN CELL BIOPROCESS IN ORBITALLY SHAKEN BIOREACTORS

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Orbitally shaken bioreactors are commonly used at an early stage of bioprocess development. In contrast to stirred-tank bioreactors, shaken flasks are usually operated in probe-independent bioprocesses without controlling the pH or dissolved oxygen concentration (DO). As a consequence, gas transfer issues, such as oxygen and CO_2 limitations, are often considered the major drawbacks of orbitally shaken bioreactors. To define optimal operating conditions for probe-independent bioprocesses, we tested the effects of the mass transfer coefficient of oxygen (k_La) on mammalian cell growth, recombinant protein production, and culture conditions (pH, DO). CHO cells stably expressing a recombinant anti-RhD IgG antibody were cultivated in 1-L cylindrical bottles. As the culture volume increased from 200 to 600 mL, the k_La decreased from 19 to 4 h^{-1} . The cells reached the highest densities in cultures with a k_La equal to or higher than 7 h^{-1}. In these cultures, the lowest pH was 6.7 and the minimal DO was about 30% air saturation. In contrast, for cultures with a k_La lower than 7 h^{-1}, the pH dropped to a value below 6.6 and the dissolved oxygen was depleted by day 4. Despite the differences observed in cell density, similar levels of biomass (as determined by the packed cell volume) and recombinant protein were found for all the cultures. To test the scalability of probe-independent bioprocesses, CHO cells were cultivated at various working volumes in 250-mL, 500-mL and 1-L bottles. Similar values and trends in terms of cell density, DO, pH, biomass, and specific productivity were observed for cultures at the same k_La but in different bottles. These experiments are being repeated with other clonal cell lines. Our results show that k_La is a crucial parameter for the scalability of probe-independent bioprocesses to ensure that the pH and DO remain within a suitable range. This study suggests that probe-independent bioprocesses may be scaled-up successfully as long as a sufficient k_La can be achieved.

P4.31. ESTABLISHING HIGH-YIELDING PROCESS FOR ANTIBODY PRODUCTION OF CHINESE HAMSTER OVARY (CHO) CELLS USING ONE-STEP OPTIMIZATION METHOD

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A high-yielding fed-batch process for Chinese Hamster Ovary (CHO) cells producing antibody has been established using one-step optimization method. According to detailed information of an animal-component-free and chemically defined batch experiment, initial medium of fed-batch was designed for supplying sufficient and balanced nutrients to match their consumption, simultaneously minimizing the accumulation of by-products. The proportions of feeding nutrition relied on the specific consumption rates analyzed and calculated in former batch. The responsive feeding strategy was based on the measurement of glucose. In the optimized fed-batch culture, 1.06×10^7 viable cells/ml and 1.02 g antibody/L were obtained, which got 4.6-fold increase of viable cell density and 20.8-fold increase of final antibody concentration compared with batch culture. The optimization from batch to fed-batch using the one-step method was proved to simplify the development of antibody production and accelerated the industrialization process.

P4.32. DEVELOPING A SCALE-DOWN MODEL FOR A CHO CELL FED-BATCH PROCESS

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Characterizing a commercial manufacturing cell culture process at the scale is not practically feasible due to cost of operation and limited availability of large-scale bioreactors. Therefore it is important to develop a scale-down model that represents the performance of the commercial scale process. Various approaches may be used to scale-down the different process parameters. It can be challenging to establish a representative scale-down model that has comparable process performance and product quality as the commercial scale process not only when the process parameters are within the operating ranges but also when a process parameter deviates outside its operating range.
In the present work, we developed a 2 L scale-down model for a 2500 L scale fed-batch process for production of a recombinant protein in CHO cells. The volume-independent parameters including inoculation cell density, temperature and pH were controlled at the same set-points in both large-scale and small-scale processes. Set-point for dissolved oxygen control was adjusted due to different pressure in reactors. Schedule for feed medium addition and rule for glucose feed remained the same. Among volume-dependent parameters, the bioreactor working volume and feed volume were scaled down linearly based on the volume difference. Similar aeration strategy was employed but gas flow rates and operational control were adjusted independently from the volume ratio. The impeller agitation rate was scaled down using equivalent power input per volume. Efforts were made to achieve a comparable pCO_2 level in the scale-down model as in the commercial scale process. We were able to increase the pCO_2 level to some extent, but it was difficult to reach the level seen at the commercial scale. This difference was accepted since comparable productivity and product quality were obtained in the scale-down model when the pCO_2 level was manipulated up to the same level as in the commercial scale process. To qualify the scale-down model, cell growth profile, metabolite concentrations, pCO_2, osmolality, productivity and product quality were compared between the 2 L scale-down model and the 2500 L commercial scale process.

P4.33. CHALLENGES IN SCALING UP A PERFUSION PROCESS

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Scale-up and transfer of perfusion process to a bigger scale and different facility has challenges because of the complexity in a perfusion process and unavailability of direct scale-up of perfusion equipment. The perfusion process was developed for a monoclonal antibody using the internal spin filter technology at 2L scale and was later scaled up to 1000L scale. Based on a risk assessment analysis, decision was made to scale up using an external Roto filter technology. The scale up resulted in poor cell retention (1/3rd maximum cell concentration) compared to that observed at the small scale. As a part of troubleshooting, the factors responsible for poor retention were identified and some changes were introduced. These changes included change in the Roto filter design, optimization of perfusion rates, changes in physical equipment such as circulation pump. These modifications resulted in comparable cell culture profiles to that of the small scale. However the product quality was impacted because of these changes. Work was carried out at both scales to troubleshoot the reasons for the change in product quality and corrective actions were taken to get comparable product quality. The results of scale up and product quality will be presented as a case study in this poster.

P4.34. PRELIMINARY EVALUATION OF MICROCARRIER CULTURE FOR GROWTH AND MONOCLONAL ANTIBODY PRODUCTION OF CHO-K1 CELLS

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Large-scale biopharmaceutical production commonly relies on suspension cell cultures that provide higher yields than adherent cultures. However, most mammalian cells grow adherently and therefore need to be adapted to suspended growth, which is not always simple or feasible. Microcarrier culture introduces new possibilities and makes achievable the practical high yield culture of anchorage-dependent cells in suspension systems. The aim of this study was to evaluate and optimize the use of microcarrier culture for the growth and antibody production of CHO-K1 cells. For this, the macroporous Cultispher microcarriers were used, and the initial cell adhesion to the microcarriers (occurring in the first 5-6 hours) and further cell proliferation were assessed. Cultures of antibody-producing CHO-K1 cells were performed in 50 ml vented conical tubes, and different conditions were tested: initial cell concentration ($2x10^5$ cells/ml and $4x10^5$ cells/ml), microcarrier concentration (1 g/L and 2 g/L), type of rocking during the first 6 hours of adhesion (pulse or continuous) and rocking after initial adhesion (no rocking and 60 rpm). Cell concentration and viability in the microcarriers were assessed periodically (hourly for the adhesion phase, and daily after that).
It was observed that an increase in the initial cell concentration does not enhance initial adhesion, possibly due to saturation of the microcarrier surface. For its turn, increasing microcarrier concentration, without further increasing initial cell concentration does not improve cell densities achieved in the culture. Concerning rocking, the most favorable type for the adhesion phase was pulse rocking and, after this, a continuous rocking provided an improved cell proliferation.
In conclusion, microcarrier cultures proved to be a viable alternative to suspended cultures for the growth and antibody production of CHO-K1 cells.

P4.35. A PLATFORM FED-BATCH PROCESS FOR VARIOUS CEMAX® PRODUCER CELL LINES

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The random nature of transgene integration harbours various pitfalls for development of production cell lines including clonal variation in expression level and growth characteristics. The CEMAX system is an expression system for targeted integration of expression cassettes via DNA double-strand break induced homologous recombination. Stable high producers are available within 4 weeks without the need of extensive clone screening.
Stable and high expression rates are ensured with the CEMAX system due to targeted integration of a single copy of the gene of interest at a transcriptionally highly active site in the host cell genome. Producer cell lines for various therapeutic product candidates were established. These cell lines produce antibodies and highly glycosylated antibody fusion proteins and show high clonal similarity. This makes a platform fed-batch process profitable.
We describe here a platform process for fed-batch cultivation of various CEMAX producer cells. The fed-batch process was optimised based on commercial available and proprietary basal and feed media formulations. It is suitable for all producer cell lines derived of a particular host cell due to highly similar growth behaviour after targeted integration of the gene of interest. The platform process allows high titers without the need of optimisation and a six month faster entry in first-in-man studies.

P4.36. COMPARISONS AND VALIDATIONS OF SCALE-DOWN HTP CELL CULTURE PLATFORMS

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Demands for chemically defined cell culture media and feeds dramatically increased for mAbs and recombinant production due to regulatory concern and the considerations of product quality and consistencies. Cell culture media are very complex and involve many components including amino acids, vitamins, polyamine, trace elements and etc. Each component is important for cell growth and productivity of cell lines. In order to systemically study those components for cell culture media and feeds, we developed and studied scale-down cell culture platforms which include 96 deep-well plates, spin tubes, Ambr bioreactor system from Automation Partners, and DASGIP bioreactors. Cell growth and mAb production in these scale-down and high throughput (HTP) platforms are comparable to DASGIP bioreactors. Media formulation ranking in these HTP platforms are similar to the ranking in bioreactors. These high throughput capabilities allow us to investigate various cell culture media components for our media and feed formulation and cell culture process development. Routine applications of these scaled-down HTP platforms enable fast and efficient cell culture media, feed and process develop.

P4.037 APPLICATION OF SEGFLOW® SYSTEM FOR CELL CULTURE PROCESS DEVELOPMENT

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SegFlow® is an automated sampling system with feed-back control capability. It can be programmed to sample up to eight bioreactors and send cell-free samples to four analyzers/collectors. It also has an internal web server to enable remote access.
It is well known that glucose plays an important role in mammalian cell culture as carbon and energy sources. Therefore, it is important to understand the metabolism of glucose in order to develop strategies to maximize recombinant protein production and control product quality. In the present study, experiment was designed to evaluate the impact of programmed feeding on culture performance by SegFlow and YSI 2700 interfaced with bioreactors. The automatic feed control resulted in smaller change intervals of feed medium as compared to daily manual bolus feeding. Our results showed that the application of SegFlow system could potentially reduce labor, improve bioreactor efficiencies. In combination with optimized cell culture media and feeds, continuous feeding control could result in conditions at which higher total protein production could be achieved, leading to more efficient protein manufacturing as compared to that with generic fermentation process. Product quality was examined and reverse phase HPLC data are presented.

P4.38. BAG-BASED RAPID AND SAFE SEED-TRAIN EXPANSION METHOD FOR *TRICHOPLUSIA NI* SUSPENSION CELLS

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Trichoplusia ni suspension cells (High Five™) used in conjunction with the baculovirus vector expression system (BEVS) are regarded as potential product system of new, recombinant virus-like particle (VLP) vaccines. In order to push vaccine development and production, biomanufacturers use single-use technology when- and wherever possible. This applies to upstream processing and in particular seed-train expansion ranging from cryopreserved vials via t-flasks, spinners (respectively shake flasks) to stirred stainless steel bioreactors. The stainless steel bioreactors deliver inoculum for seed bioreactors and have been increasingly replaced by wave-mixed single-use bag bioreactors during the last 5 years (1).
The approach presented for seed-train cell expansion of High Five suspension cells is based on the Biostat CultiBag RM50 optical (Sartorius Stedim Biotech). It was used for the production of cells for long-term storage and for the expansion of cells for subsequent production experiments. For long-term storage the cells were frozen at high cell concentrations (20 – 40 x 10^6 cells x mL^{-1}) in 60 mL Cryobags and stored in nitrogen at -196 °C in vapour phase.
Initial experiments were aimed at the growth characterization of High Five suspension cells from a vial working cell bank (WCB). The High Five cells were grown in batch mode and in 250 mL single-use shake flasks (Corning and Sartorius Stedim Biotech) on a Certomat® CT Plus shaker (Sartorius Stedim Biotech) during six days (triplicates, 27 °C, 100 rpm, 25 mm shaking diameter). Afterwards a procedure was developed in which thawed cells from a Cryobag were directly transferred into and expanded in a Biostat CultiBag RM. Under optimal process conditions (500 mL starting volume, a starting cell density of 1 x 10^6 cells x mL^{-1}, 27 °C, rocking angle of 6 °, 20 – 30 rpm, 0.2 vvm, DO set point 50%) growth rate (0.039 – 0.040 h^{-1}), doubling time (18 – 20 h) and maximal cell density (7.8 – 8.9 x 10^6 cells x mL^{-1}) showed good correlation with results arising from CultiBags which were inoculated with cells from shake flasks. This bag-based seed-train expansion allows time saving of about one week and reduces cross-contamination, both advantages being due to omitted intermediate cultivation steps in shake flasks.
(1) Eibl, R. and Eibl, D. (2010): Single-Use Technology in Biopharmaceutical Manufacture, Wiley VCH

P4.39. ASPECTS OF SOLID-LIQUID SEPARATION IN PHARMACEUTICAL BIOTECHNOLOGY - CHARACTERISATION, OPTIMIZATION AND SCALE DOWN OF THIS PROCESS STEP

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Continuous solid-liquid separation mostly represents one of the final steps in cell culture fermentation processes in the biopharmaceutical industry. Besides aspects like medium additives and cell disruption during fermentation it is of crucial importance when it comes to deliver constant and best possible starting conditions for the following purification process. In large scale production, continuously working disc stack centrifuges are mainly used for this separation process.
Especially in mammalian cell culture processes producing antibody and recombinant proteins issues like cell disruption by shear stress and product loss during discharge of solid components have a detrimental effect on the rate of yield. Thus, detailed equipment characterisation combined with suitable online and offline process monitoring are required for optimization of the solid-liquid separation step.
Another challenge is the scale down of the large scale continuous separation using disc stack centrifuges from production to bench top scale. Bench top scale down models for bioreactors and most downstream processing steps are widely established. However, the separation step often represents the missing link to complete small scale platforms, thus, often preventing exploitation of the scale down approach in modern process development. Due to the harsh conditions during this process step adequate small scale systems are of crucial importance to prevent scale dependent issues during scale up. Physical limitations make the determination of adequate scale down and scale up parameters very challenging.
Besides methods for the characterisation of separation processes, online and offline monitoring techniques will be discussed in this poster. Process optimization conducted after detailed equipment characterisation will be presented. Moreover, possible scale down setups including scale down criteria on technological and cell physiological level are compared and discussed.

P4.40. CHEMICALLY DEFINED BASIC FEED CONCEPT FOR FAST DEVELOPMENT OF MAMMALIAN FEDBATCH CULTURE

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Fedbatch processes are the common mammalian cell culture for the industrial production of recombinant proteins. The development of these processes is still time consuming and complex. Different cell lines and clones have unique metabolic profiles requiring an optimization of feed solutions and strategies for high-end fedbatch processes.
Based on our proprietary chemically defined batch medium platform technology, we designed a new high performance basic feed concept. For this development we first established accurate quantitative analytical methods for more than 40 of the components in our media. This spent media analysis was used for the estimation of cellular needs of various mammalian cell lines including a variety of CHO cell lines and clones. For components without analytical characterization, empirical determination of cellular requirements was done by cultivation.
Merging of the cell characteristics and using a model-based approach addressing specific rates led to a feed solution for a virtual cell line. Its composition was further modified in respect to chemical stability, applicability and inhibitory effects of single components. As a result, an animal component free, chemically defined, protein, peptide and hormone free basic feed for fast development of fedbatch processes was designed. This platform technology can be used as a stand-alone solution for fedbatch cultivation or as a starting point for fast optimization leading to highest performance.
The results of cultivations using this new chemically defined basic feed concept for CHO-cell lines will be presented, showing its potential to obtain cell densities of about 2.5x10^7 cells/mL and high product titres.

P4.41. HIGH-END PH-CONTROLLED DELIVERY OF GLUCOSE EFFECTIVELY SUPPRESSES LACTATE ACCUMULATION IN CHO FED-BATCH CULTURES

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A simple method for control of lactate accumulation in suspension cultures of Chinese Hamster Ovary (CHO) cells based on the culture's pH was developed. When glucose levels in culture reach a low level cells begin to take up lactic acid from the culture medium resulting in a rise in pH. We will describe a nutrient feeding method that has been optimized to deliver a concentrated glucose solution triggered by rising pH. Data will be presented which shows that this high-end pH-controlled delivery of glucose can dramatically reduce or eliminate the accumulation of lactate during the growth phase of a fed-batch CHO cell culture at both bench scale and large scale (2,500-liter). The method has proven applicable to the majority of CHO cell lines producing monoclonal antibodies and other therapeutic proteins. Using this technology to enhance a 12-day fed-batch process that already incorporated very high initial cell densities and highly concentrated medium and feeds resulted in an approximate doubling of the final titers for eight cell lines. The increase in titer was due to additional cell growth and higher cell specific productivity.
The technology has been used to dramatically increase the productivity for two processes producing two therapeutic proteins for clinical trials. Process performance in terms of increased cell growth, titer increases, and product quality will be presented for the improved processes for the two campaigns at the 2,500-liter scale and compared directly with the original processes previously run at the same scale.

P4.42. ANALYTICAL CHEMISTRY APPROACHES TO MODERN PROCESS DEVELOPMENT CHALLENGES

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Analytical chemistry has been employed in cell culture process development for decades. The advent of superior instrumentation and interrogating samples using multiple methodologies has changed the landscape for what can be detected or predicted. In this presentation, we will discuss three new capability areas: 1) the ability to link chemical profiles of cell culture media samples with biological performance; 2) the ability to evaluate accelerated stability studies of raw materials and formulations; and 3) the ability to create protein expression fingerprints for high producing CHO cell lines. Chemical profiling of media uses the orthogonal platforms of liquid chromatography-mass spectrometry (LC-MS), nuclear magnetic resonance spectrometry (NMR) and Fourier transform infrared spectrometry (FTIR). Differences in chemical composition of media samples are determined by direct analyses, employing the combination of LC-MS, NMR, FTIR and statistical evaluation. To demonstrate developed methodologies, a series of artificial chemically defined media lots were created with slight variations. Differences were noted and comparison of chemical profiles demonstrated direct correlation to biological performances. The ability to accelerate the testing, and analyses, of formulated media stability would allow for an efficient discovery of media component behavior that would normally take months to evaluate. Through a system of controlled heating, destabilization of media components were accelerated to a number of weeks. LC-MS analysis permitted the identification of media components that may be reactive or labile to degradation during long term storage. This high throughput analysis has application in the development of new media formulations that have required performance characteristics, including storage stability. Spent medium is a complex matrix of proteins, peptides and small molecules resulting from secretion or cell lysis. Despite the challenges of spent media analysis, new analytical methodologies enable discovery of unknown components evolved in cell growth, viability and recombinant protein quality. One developed method introduces a "click chemistry" handle to the O-linked glycans of secreted host cell proteins, allowing for their affinity purification and the direct monitoring of proteins in media that are reflective of the cellular condition (secreted proteins) and avoiding the large background of proteins occurring from cell lysis. Significant changes in this "secretome" were demonstrated to relate to expression titers of IgG in DG44 CHO clones.

P4.43. ACCELERATING PROCESS DEVELOPMENT THROUGH ANALYSIS OF CELL METABOLISM

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Network models of cell metabolism upgrade metabolomics data by enabling predictions of cell behavior from concentration time series of extracellular and/or intracellular metabolites measured in industrial fermentations. Model simulations can be used for rapid hypothesis testing, e.g. to evaluate the impact of changes in feeding (media composition/feed rates) on intracellular metabolism, growth, or (by)product formation. Identifying suitable metabolic target genes for engineering host cell lines represents another application area of such models.
Here, we illustrate this approach using the prediction of optimal media compositions for a CHO cell line employing a genome-scale CHO network model as example. Intracellular flux distributions were determined from concentration time-series of extracellular metabolites during a fermentation run. Distinct optimal media compositions matching the observed nutrient demand of the clone were computed for different phases of the fermentation through stationary and dynamic model simulations on high-performance computing clusters. The optimized media were implemented in a fed-batch process using two continuous feed streams and resulted in a 50% increase of final product titer and in reduced ammonium release.
The combination of metabolomics data and network models not only improves our quantitative understanding of cell physiology, but can also support and accelerate multiple steps of rational process development strategies:

- Tailor media compositions to specific clones and curtail the time and experimental effort required for medium optimization compared to standard DoE techniques
- Identify highly productive and robust clones for scale-up during selection at small scales
- Devise cell line engineering strategies for overcoming metabolic bottlenecks in cell growth and product formation
- Employ metabolic network models for controlling feed additions at the production scale

This methodology is readily transferable to other cell lines.

P4.44. IMPROVED FERMENTATION PROCESS AND STRAIN DEVELOPMENT USING HIGH-PERFORMANCE LABEL-FREE LC-MS QUANTIFICATION FOR BIOPROCESS ANALYTICS

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The development of bioprocesses is time and resource intensive and the impact on product quality and economic value is highly important. A key to optimized and faster development are new analytic methods which do support process understanding beyond conventional macroscopic process parameters such as pH and temperature.
Here, we employ a dedicated label-free LC-Electrospray mass spectrometry based (LC-MS) protein quantification strategy (Q-ProM) for fast and time-resolved quantitative analysis of proteins during fermentation. This allows to study process parameter influence on fermentation with different conditions in time resolved manner and on protein level.
As an example, the effect of changing pH during fermentation was analysed in a CHO based Antibody production using Q-ProM technology. The results show, that the central enzymes of cellular metabolism and production can be identified and quantified over several time points during fermentation. This forms a basis for tracking the influence of process parameter changes at the molecular level. Clustering of protein regulation allows detection of co-regulated proteins pointing to dependencies within the intracellular regulatory network.
Besides identifying and quantifying hundreds of proteins in fermentation samples over several time points the technology was used also to monitor the product (antibody) quantity at several time points. Using the presented quantification approach, product quality parameters (e.g. glycosylation) may be directly and quantitatively accessible during fermentation as well.
The label-free Quantification method is also used to monitor the effect of stepwise strain development in yeast based production. As expected, developed strains show major quantitative changes in the enzymes of the engineered production pathway. Further quantitative differences on the protein level are detected. This can give further insight into production capabilities and optimization potential of the strains.

P4.45. INFLUENCE OF CULTURE CONDITIONS ON THE KINETICS AND METABOLISM OF SUSPENDED HeLa CELLS

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In the past few years, HeLa cells have been employed for many purposes such as vaccine and recombinant protein production, cancer research and citotoxicity assays. Nevertheless, information on process variable influence on cell growth and metabolism is still scarce.
In this study, the effect of dissolved oxygen level (pO_2: 5 to 50%), and initial concentrations of glutamine (0.175 to 1.05 g/L) and glucose (2 to 16 g/L) on growth and metabolism of a HeLa cells adapted to suspension cultivation and serum free medium (Hyclone CDM4CHO) was evaluated. Runs were carried out in a 2L bubble-free bench bioreactor, operated in batch mode, with automatic control of pH (7.4), temperature (37 ^{o}C) and pO_2 (variable).
For most conditions tested, cell growth and viability were limited by glucose, and glutamine starvation proved to have no influence on exponential phase. No significant inhibitory effects by ammonia or lactate were observed for these runs, except for higher glucose initial concentration where lactate apparently inhibits cell growth.
Optimum cell growth was achieved at 30% pO_2, which was up to 34% higher in comparison to other condition tested. Apparently, this behavior was a consequence of metabolic changes: data showed values up to 50% higher for glucose to cell yield factor ($Y_{X/GLC}$) and up to 17% higher for the maximum specific growth rate ($\mu_{X,MAX}$) for this condition.
For glutamine supplies superior to 0.35 g/L, $\mu_{X,MAX}$ values was unaffected (0.0241 h^{-1}), but for initial concentration of 0.175 g/L a significant decrease of 80% was observed. Maximum cell growth ($2.8x10^6$ cel/mL) were obtained for GLN_0 between 0.35 and 0.7 g/L, and were many times higher than values observed 0.175 g/L (88x) and 1.05 g/L (1.6x). The poor performance of the system at lower GLN_0 was apparently due to a higher inhibitors formation (lactate and ammonium).

P4.46. STEADY STATE AND DYNAMIC CONTROL PERFORMANCE OF THE AMBR™ AUTOMATED MICRO BIOREACTOR SYSTEM IN A CHO CELL BATCH CULTURE

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Implementation of high throughput automated systems is recognised as a valuable approach in the field of bioprocess development, with broad acceptance that an efficient multi-parallel microscale bioreactor will become an important enabling technology. A standardised micro bioreactor system can unlock development bottlenecks in a variety of common bioprocess applications, such as cell line screening, media development, feed and process optimisation, QbD and DoE studies.
In this study we investigate the process control performance of the ambr™ micro bioreactor system, using 24 disposable micro bioreactors in parallel for a combination of steady state and dynamic control tests. We examine process control performance for three key process parameters (seeding cell density, pH, DO) and review cell count and glucose concentration profiles. Dynamic control tests challenge the capability of the ambr system to deliver common industrial process requirements such as pH and temperature shifts.
The results demonstrate the capability of the ambr system to support a range of typical steady state and dynamic control requirements for mammalian cell culture processes. The accurate and precise automated liquid handling and process control systems result in very low culture variation between replicate bioreactor conditions. Clear resolution in culture response (cell count, metabolism) is observed between different test conditions. The combination of tight process control performance, low replicate variation and high resolution between test results, illustrates the suitability of the ambr micro bioreactor system for large numbers of parallel bioreactor tests in a wide range of cell culture applications.
For questions about this poster, please contact barney.zoro@tapbiosystems.com

P4.47. EXPRESSION AND PURIFICATION OF TGF-ß-1 IN CHO-CELLS

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The development of chemically well defined media is a demanding task in order to create the optimal conditions for an in vitro stem cell (SC) proliferation and differentiation system. Signals that govern SC differentiation into multiple mature cell types are provided by growth factors, namely cytokines.
TGF-beta regulates a plethora of biological processes - including cell differentiation and proliferation, embryonic development, apoptosis and immun responses - in concert with cell surface receptors and signal tranduction molecules within the cell. The whole signal transduction pathway leads to the specific expression of distinct proteins.
This work aims at the optimization of the purification process of a fragment of the transforming growth factor-ß-1 (TGF-ß-1) expressed in Chinese Hamster Ovary cells (CHO) grown in serum free Pro CHO-5 medium.
Our TGF-ß-1 fragment (A280 - S391) is a secreted glycoprotein with a calculated molecular weight of 12794.6 Da. The transfection of TGF-ß-1 was performed using the transfection reagent IL 2 SP. The protein contains also a His-tag to simplify the purification of the protein.
For detection of the target protein in the supernatant we used Western-Blot. Intracellular protein was analyzed by flow cytometry. After detection of the protein different affinity chromatography methods were applied to optimize protein purification with HPLC.

P4.48. PHYSIOLOGICAL RESPONSE OF CHO CELLS CULTIVATED IN CONTINUOUS BIOREACTOR AT VARIOUS AGITATION RATES

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Mammalian cells are known to be sensitive to hydrodynamic stresses. Therefore, "soft" agitation and aeration are generally recommended in culture bioreactor to prevent cell damages. Nevertheless, at the industrial scale, this may induce CO_2 accumulation, high mixing times, poor air dispersion and concentration gradients. The aim of this study was to jointly examine the hydrodynamics of the bioreactor and the physiological response of CHO cells cultivated under increasing agitation, in order to determine the agitation thresholds tolerated by cells.
CHO 320 cells were cultivated in continuous mode in a 2 L stirred bioreactor equipped with a 4-pitched blade turbine and a porous sparger. Cell physiological response was characterized by kinetics of cell growth, cell death, substrate consumption and metabolite production and, by the identification of the specific mechanisms involved in cell death. Several continuous cultures were carried out during more than 900 hours to study the response of cells to agitation steps, with a particular focus on the effects of the medium composition. To do this, the medium used during the culture process was supplemented with two concentrations of glutamine, 6 and 12 mM. In addition, the hydrodynamics of the culture bioreactor was numerically simulated using Computational Fluid Dynamics (CFD) to predict power dissipation and velocity fields at each agitation rate.
Under glutamine limitation, an agitation rate step from 0.32 $W.kg^{-1}$ (300 rpm) to 2.5 $W.kg^{-1}$ (600 rpm) was applied to cells, when the steady state of cell density was reached. As a result, an intense necrosis and apoptosis phenomena occurred. The cell death followed a zero-order kinetics compared to cell concentration. After the power dissipation was stepped back to 0.32 $W.kg^{-1}$, a second step to 2.5 $W.kg^{-1}$ on the same culture resulted in the same cellular response. Such fast cell death at 2.5 $W.kg^{-1}$ was not observed in a batch culture performed with the same power dissipation, but without glutamine limitation. So, these results suggested that cells subjected to a glutamine limitation were more sensitive to hydrodynamic stress that cells supplied with an excess of this substrate, as observed during the batch phase preceding the steady state of continuous culture. This hypothesis was strengthened by the fact that cells exhibited an increased resistance even to higher dissipation rate (5.6 $W.kg^{-1}$ or 800 rpm) when cells were cultivated in continuous mode without glutamine limitation.
Finally, cells grown under glutamine excess and high dissipation rate during the preliminary batch phase (2.5 $W.kg^{-1}$) appeared to be resistant to higher dissipations (11 $W.kg^{-1}$ or 1000 rpm), compared to those initially grown under glutamine starvation and lower dissipation (0.32 $W.kg^{-1}$). Indeed, in this culture, only cell death by lysis (violent break-up of the cell membrane) was observed while neither necrosis nor apoptosis were significant.
Apoptosis seems to be a physiological response of CHO cells cultivated under glutamine limitation and hydrodynamic stresses in continuous bioreactor and a glutamine excess may improve cellular resistance to high agitation rates (up to 1000 rpm). Moreover such resistance capability seems to be strengthened when high agitation rates were applied to the cell culture during the exponential cell grown phase, suggesting a potential adaptation of cells.

P4.49. OUR COMPARATIVE ANALYSIS OF SINGLE USE BIOREACTORS AND UNDERSTANDING THE MOST EFFECTIVE IMPLEMENTATION

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Single-use bioreactors are now commonly used for seeding stainless steel bioreactors or for producing material. The profitability of these equipments has been well demonstrated on more that decade. But only a few data on their scalability are published.

In 2010-2011, Merck Serono Biodevelopment performed a study in order to evaluate the performances of disposable bioreactors. As different technologies were available, this study compared performances of several types of mixing in single-use bioreactors. The evaluation was performed both for seeding application and for clinical material production.

The feature of this study is the use of 3-250L disposable bioreactors with gas flow rate scaled-down from seeding and production bioreactors.

A fed-batch process producing a highly glycosylated molecule was performed in 6 different types of disposable bioreactors. The quality of the molecule together with the molecule titer and the cell growth was compared between the 6 single use technologies. These process performances were also compared to 250L and 1.25kL bioreactors and to a 3.6L glass development bioreactor. This study was completed by a characterization of liquid/liquid and gas/liquid transfers inside each disposable bioreactor in order to estimate their potential in terms of cell culture. An evaluation grid was applied to choose the best disposable bioreactors. All these comparisons allowed Merck Serono Biodevelopment to conclude on disposable bioreactors uses and on the scalability (up and down) of these disposable systems

P4.50. IMPROVEMENT OF CELL-FREEZING TECHNOLOGIES ALLOW TO PERFORM FULLY CLOSED PROCESS

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Cell culture from one vial to containers such as T-flasks or shake flasks is usually an open phase. This critical step may take several days or even weeks and thus can delay production timeline due to contamination. By coupling traditional technologies (bags) and new technologies (NovaCase®, Disposable bioreactor and Aseptic Transfer Cap), Merck Serono Biodevelopment succeeded in freezing mammalian cells in bags with direct thawing in closed cell culture containers. A study on bags and freezing conditions was performed. The best parameters have been applied to different cell lines and media. With this technique, cell amplification and cross contamination were reduced.

P4.51. COST OF GOODS REDUCTION IN COMMERCIAL BIOPHARMACEUTICAL PRODUCTION - A COST EFFECTIVE HARVEST FILTRATION SETUP

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Current clarification of harvest requires an average of 28 filter housings for a 60-day fermentation process, each containing 5 membrane filter candles. An evaluation of the current harvest clarification costs shows that the membrane filters alone are responsible for 64% of the total harvest filtration costs. To reduce the harvest filtration costs, alternative clarification systems were investigated. Six vendors were invited to propose a cost effective harvest filtration setup.
Three of the six proposed alternatives were chosen for further investigation on small scale and the alternatives were based on depth filtration and the use of disposable filters. The poster describes the evaluation of the filter capacity using a Pmax versus Vmax analysis. In addition, the filtration efficiency is evaluated for the three alternatives.
The filter capacity shows an increases with a factor 11.8 for depth filtration with respect to the current capacity (capacity = the amount of harvest that can be filtered). This results in a significant reduction for the number of filter change-outs (from 28 to 5) for a 60-day fermenter batch. Cost savings were evaluated for the disposable options versus the semi-disposable alternatives in terms of labour, materials, assembly and harvest hold-up.

P4.52. PLATFORM PROCESS FOR PRODUCTION OF MONOCLONAL ANTIBODIES FOR RESEARCH PURPOSES

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In 2006 Novo Nordisk decided to invest in building a pipeline within inflammatory disease management. To support this strategy the cell culture units had to establish technology for expression and production of monoclonal antibodies in CHO cells. A number of technology providers at that time offered proven platform processes for this purpose. It was decided to in-license one of these technology platforms, the one developed at Lonza Biologics, and focus research resources on product innovation rather than development of an in-house production system. The platform has now been fully implemented and the work flow optimised and standardised. A proven generic task list and timeline now allow us to provide very reliable lead time estimates for delivery of cell lines and research batches of monoclonal antibodies to the projects.
The platform process has been applied for cell line development and antibody production for R&D purposes for five years. During this period 11 monoclonal antibodies have been transferred from laboratory scale to pilot plant production. The performance of the process platform across projects has been reviewed and benchmarked against the industry standard. An improvement option: Upgrade of the medium, feeds and process protocols offered by Lonza Biologics, has been identified. A β-version of the latest process from Lonza has been tested and compared to the previous version in a study including five cell lines. The study results indicate that significant improvements can be achieved.

P4.53. FURTHER OPTIMIZATION OF CELL CULTURE PRODUCTION PROCESSES – WHERE DO WE GO NEXT?

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The drive for more efficiency and cost reduction in the development and manufacturing of recombinant protein drugs has resulted in significant progress towards speed of development, improved productivity and better control of product quality aspects of cell culture processes. A push towards process intensification has enabled multiple teams over the last 5 years or so to demonstrated the possibility to achieve antibody titers in the 10 g/L range using conventional fed-batch technology – something many of us would have thought to be impossible maybe as recent as 10 years ago!
Beyond the improvements in titer and volumetric productivity much focus nowadays is on aspects of process automation and control, disposable reactor systems and flexible manufacturing facilities. A combination of these elements – an intensified culture process that is controlled by internal sensors and conducted in disposable reactor equipment maybe what the culture process of the not too far future will look like.
At Biogen Idec we have done work in these areas to be ready for the next quantum leap in process efficiency. The presentation will lay out the elements under development including process intensification, evaluation of disposable reactor equipment and development of MIR based process control. Results presented will include the approach to an intensified high productivity process platform (doubling productivity in a CD process format), exploration of a high titer process in a 1000L disposable reactor configuration (yes, 8 g/L is possible) and the development of a Raman Spectroscopy based process control system that we expect to allow process control with elimination of the need for process sampling and off-line analysis in the near future.

P4.54. THE EFFECTS OF INOCULUM DEVELOPMENT ON PILOT SCALE BIOREACTORS

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The quality of the inoculum can have a significant impact on the performance of a Bioreactor at the large scale of operation. Differences in growth characteristics of selected clones can also greatly influence production potential at pilot scale. A case study is presented where the inoculum was seen to have a significant impact on cell growth and productivity at the Pilot Plant scale of operation. Troubleshooting identified the inoculum quality as having a major impact on the process at scale. Data will be presented showing the critical steps identified during the development of the inoculum. We will also present data showing the effects on growth and productivity at different scales of operation and with different inoculum development strategies.

P4.55. PRODUCTION AND PRODUCT CHARACTERIZATION OF THERAPEUTIC PROTEINS USING THE NEW SINGLE-USE BAYSHAKE® FERMENTATION TECHNOLOGY FOR THE CULTIVATION OF MAMMALIAN CELLS

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Single-use technologies play an increasingly important role in manufacturing of therapeutic proteins. They offer big advantages compared to conventional process technology in stainless-steel vessels and reactors housed in expensive, highly automated production facilities. The biggest benefits expected by single-use technologies are shorter lead times for plant investment, less capital invest cost and time savings for utilities including sterilization, cleaning and cleaning validation, higher flexibility and speed of product changeover in biopharma plants operated under cGMP. On the other hand, aspects like equipment robustness and reliability and comparability of therapeutic protein "products by process" need to be considered carefully.
This presentation gives an overview of the ongoing development of an innovative, single-use bioreactor system for the cultivation of mammalian cells, BaySHAKE (TM), which operates without integrated agitator and sparging. This system excels by extreme simplicity, i.e. low cost of disposables, full scalability in a range of fermenter working volumes from liters to cubic meters and a high flexibility in terms of building space consumption. The system has been developed in a partnership between Bayer Technology Services (process technology), Bayer Schering Pharma (biological evaluation) and Sartorius Stedim Biotech (single-use components). Developmental fed-batch fermentations of therapeutic monoclonal antibodies and MoAb-derivatives have shown excellent performance and scalability of the BaySHAKE (TM) up to 1000 L fermenter volume without compromising product quality compared to fermentations using standard agitated and sparged stainless steel systems. In addition to basic fermentation parameters product characterization results including glycosylation of the purified protein will be shown which prove excellent comparability.

P4.56. ON-LINE MONITORING OF MAMMALIAN CELL CULTURE USING SINGLE PHASE AND SCANNING CAPACITANCE MEASUREMENT

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Capacitance measurement is generally regarded as the most reliable and robust method for the online measurement of viable biomass during fermentation and cell culture. Performed at a single frequency this method uses the fact that under the influence of an electric field cells with intact cell membranes are polarised and act as tiny capacitors. Therefore permittivity measurements correlate well with the biovolume enclosed by the cell membrane and thus the viable cell concentration.

However, in principle this method seems to hold more potential than has been exploited up to now, especially when 'capacitance spectra' are evaluated with advanced mathematical and statistical methods. This presentation will show that by increasing the number of scan frequencies from one to twenty-five (0.1 MHz to 20 MHz) additional information on the physiological state of the cells in culture can be generated. The generation of this type of process information increases process understanding and assists in the scale-up and technology transfer of bioprocesses.

P4.57. ELECTRICAL RESISTANCE TOMOGRAPHY AS A TOOL FOR ASSESSING TRADITIONAL AND DISPOSABLE STIRRED TANK MIXING PERFORMANCE

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Fluid mixing is an important parameter in the design of bioprocesses. Mixing time contains useful information about flow and mixing within the vessel and can be important for successful process scale-up and scale-down. The mixing time, T_m, denotes the time required for the tank composition to achieve a specified level of homogeneity following addition of a tracer pulse at a single point in the vessel.

Normally mixing times are calculated by using point-conductivity probes in different areas of the vessel and using these to determine when mixing is complete. This presentation will show mixing data from traditional glass and SIP bioreactors and HyClone SUB's that shows by using linear electrical resistance tomography (ERT) the mixing performance of the bioreactor, under gassed and non-gassed conditions, can be visualized across the entire cross-section of the bioreactor meaning mixing can be assessed much more effectively across all zones of the bioreactor.

P4.58. ENGINEERING CHARACTERISATION OF SINGLE-USE, WAVE-TYPE BIOREACTORS AND SCALE-UP CONSIDERATIONS FOR EARLY PHASE CELL CULTURE PROCESS DEVELOPMENT

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Single-use wave-type bioreactor systems have gained increasing popularity for application in industrial cell-culture processes in the last decade. The combined benefits of these systems including increased flexibility, increased experimental throughput and cost savings, together with process monitoring capability (pH and dissolved oxygen), makes them highly attractive for cell culture. Mixing and mass transfer is achieved by varying the one dimensional rocking motion and rocking angle; this induces a wave-like motion of the liquid in the chamber enhancing bulk mixing and oxygen transfer. Given the unique mode of operation of wave-mixed bioreactors, engineering characterisation of these systems is essential in order to better understand the mixing mechanisms and to enable comparison with other bioreactor geometries such as stirred tanks and shaken systems (microwell and shake flasks).
In this work we apply engineering characterisation of wave-mixed bioreactors for the selection of an appropriate scale-up methodology and apply the selected approach in parallel cultivations performed in shaken microwells, wave and stirred tank bioreactor systems. The 2L (1L working volume) BIOSTAT CultiBag system has been characterised in terms of mixing time, T_m, using the iodine decolourisation method. The bioreactor mixing times have been evaluated as function of rocking rate and rocking angle, suitable for cell culture operations. Mixing times as low as 2.3s can be achieved. Subsequent scale-up studies from microwell (24-standard round well, 24-SRW) (850 µl) scale to wave-bioreactor (1 L) at matched mixing times of ~5s were performed for the growth and productivity of industrially relevant GS-CHO (Glutamine Synthetase-Chinese Hamster Ovary) cell line in fed-batch mode. Initial results have shown good agreement of peak viable cell densities of greater than 10×10^6 cells mL^{-1} in both 24-SRW plates and wave-bioreactor. Final titres of whole IgG, and metabolite profiles were also highly comparable, thus demonstrating the potential for mixing time to be a viable basis for scale-up across different vessel geometries. The methods developed in this work are also currently being applied for viral vaccine production.

P4.59. CHARACTERISATION OF GAS TRANSFER PROPERTIES IN SHAKE FLASKS USING DISPOSABLE PH AND DISSOLVED OXYGEN SENSORS AND THEIR APPLICATION IN MAMMALIAN HIGH CELL DENSITY CULTURES

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Oxygen transfer rates have been systematically analysed in shake flasks at various shaking frequencies and with different liquid volumes. Even at low shaking frequencies and high liquid volumes oxygen transfer was shown to be sufficient to compensate for the demand of mammalian cell cultures. The suitability of shake flasks for the cultivation of mammalian cells at high cell concentrations has been tested. Cell densities increased $300x10^5$ viable cells/ml with high viability in standard batch cultivations of CHO cells. Even this amount of cells could be sufficiently supplied with oxygen at modest shaking frequencies. In addition to the partial pressure of oxygen, the pH value has been monitored over time and its shift towards more acidic conditions has been analysed, especially at high cell concentrations. The long term stability of both, pH and dissolved oxygen signal were evaluated.
Pre-sterilised shake flasks with sensor patches containing fluorescent dyes, sensitive to dissolved oxygen and proton concentrations, were used for this study. The read out unit underneath the shake flasks guided light for excitation and the emitted fluorescence signal by optical fibres to the amplifier (SENSOLUX system, Sartorius Stedim Biotech, Göttingen, Germany). Results of the relevant physical parameters of the shake flasks as well as the culture conditions and growth curves will be presented.

P4.60. LARGE PILOT SCALE CULTIVATION PROCESS STUDY OF ADHERENT MDBK CELLS FOR PORCINE INFLUENZA A VIRUS PROPAGATION USING A NOVEL DISPOSABLE STIRRED-TANK BIOREACTOR

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Influenza is one of the major viral diseases and has tremendous importance for human and animal health all over the world. Pigs are susceptible for a number of Influenza A viruses from different species (human, avian, porcine); in the case of multiple infections of swine new and dangerous virus strains can be assembled by reassortment of genes (antigenic shift). The strain which was used in this study is part of a trivalent inactivated vaccine against porcine Influenza (IDT Biologika GmbH). The cultivation of Influenza virus for the production of vaccines is often still done in embryonated hens eggs, although there are a number of cell lines available. Egg production processes are limited in terms of capacity whereas cell culture based processes are much more flexible and easy to scale up when facing pandemic situations which can not be foreseen (examples: H5N1 occurrence in 2005 and H1N1 pandemic strain occurrence in 2009/10). Currently adherent Madin-Darby bovine kidney (MDBK) cells are used for the production of porcine Influenza A viruses at commercial scale (IDT Biologika GmbH). The main goal of this study was to explore options for direct transfer of this process into a novel disposable stirred-tank bioreactor (Biostat Cultibag STR, Sartorius Stedim Biotech GmbH) using microcarriers for the attachment of adherent MDBK cells. The advantage of this system is its scalability (up to 1000 l with similar reactor geometry; thus known scale up factors), better process control and presumably higher virus yields.
The system was set up with 35 l wv using a serum containing minimal medium. Seeding cell number was 2.0×10^5 cells/ml and a microcarrier concentration of 2 g/l Cytodex 1 was used. Analytics for cell counting, flow cytometric assays, metabolism and virus titres was performed. At first analytics after 24 h post seeding cells showed a good distribution on the microcarriers, also the cell morphology was typical for MDBK cells on microcarriers. Cell number increased up to a maximum of 2.13×10^6 cells/ml with a viability of 92 % after 120 hours. The viral infection was started at that time and proceeded very rapidly with first signs of cytopathic effect (CPE) after 24 h p. i., leading to virus yields of maximum log 3.0 HA units and $10^{8.00}$ $TCID_{50}$/ml after 48 h post infection. The virus yields were 2-4-fold higher compared to standard roller bottle cultivations. For a non optimized process, these results are very promising and sufficient for vaccine production. Very likely, by finetuning of infection parameters even higher titers are achievable. One of the most important advantages of a bioreactor system is its controlled surrounding for the cells in terms of pH and oxygen level. In this study that fact was proven very well by the good performance of the control system. Temperature and stirrer speed control was not a problem throughout cultivation, also control of pH value at 7.40 and oxygen level at > 40 % pO_2 worked very well. The results of this study prove a successful tech transfer for a porcine Influenza A virus production process from roller bottles into a novel disposable STR bioreactor system with advances in both cell and virus yields and process control possibilities. These are important factors in near future, keeping upstream processing competitive in terms of prices, productivity and surely also from a regulatory point of view. It is a further step in order to be prepared for pandemic situations as it was seen for 2009 H1N1 occurrence. There should be no obstacles for the implementation of such systems into GMP surrounding for vaccine production purposes. As result of the study also a lot of data were generated that can be used for establishment of descriptive mathematical models for a deeper understanding of vaccine production processes.

P4.61. CHARACTERIZATION AND PERFORMANCE OF THE MOBIUS® CELLREADY 250L BIOREACTOR SYSTEM: THE NEXT GENERATION OF SINGLE-USE BIOREACTORS

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Successful bioprocessing relies on the ability to accurately and effectively monitor and control critical process parameters. The Mobius CellReady 250L single-use bioreactor is uniquely designed with the novel SenorReady technology that enables configurable, flexible and functional monitoring and control. Bioreactor characterization properties such as mixing time, volumetric mass transfer capabilities, temperature mapping and power input define the process design space wherein accurate and effective monitoring and control can occur. Each of the aforementioned characterization properties were explored and evaluated for the Mobius CellReady 250L single-use bioreactor system. Mixing studies were performed using both conductivity and pH response curves. Mass transfer capabilities were studied using the static gassing out method for determining the volumetric mass transfer coefficient (k_La) for oxygen and by the demonstration of CO_2 stripping in live cell culture material using the microsparger and open pipe sparger, respectively. Heating and cooling capabilities were measured directly using inserted probes; power input was determined based on the wattages measured within the agitator motor box over varying agitation rates. In addition to physical characterization, cell culture capabilities were demonstrated with the execution of a cell culture process that achieves a peak cell density of 20e6 cells/mL. The results of these studies support that the Mobius CellReady 250L single-use bioreactor system is capable of supporting a wide range of biomanufacturing processes.

P4.62. NOVEL, ROTARY OSCILLATED, SINGLE-USE BAYSHAKE® BIOREACTOR FOR THE CULTIVATION OF MAMMALIAN CELLS

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Single-use technologies play an increasingly important role in manufacturing of therapeutic proteins. They offer big advantages compared to conventional process technology in stainless-steel vessels and reactors housed in expensive, highly automated production facilities. The biggest benefits expected by single-use technologies are shorter lead times for plant investment, less capital invest cost and time savings for utilities including sterilization, cleaning and cleaning validation, higher flexibility and speed of product changeover in biopharma plants operated under cGMP. On the other hand, aspects like equipment robustness and reliability and comparability of therapeutic protein "products by process" need to be considered carefully.
The poster gives an overview of the ongoing development of an innovative, single-use bioreactor system for the cultivation of mammalian cells, BaySHAKE®. The system has been developed in a partnership between Bayer Technology Services (engineering and process technology), Bayer Schering Pharma (biological evaluation) and Sartorius Stedim Biotech (single-use components and control units).
The BaySHAKE® bioreactor is agitated by the rotary oscillation of the bag itself to induce a fluid flow suited for efficient mixing and surface aeration. The hydrodynamic characterization of mass transfer, particle stress and mixing properties of the BaySHAKE® system shows linear scalability in a range of working volumes from 30 L to cubic meter. The system excels by its low shear performance, its simplicity caused by absence of internals and the ability to avoid foam formation. The fed-batch process may be started at low filling levels of only 5 - 10 % final working volume offering enhanced flexibility and a reduced no. of seeding steps. Developmental fed-batch fermentations of therapeutic mAb's and its derivatives have shown comparable performance of fermentation and product quality parameters (e.g. growth, viability, productivity, gycosylation) to fermentations using standard agitated and sparged stainless steel systems up to the 1 m^3 working volume.

P4.63. EVALUATION OF A NEW DISPOSABLE 200 L STIRRED TANK BIOREACTOR EQUIPPED WITH DISPOSABLE SENSORS FOR PH AND DO FOR THE CULTIVATION OF CHO-CELLS

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Disposable bioreactors are increasingly gaining acceptance for cell culture applications due to a number of advantages including ease of use and reduced labour costs, reduced requirement for utilities such as steam and purified water, no need for cleaning and cleaning validation and reduced clean room footprint. Integrating disposable sensors for pH and DO monitoring into the bioreactor bag are expected to reduce the risk of contamination due to manipulation during introduction of conventional electrodes.
We have evaluated a disposable 200 L bioreactor (BIOSTAT CultiBag STR 200L, Sartorius-Stedim Biotech, Göttingen, Germany) equipped with disposable optical sensors for DO and pH for suspension cultivation of CHO cells in a protein free medium. Agitation and aeration of the culture was performed by a pre-installed magnetic driven stirrer with two impellers and a ring-sparger, respectively. The signal quality and accuracy of the optical sensors for pH and D0 was monitored by additionally installed conventional probes. Each cultivation was carried out as a batch process with feeding over 7 days. The results are comparable to previous results obtained in a stainless steel stirred tank bioreactor. Besides data on cell growth and metabolism, we will present a detailed comparison of the results obtained with the two different types of sensor probes (optical and conventional).

P4.64. ENGINEERING CHARACTERISATION OF A SINGLE-USE PNEUMATIC BIOREACTOR FOR CELL CULTURE BY EXPERIMENTAL FLUID DYNAMICS STUDIES

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Single use technology is becoming increasingly popular in biopharmaceutical manufacturing, due to its benefits of low capital cost, manufacturing flexibility and reduced validation costs. These single-use devices include bags for storage, mixers, filter systems and bioreactors and are now widely used in R&D, pre- and clinical sample production and biomanufacturing sectors. A novel single-use bioreactor system has been recently developed by PBS Biotech (www.pbsbiotech.com) in the US by utilizing a pneumatic mixing mechanism which converts the buoyancy of inlet gas bubbles to a rotational energy by an air-wheel™. This technology achieves fast and homogeneous liquid mixing with low shear stress and high mass transfer rates without requiring an external mechanical device. Consequently, the pneumatic mixing mechanism can offer a simple, compact and scaleable bioreactor system. Cell culture performance has been studied using CHO cell lines expressing recombinant monoclonal antibodies and it has been compared to conventional laboratory-scale stirred bioreactor results. However a deeper understanding of the mixing and the shear conditions that cells experience inside the bioreactor is necessary to rationally define scale-up parameters and validate its use to inform larger scales of operation. Particle Image Velocimetry (PIV) is a non-intrusive technique to measure 2-dimensional velocity profiles by image visualization and allows studies of mixing and shear within transparent geometries. Experimental fluid dynamics studies in the PBS bioreactor are ongoing to quantify the shear stresses cells are subjected to during culture and to evaluate the size distribution of the air bubbles produced by the built-in microsparger. The results obtained will also provide valuable information on the suspension of microcarriers' type particles and on their local concentration profiles within the pneumatic bioreactor. Comparisons of shear stress, mixing effectiveness and gas phase distribution with a laboratory-scale conventional bioreactor for cell culture will be presented.

P4.65. UTILITY OF THE MOBIUS® CELLREADY 3L SINGLE-USE BIOREACTOR FOR UPSTREAM PROCESS DEVELOPMENT APPLICATIONS

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Single-use processing solutions have become an important trend in the biopharmaceutical industry spanning both upstream and downstream applications. The advantages of the single-use approach to industrial cell culture versus traditional stainless steel and/or glass bioreactors has resulted in the recent commercialization of several single-use bioreactors (SUBs) at small (3L-15L), intermediate (50-500L) and large scales (>1000L). EMD Millipore has recently introduced the Mobius CellReady 3L bioreactor, a SUB that shares the features of conventional bioreactors but offering ease-of-use benefits associated with single-use technology that was developed for the optimization of mammalian cell growth and expression. Detailed characterization of this vessel, both in terms of function for CHO cell growth and expression, and volumetric mass transfer coefficients (k_La) for oxygen, as compared to glass bioreactors was performed. The results demonstrate similar cell culture performance and k_La values. Based on these results, we have been able to streamline our cell culture work by using the 3L CellReady bioreactors to scale up inoculum (i.e., seed-train) for large scale bioreactor experiments. By pooling the cultures from 2-3 small scale single-use bioreactors, sufficient biomass was readily achieved to inoculate directly into the 250L CellReady Bioreactor. There, a 40L n-1 step preceded the 200L production experiments. Subsequently, the 3L CellReady bioreactors were used to monitor performance of the 200L large-scale cell culture experiments as satellite bioreactors. Tracking of cell culture performance in this fashion was both effective and convenient. In addition to its use in process characterization and development work, this study demonstrated that the small size and portability of the 3L CellReady bioreactor make it an ideal platform for both seed-train and satellite applications.

P4.66. HIGH CELL DENSITY GROWTH OF HIGH FIVE SUSPENSION CELLS IN DO-CONTROLLED WAVE-MIXED BIOREACTORS

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Insect cells such as High Five cells used in the manufacture of biopharmaceuticals are best grown in wave-mixed bioreactors (1). This is due to the continual blending of foam with the culture medium which results from the wave-induced mixing and permanent renewal of the medium surface. Even the addition of an antifoam agent is not required.
Process conditions which ensure maximum High Five cell densities and which have been reported to be about 8×10^6 cells x mL^{-1} (2) were determined in Biostat CultiBag RM50 optical experiments for batch mode and 1 L culture volume. Seed inoculum for these experiments was generated in single-use shake flasks (Corning) incubated in an Infors`Multitron shaker (27°C, 100 rpm, 25 mm shaking diameter). Biostat CultiBag RM50 optical was controlled in four different modes: non-pH- and non-DO-controlled, DO- controlled, pH-controlled, DO- as well as pH-controlled. The DO level was guaranteed by increasing the rocking rate up to 28 rpm and, if required by addition of pure oxygen. In process control was supplemented with off-line analyses of cell density, viability, metabolites (glucose, glutamine, glutamate, lactate, ammonium) and pH.
While the influence of the type of bioreactor`s control on the maximum growth rate (0.041-0.044 h^{-1}) and doubling time (15.6-17.7 h) was negligible, maximum cell densities were achieved with DO regulation (set point 50%). Maximum cell densities ranged between 8.2 and 9.4×10^6 cells x mL^{-1} and represent the highest values described for High Five cells so far in the literature. They are 35% higher compared to those seen in pH-controlled and non-controlled experiments. Controlling both DO and pH level did not lead to any further improvement of cell growth i.e. the range of growth parameter values was the same as that observed in the previous experiments. For High Five cell-based biopharmaceuticals this knowledge enables optimized seed inoculum/seed train production in wave-mixed bag bioreactors.
[1] Werner, S. et al. (2010) Innovative, Non-stirred Bioreactors in Scales from Millilitres up to 1000 Liters for Suspension Cultures of Cells using Disposable Bags and Containers - A Swiss Contribution, CHIMIA 64/11:819-823
[2] Rhiel, M., Mitchell-Logean, C.M. and Murhammer, D.W. (1997). Comparison of *Trichoplusia ni* BTI-Tn-5B1-4 (High Five™) and *Spodoptera frugiperda* Sf-9 Insect Cell Line Metabolism in Suspension Cultures, Biotechnology and Bioengineering 55/6:909-920

P4.67. CHARACTERIZATION AND OPTIMIZATION OF SINGLE USE BIOREACTORS FOR SEED TRAIN AND PROCESS DEVELOPMENT APPLICATIONS

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With the advent of single-use (disposable) bioreactors, downtime of bioreactors due to cleaning, assembly and sterilization can be reduced significantly in R&D and/or process suites. On the other hand, those bioreactors are preconfigured and sterilized by the supplier which allows only for few modification by the end-user. Therefore, care has to be taken during the design of the bioreactor that important process parameters, e.g. mixing time, k_La-value, are suitable for the application. Most existing disposable bioreactors for seed train and development purposes differ from the well-characterized, reusable stirred tank bioreactors and hamper the process transfer from development to process scale. A disposable bioreactor of 2 L working volume with a classical stirred-tank design was developed to facilitate this transfer.
The study shows the optimization of stirrer design and positioning with respect to mixing time and k_La-values at different gassing rates. Growth curves in disposable and reusable bioreactors show the applicability of the bioreactor for seed and development applications.

P4.68. VERO CELL GROWTH IN THE CELL-TAINER® DISPOSABLE BIOREACTOR

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Rapid and simple cell cultivation can currently be carried out using disposable bioreactors. Various reports have described growth of adherent cells in rocking-type bioreactors which rely on the rocking motion for both mixing and mass transfer. The CELL-tainer® (CELLution Biotech BV) disposable bioreactor is a rocking-type bioreactor which not only has vertical movement but horizontal displacement as well. Due to this 2 directional movement very high mass-transfer capacities can be reached. Mass transfer coefficients (k_La) from 100 h^{-1} to 700 h^{-1} have been described (Oosterhuis and van der Heiden 2010). Using the Design of Experiments (DoE) approach we have developed models for the mixing times in the CELL-tainer® and the BIOSTAT® CultiBag RM (Sartorius Stedim Biotech) bioreactor (standard rocking-type). Based on comparable mixing times, the settings for cultivation of Vero cells in the CELL-tainer® bioreactor were chosen.Vero cells growing adherent to Cytodex 1 microcarriers were cultivated in the CELL-tainer® and BIOSTAT® CultiBag RM. Cells were grown in animal component free (ACF) media. Both bioreactors were controlled with regard to T, pH and DO%. Vero cell growth in both bioreactors was comparable with respect to the growth characteristics and main metabolite production and consumption rates.
Conclusions: The applied strategy to select cultivation conditions for implementation of a new rocking-type bioreactor based on comparable mixing times was successful. The CELL-tainer® supports growth of Vero cells adherent to microcarriers comparable to conventional rocking-type bioreactors. At present these bioreactors are compared with respect to virus production performance.

References

Oosterhuis, N. M. G. and P. van der Heiden (2010). Mass Transfer in the CELL-tainer® Disposable Bioreactor. Cells and Culture. T. Noll, Springer Netherlands. **4:** 371-373.

P4.69. COMPARISON OF DIFFERENT SINGLE USE BIOREACTOR SYSTEMS AGAINST STANDARD GLASS VESSELS

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Single use bioreactor systems for mammalian cell cultures have gained more and more in significance during the last couple of years. However, the scale-up and feasibility is still questioned.
In our laboratory, we compared a standard fed-batch process in a conventional 5 L glass vessel with cultivations at 2.5 L scale in a stirred plastic tank and 150 L scale cultivation in a stirred bag system. We present data on growth, productivity and metabolism of the cells in the different systems. Furthermore, we will go into practicability of the handling of the systems and the scalability of the process.

P4.70. HIGH CELL DENSITY XD® CULTIVATION OF CHO CELLS IN THE 50 L CULTIBAG STR SINGLE USE BIOREACTOR WITH NOVEL MICRO-SPARGER AND DISPOSABLE EXHAUST COOLER

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Based on the existing Cultibag STR concept, Sartorius has developed a number of pivotal innovations that allow for safe and well controlled high cell density mammalian cultures in single-use stirred tank bioreactors.
First-of-all a single-use exhaust cooler has been designed that mitigates exhaust filter clogging. Secondly, a 150 µm drilled hole micro-sparger was developed to enhance mass transfer whilst maintaining acceptable power input and tip speeds.
To test the performance limits of the Cultibag STR equipped with the novel features, a very high cell density CHO XD® culture was performed in the Cultibag. XD® culture is a proprietary DSM technology that is characterized by an extended exponential growth phase resulting in a short process with extremely high viable cell densities and viabilities and a 3 - 10 fold titer boost.
Using a CHO based XD® process, during 9 days a maximum viable cell density of almost 90 mln cells/mL at a viability >98% was achieved in the Cultibag STR. Temperature, pressure, agitation, as well as oxygen partial pressure and pH control (using disposable probes) were well maintained to set point at these very high cell densities.
Despite an increasing gas flow rate, no exhaust filter clogging as indicated by a pressure increase was observed at all due to the exhaust cooler. Moreover, even at peak viable cell density, the oxygen transfer capacity of the newly developed micro-sparger had not reached its limit. A flow rate of 1,5l/min (0.03 vvm) pure oxygen was sufficient to maintain the DOT whilst no CO2 stripping was necessary.

P4.71. SCALE-UP OF HIGH CELL DENSITY CULTURES IN SINGLE-USE BIOREACTORS: SEAMLESS TECH TRANSFER AND SCALE-UP OF A HIGH CELL DENSITY PER.C6® FED-BATCH PROCESS TO 1000 L SUB SCALE AND SCALE-UP OF EXTREME CELL DENSITY CHO XD® CULTURE

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The use of disposable bioreactor systems has been a recent addition to the biopharmaceutical manufacturing sector with larger scales continually being introduced onto the market. This presentation will highlight the scalability of disposable Single Use Bioreactors from bench scale to 1,000L scale for standard fed-batch processes with high cell-densities (25 mln cells/mL) and it will highlight the bioreactor requirements to run extreme cell density processes such as XD® in disposable systems.
Data will be presented of the introduction of a 1000 l disposable bioreactor into the GMP facility for fed-batch as well as scale-up data of XD® processes (that can reach up to 240 mL cells/mL) in Single-Use Bioreactors.

P4.72. CHARACTERIZATION OF METALLOPROTEASE AND SERIN PROTEASE ACTIVITIES IN BATCH CHO CELL CULTURES: CONTROL OF HUMAN RECOMBINANT IFN-γ PROTEOLYSIS BY ADDITION OF IRON CITRATE

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During the production of any recombinant proteins, an evaluation of the product quality is crucial. Proteolytic events may occur during the process and could influence the product quality. Indeed, proteolysis is an unpredictable process and relatively little is know regarding the proteolytic enzymes produced and released by mammalian cells. In fact, proteases originating from the host cell line cannot be avoided in cell culture. Due to regulatory and safety prospects, the addition of serum, fetuin or albumin that usually limit protease activities, is not desirable. Thus, in serum-free cultures of mammalian cells, control of protease activity constitutes a major challenge.
In the present work, the presence of proteases in batch cultures performed using a recombinant CHO cell line producing IFN-γ in a stirred-tank bioreactor was studied. Cultures were carried out in two different media, namely RPMI and BDM. Whereas RPMI is a classical medium containing serum, BDM is a chemically defined medium without any proteins or serum addition, but supplemented with Pluronic F-68, ethanolamine and iron citrate. Using zymogram analysis, gelatinase and caseinase activities in CHO batch cultures performed with or without serum were detected, and belong most likely to the metalloprotease and serine protease families. During a culture carried out in RPMI with serum, a degradation of recombinant IFN-γ was observed, while no IFN-γ proteolysis was observed in culture performed with the BDM medium. Furthermore, our results showed that despite the medium used (RPMI, BDM, with or without serum), addition of iron minimized IFN-γ proteolysis, probably due to the inhibition of a 90 kDa metalloprotease activity. Thus, we demonstrated that the addition of iron citrate can be advantageously considered for industrial processes to prevent the proteolysis of a recombinant protein, in particular if one or several metalloproteases are present in the culture.

P4.73. ENGINEERING ANALYSIS OF MIXING PROCESS IN ATMI SINGLE-USE CUBICAL BIOREACTORS USING COMPUTATIONAL FLUID DYNAMICS

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Most industrial-scale bioreactors are made of stainless steel and follow a traditional design, usually a cylindrical shape with a 3:1 aspect ratio. Whilst this design fulfils specific process requirements; it is also governed by constraints such as CIP and SIP. As single-use technology eliminates the need for cleaning and sterilisation, it offers greater flexibility for bioreactor design optimisation.
Some of ATMI's single use bioreactors: the Nucleo™ (co-developped with Pierre Guerin) and PadReactor consist of a cubical vessel agitated with a paddle-shaped impeller rotating in an elliptical motion .
Engineering characterisation of bioreactors, via CFD modelling, can serve many purposes such as process transfer and scale-up, process optimisation and process validation. This poster discusses one part of this study: the mixing characterisation of the Nucleo™ and PadReactor

P4.74. CHARACTERIZATION OF NOVEL PNEUMATIC MIXING FOR SINGLE-USE BIOREACTOR APPLICATION

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Novel pneumatic mixing mechanism has been developed which converts buoyancy of gas bubbles to rotational energy to achieve an efficient liquid and gas mixing in bioreactors. The system achieves fast mixing and high mass transfer without any external mechanical mixing device, and is broadly scalable from 2L to 5000L. Computational fluid dynamics (CFD) analysis indicates that the system offers more homogenous mixing with lower shear stress than the conventional stirred bioreactors. The pneumatic mixing system is ideal for single use bioreactor application due to its efficient mixing characteristics and simplicity of the mechanism. Evaluation of biological performance using CHO cell lines in the pneumatic single-use bioreactors up to 250L scale yielded comparable results with stirred bioreactors including similar peak cell densities, cell viability, monoclonal antibody productivity, and product quality profiles. The pneumatic bioreactor system will be useful to grow not only robust industrial cells, but also shear-sensitive cells, and has potential applications in cell therapy, personalized medicine, viral production, and cultivating adherent cells on micro-carriers at various scales. In this presentation, the results of liquid mixing, mass transfer, shear stress, kinetic dissipation energy distribution, and cell culture performance will be discussed for various sizes of the system.

P4.75. LATE STAGE PROCESS CHARACTERISATION AND CONTROL STRATEGY DEFINITION FOR VALIDATION OF A FULLY DISPOSABLE BIOREACTOR PROCESS FOR MONOCLONAL ANTIBODY MANUFACTURE

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Disposable production systems for the manufacture of biopharmaceuticals are becoming more widespread, but few (if any) examples exist of process validation for commercial supply. Process control capability for some disposable systems may be less than that obtainable with fixed stainless steel systems, due to probe technologies and the degree of automation, which may present challenges for definition of robust control strategies for process validation.

We describe late stage process characterisation, development, and subsequent definition of an integrated control strategy (process, analytical, microbiological) for validation of a monoclonal antibody (MAb) cell culture (fed-batch) manufacturing process based on a fully disposable production platform (Wave System). The MAb was co-developed with MacroGenics, Rockville, MD. Process characterisation studies and risk assessments (FMEA) identified control of production bioreactor temperature and inoculation seed density as critical controls for maintaining consistency of product critical quality attributes (CQAs). These two factors are interacting, and a bivariate design space defines their proven acceptable ranges. Gas control (dissolved oxygen, PCO_2) and pH were determined to have no measureable impact on product CQAs within specified ranges. The behaviour of product- and process-related impurities were assessed from analysis of full scale batch data and used to inform placement of in process analytical controls, and the setting of process validation acceptance criteria, for validation. Evaluation of bioburden and endotoxin sampling and testing performed during manufacture of clinical lots, in conjunction with process risk assessments, informed selection of microbiological controls. Classification of critical (impacting CQAs or process consistency) and non-critical (GMP) controls is described. Control of process parameters is achieved through a combination of direct control by the bioreactor system (temperature, gas mass transfer), offline analysis (gas analysis, cell counts), and GMPs. Necessary optimisations to vendor supplied control systems to enable a robust and validatable process are described. Limitations of vendor supplied control elements (e.g., probe technologies) are discussed. Additionally, variability assessment and reduction exercises were undertaken to increase control capability for certain (non-automated) process control elements.

In summary, a fully disposable process for MAb production has been characterised, risk assessed and optimised leading to definition of an integrated control strategy for process validation and commercialisation. Certain vendor supplied process control elements required optimisation to ensure robust control for process validation.

P4.76. REPORT AND CHARACTERIZATION ON MAB PRODUCTION USING THE CUBICAL ATMI SINGLE USE BIOREACTOR AT 1000L SCALE

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The trend towards disposable processing systems in biopharmaceutical manufacturing is driven by the considerable economic and operational advantages of these systems. This presentation describes an enabling cubical shaped single-use bioreactor system that has been developed by ATMI LifeSciences using its patented PadMixer technology.

This presentation demonstrates the suitability of this single-use bioreactor system for mammalian CHO cell culture at 1,000L scale and thus scalability. A mixing characterization study that demonstrates superior mixing properties of the low shear mixing mechanism will also be presented.

Scaleable mass-transfer characteristics, cell growth, metabolism and secretion data from batch culture using 1,000 liter Nucleo™ / PadReactor™ are presented and compared to comparable cultures in classical stainless-steel and single-use stirred tank bioreactors.

P4.77. EVALUATION OF DISPOSABLE BIOREACTORS FOR VACCINE MANUFACTURING. LARGE SCALE EXPERIENCE FOR NEW RABIES VACCINE DEVELOPMENT CASE STUDY

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Several data have been reported on the benefits of disposable bioreactors for suspension cell culture such as CHO for recombinant protein production, however very little applications have so far been reported for vaccine manufacturing and anchorage dependent cell line like Vero. Taking into account general shear and mixing design specificities associated with the development of anchorage dependent cell cultures, current range of vaccine manufacturing scale, makes disposable bioreactors an attractive solution. Within the prospect of a new rabies vaccine manufacturing process, we have developed serum protein free media formulations allowing to achieve comparable results than what obtained with serum supplemented culture. Further media development strategies based on design of experiments, metabolic studies and FACS analysis of cells prior and after infection have resulted on average two fold viral productivity improvement. In parallel, disposable bioreactor process evaluation was combined and up to the 500L scale with PG-ATMI Nucleo bioreactors series. Efficiency of mixing and transfer (Kla) is investigated across scales including 5, 20, 200 and 500L scale. When compared to stainless steel bioreactor, comparable cell growth is obtained at all scales, with however higher viral productivity, and attributed to better gas transfer. In summary, we have demonstrated the feasibility and higher productivity of Vero cells for viral vaccine manufacturing within disposable bioreactors and up to an industrial scale of 500L, largest reported so far with such equipments. Preliminary operating cost evaluations indicate favorable figures for disposable bioreactor implementation versus stainless steel equipment.

P4.78. ESTABLISHMENT OF DISPOSABLE FILTRATION SYSTEMS FOR CELL SEPARATION IN CELL CULTURE HARVESTS OF CHO HIGH-CELL DENSITY FED-BATCH PROCESSES FOR THE PRODUCTION OF BIOPHARMACEUTICALS

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Disposable technologies in the biopharmaceutical sector offer important advantages compared to conventional clinical manufacturing making them a good choice for fast and economic production of biopharmaceuticals. Ready-to-us disposable materials allow a faster implementation and qualification of equipment thus reducing costs while increasing production capacity due to shortened cleaning and maintenance procedures. A major benefit is the flexibility of single-use equipment which enables the easy combination of various process steps and offers the possibility to utilize critical cell lines (e.g. hybridoma) and media components (e.g. serum). To meet the increasing demand for disposable manufacturing techniques in biotechnological production, Rentschler Biotechnologie GmbH is building two 1000 L fully disposable upstream and downstream manufacturing lines.

In the underlying study we evaluated different single-use filtration systems for cell separation in 1000 L scale with regard to their capability to replace the traditional harvest procedure containing separators or centrifuges by a robust filtration system. A screening of different depth and 0.2 µm filters was carried out with various filters of the four suppliers Cuno (3M), Pall, Millipore and Sartorius Stedim. High cell density fed-batch cultivations of a mAb expressing CHO cell line were clarified by constant flow depth filtration and subsequent 0.2 µm filtration. Evaluation of results focussed on the key parameters product loss, turbidity, capacity and costs of a filtration in 1000 L scale. In total, we included 29 different depth filter combinations and 85 single filtrations and 8 sterilizing-grade 0.2 µm filters in the screening. Out of that, two depth filtration systems were chosen and tested in 200 L scale by harvesting two fed-batch production processes in disposable bioreactors. Based on the results of this study suitable disposable depth filter systems were suggested for harvesting large-scale fed-batch processes in disposable manufacturing lines.

P4.79. HIGH CELL CONCENTRATION PROCESS IN DISPOSABLE 30 LITRE ROCKING MOTION BIOREACTOR EQUIPPED WITH DISPOSABLE SENSORS FOR DISSOLVED OXYGEN AND PH MEASUREMENTS

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Modern protein production processes generate high cell concentrations of several 10 million cells/ml. Oxygen supply and pH control become challenging under these conditions, especially if surface aeration is being used, e.g.in case of disposable rocking bioreactors in production scale cell cultures.
In a first step, the relevant physical parameters of a rocking bioreactor system equipped with disposable sensors for dissolved oxygen and pH measurement (Cultibag RM optical, Sartorius-Stedim Biotech, Göttingen, Germany) have been characterised. This includes sensor signal stability, a comparison of disposable optical sensors with conventional electrodes as well as measurements of oxygen transfer rates at different rocking frequencies and rocking angles.
To show the practical relevance of the obtained data, CHO cells were cultivated generating cell concentrations of more that 300•105 viable cells per ml in a 30 litre fed batch process. The composition of substrate concentrates for daily feeds was based on measurements of media components (Cubian XC bioanalyser, Optocell Technology, Bielefeld, Germany) in process samples. The concentration of recombinant protein being produced in this fed batch process was significantly increased compared to a standard batch process. Dissolved oxygen concentrations and pH values have been monitored on-line. High rocking frequencies and angles were required to maximise gas exchange to control dissolved oxygen levels and pHvalues. The known advantages of rocking disposable bioreactors like low shear rates, fast and easy handling, no cleaning and sterilization and a short setup time can now be combined with efficient controllers, based on disposable optical sensors, to achieve extremely high cell and product concentrations. Results of the relevant physical parameters of the bioreactor system as well as the culture conditions, growth curves and product concentrations will be presented.

P4.80. PROCESS INTEGRATION BY DIRECT CAPTURE OF MABS FROM CRUDE CELL CULTURE HARVEST: NOVEL, SECOND GENERATION EXPANDED BED ADSORBTION TECHNOLOGY (RHOBUST™) DEMONSTRATES UNIQUE SUITABILITY FOR DIRECT CAPTURE OF MABS FROM EXTREME CELL DENSITY CHO XD® CULTURES

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DSM Biologics' XD® technology provides an intensified Upstream Process delivering high cell density, high titer harvests compared to traditional Fed Batch operations. These harvests pose a challenge to start clarification unit operations like filtration & centrifugation. The benefit of highly concentrated harvest would be lost as standard unit operation would require dilution of the harvest. Before a standard packed bed capture step can be performed.
Robust™.(Expanded Bed Adsorption (EBA)) provides an elegant solution as a first unit operation to combine clarification and capture step. It is the alternative for future purification of high titer monoclonal antibody and recombinant protein processes. Here, we present a second generation EBA approach as the ideal solution for further Downstream Processing of XD® harvests. High density tungsten carbide-containing agarose beads were modified with traditional Protein A and innovative Mixed Mode ligands. Cell harvests with more than 100 million cells/mL even as high as 170 million cell/mL were easily clarified and product captured in one single step while still retaining the same product quality when compared to traditional methodologies.

P4.81. EXTRACTS OF PHYTOPLANKTON SUPPORT GROWTH AND PRODUCTIVITY OF RECOMBINANT CHO CELLS

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Serum-free cell culture media is required for the production of biotherapeutics such as recombinant proteins and monoclonal antibodies to maintain high cell yields, high protein productivity and consistent quality. Historically, cell culture media has been enriched with animal products such as serum, and more recently with animal hydrolysates such as Primatone. The possibility of contamination of products with mycoplasma, prions and viruses has increased the pressure to eliminate animal-derived components from cell culture media. This has lead to alternate sources of supplements for enrichment of basal media. Many plant and yeast hydrolysates are now commonly used as media supplements. In the study we have investigated extracts of marine phytoplankton as potential supplements for recombinant protein production from Chinese hamster ovary (CHO) cells. Three phytoplankton preparations, Celloments, Cellankton and UMAC, were obtained in a powder and slurry form. Growth and productivity of a CHO cell producing a marker recombinant protein (luciferase) were determined following addition of phytoplankton powder and slurries to a base of Biogro-CHO media.
Analysis of three phytoplankton extracts and a raw algal slurry showed that Cellankton had the highest bioactivity to support the cell growth and the production of luciferase. Further enhancement of bioactivity was observed by sonication of the Cellankton slurry which improved both growth and productivity of the CHO cells over several culture passages. Treatment of the slurry for 24 h at 37°C resulted in a browning reaction measured by enhanced absorbance at 350 nm and concomitant with a further increase in bioactivity. The bioactivity was shown to be thermally stable at varying exposures to high temperature. Treatment of the Cellankton by a novel protocol of sonication and subsequent heating produced a supplement for mammalian cell culture that resulted in a high cell yield ($>2x10^6$ cells/ml) and recombinant protein production equivalent to a positive control.

P4.82. PROTEIN QUALITY ANALYSIS AND DOWN STREAM PURIFICATION STUDIES DURING EARLY HTP CELL CULTURE MEDIA AND FEED DEVELOPMENT

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Demand for high titer and high quality of mAbs and other recombinant proteins from cell culture processes increased dramatically due to dramatically increase in demand for biotherapeutics. Titer is usually the most important factor when it comes to cell culture media, feed and process development. Quality of mAbs or recombinant proteins is usually investigated at much later stages of process development due to throughput and material constraints. However, the game has changed recently. In the past several years, many companies have considered quality of mAbs or recombinant proteins as an important factor during development period of the protein products. Many analytical tools and methods were developed and implemented in early stage of stable cell line development and cell culture process development. The similar demands also exist for early understanding on the impact of cell culture media and feeds on downstream purification efficiency. New technologies and high throughput purification technologies allow us to study the efficiency of whole therapeutic production process at early stages of cell culture development.
At EMD Millipore, a division of Merck KgaA, we recently developed several HTP analytical tools to investigate aggregation and glycosylation of mAb quality during cell culture media development. Those tools provide great deal of insides in cell culture media and feed development. The information obtained is used to guide further media and feed development and to investigate potential factors causing mAb aggregation and study glycosylation pattern at early stage of cell culture media, feed and process development, which can significantly reduce the risk and shorten the timeframe of cell culture media and cell culture process development. With our strong capabilities in the whole therapeutic production processes including upstream and downstream, we also studied the impact of media and feeds on purification efficiency. These studies enable us to supply integrated solutions with early consideration of protein quality.

P4.83. METABOLIC ENHANCERS: A NEW PARADIGM IN CELL CULTURE MEDIA OPTIMIZATION

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Cell culture media development is ever-evolving, driven by demand for higher yields, shorter timelines, and dynamic global regulatory policies. It is also very personal, in that each clone, each product, and each process has unique requirements and targets that often make elusive the successful implementation of a single solution or platform approach. In such a challenging field, it is critical to identify tools that can be applied across a variety of media strategies, be them defined or undefined in character. It is also of unique advantage to utilize components which act through different mechanisms than most traditional media components and supplements. Here, we present data demonstrating the application of novel, non-nutritive metabolic enhancers, which act on ubiquitous cellular pathways to increase process yield and robustness. These supplements are chemically defined and comprised of mixtures of small organic molecules. Antibody titer improvements in CHO cells averaged >30% in 6 different commercially available media. Data showing the impacts of passage number, the persistence of metabolic effects, and synergy with nutritive feeds will be discussed. Data also demonstrate similar improvements in hybridomas, as well as unique improvements in performance of MSC and ESC, promoting expansion while limiting differentiation. Data demonstrating compatibility with serum-containing, serum-free, hydrolysate-containing, and chemically defined media will also be presented. Future work will evaluate whether such supplements positively impact product quality and consistency.

P4.84. ACCELERATING CELL CULTURE MEDIA AND FEED PROCESS USING HIGH-THROUGHPUT PLATFORMS AND WORKFLOWS

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Mammalian cell cultures have become the main platforms for biotherapeutic productions, with cell culture media, cell culture feeds, and process being the critical drivers in achieving high quality protein and productivity. Recently, chemically defined media have become the desired method by the industry for high cell density and cell specific productivities. This requires efficient workflow and testing systems for the synergy of chemicals formulated in a media and feeds. In an effort to effectively formulate and test our chemically defined media and feeds, we have developed high-throughput (HTP) platforms and robust workflows to reduce time and increase overall cell culture performance. Our HTP platforms include the usage of liquid handling systems to design and formulate many variations of media and feeds using predetermined stock solutions. These platforms also include culturing cells with the formulated media and feeds using 96 deep-well plates, spin tubes, and shaker flasks. In addition, high-throughput assays on cell growth, cell viability, productivity and spent media analysis are developed and incorporated into the workflow. Furthermore, a liquid handling system has also been used for cell seeding, transferring and preparing samples for HTP analytical measurements. The analytical outputs are then analyzed and as a result we are given a better understanding of the contribution of media and feed composition on cell culture's performance. The results and knowledge gained through the use of HTP have successfully led to our CHO cell culture media and feed platforms within a short time frame.

P4.85. STRATEGIES FOR ADAPTATION OF MAB-PRODUCING CHO CELLS TO SERUM-FREE MEDIUM

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Large-scale production of biopharmaceuticals commonly requires the use of serum-free medium, for safety and cost reasons. However, serum is essential to most mammalian cells growth, and its removal implies a very time-consuming process for cell adaptation. Thus, the aim of the study was to evaluate different strategies for cell adaptation to serum-free medium.
Three cell types were used to assess the impact of transfection on adaptation: one common CHO-K1 cell line and two CHO-K1 cells transfected with different technologies for antibody production. Cultures were started with a known cell concentration in Dulbecco's Modified Eagle Medium supplemented with 10% serum. The effect of five combinations of supplements, that could support cells during adaptation, was tested. These supplements included insulin and trace elements (copper sulfate, zinc sulfate, sodium selenite, ammonium iron citrate, ferrous ammonium sulfate, ammonium metavanadate, nickel chloride and stannous chloride). A methodology of gradual adaptation was followed, consisting on sequential steps of serum reduction, after assuring good cell adaptation from the previous step. After reaching 0.625% serum, medium was gradually switched to the chemically defined serum-free EX-CELL CHO DHFR- medium.
It was observed that supplements influence cell adaptation to serum-free medium. Indeed, the combinations containing the trace element ammonium iron citrate gave the worst results, with cell death at 2.5% serum. In contrast, the combination of ammonium metavanadate, nickel chloride and stannous chloride proved to be the most favorable to the three cell lines. Comparing the cells, it seems that the ability to produce antibody and the transfection methodology used does not have a great impact on adaptation.
During the study, some procedure details were identified as particularly important and should be carefully considered in the process of cell adaptation to serum-free medium. These include the use of a higher initial cell concentration that will allow the survival of an increased number of cells during the process; avoiding harsh procedures to the cells such as centrifugation and the use of enzymes (i.e. trypsin), due to a higher cell sensibility during adaptation; and to give enough time for a full cell adaptation at each step.

P4.86. APPLICATION OF COMPLEX AND CHEMICALLY-DEFINED MEDIUM SUPPLEMENTS TOWARD CELL LINE SPECIFIC PERFORMANCE ENHANCEMENT OF BIOPHARMACEUTICAL PRODUCTION SYSTEMS

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Medium optimization is an integral part of biopharmaceutical process development, and commonly involves the addition of various additives to an existing basal medium formulation. There is an on-going debate within the industry as to the various advantages and disadvantages of both defined and undefined media and media components. The choice of which type of system to employ is often motivated by risk mitigation with respect to consistency of performance, as weighed against the underlying goal of achieving the highest possible product titers for any given system. Process development scientists must make these assessments within the context of their own targets and timelines, as well as the tools available to achieve these goals. These supplementation solutions may not be mutually exclusive. To that end, a series of cell-line specific complex media supplements have been developed for enhancing performance of various biopharmaceutical production systems. Created using in-house media optimization methods, these supplements are manufactured using innovative, proprietary process technology which allows for the combination of complex and/or chemically defined animal-component free media additives into a single homogeneous functional supplement. These supplements have been optimized for individual cell lines, and have proven to be suitable for use in a range of basal media. Data are presented which demonstrate the effectiveness of these optimized supplements as performance enhancers for application in CHO and SP2/0 batch culture, and as feed supplements in fed-batch systems. Examples will also be given where these supplements have been employed as a vehicle for serum-reduction in MDCK, BHK and CEF media. Preliminary data will also be presented on a parallel series of strictly chemically-defined supplements, similarly optimized for specific cell lines, providing the opportunity for comparison with their partially-defined counterparts.

P4.87. A COMPARISON OF PERFORMANCE ENHANCING SYNERGY AMONG ULTRAFILTERED YEAST EXTRACTS AND RECOMBINANT HUMAN SERUM ALBUMIN IN CHO-K1 CELLS

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The performance benefit provided by any medium supplement is subject to its interaction with other medium components present in the basal formulation, as well as other supplements being employed. In some instances, a combination of supplements may provide better performance than is seen when supplementing with the individual entities. We have previously demonstrated a synergistic reaction between a wheat hydrolysate and recombinant human serum albumin used to supplement a chemically defined growth medium for SP2/0 hybridoma cells. The data presented here illustrate the synergystic performance enhancing effect obtained when ultrafiltered yeast extract and recombinant human serum albumin are co-supplemented in CHO cell media. Each combination has its own distinctive effect on the growth and productivity of transfected cells. Cell viability, cell proliferation and target protein production all may be improved, yet these effects are not necessarily observed concurrently in a given system. In three separate basal media, cell response to co-supplementation for each of the yeast extract/recombinant albumin tested is shown to be both medium and dosage dependent.

P4.88. RAW MATERIAL CHARACTERIZATION OF MEDIA COMPONENTS: A CASE STUDY WITH SOY HYDROLYSATE

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Today's biopharmaceutical and biotech industries require comprehensive knowledge of raw materials to achieve consistent culture process performance and product quality attributes. In response, SAFC launched a raw material characterization program to evaluate raw materials used in media preparations, employing biological and analytical methods. Soy hydrolysate was a key target in the program because it is a complex, undefined medium component and a recognized source of variability in animal cell culture processes.

SAFC has investigated variability in soy hydrolysates using biological assays to characterize growth, production and product quality using three industrially relevant cell lines. The study included four vendors and at least twenty-five lots of soy hydrolysate at a range of concentrations. The individual cell line responses to soy in the assay ranged from limited to highly dependent. While variability in cell growth and productivity was observed among soy lots from the same vendor, differences were more pronounced comparing soy among different vendors. Analytical methods were also employed to study product glycosylation resulting from different soy concentrations in the medium among all the lots. The lots demonstrated a soy concentration-dependent shift in glycosylation patterns.

Soy hydrolysate is just one of many components that SAFC continues to evaluate using various biological and analytical methods to characterize variability, impurities and identify markers for performance in complex raw materials.

P4.89. OPTIMIZATION OF CELL COUNTING THROUGHPUT FOR THE SAFC RAW MATERIALS CHARACTERIZATION PROGRAM

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The development of cell culture media and processes typically requires screening large numbers of cultures or conditions. Monitoring cell density is a key parameter for evaluating culture performance. The development of automated viable cell counters based on the trypan blue exclusion method has significantly increased throughput, improved consistency and reduced the labor in this process. However, these cell counters generally have relatively limited sample queues (up to 20 samples) and long processing times (>3 minutes per sample). SAFC's raw material characterization (RMC) program, for example, requires analysis of more than 200 samples per day, exceeding 11 hours of instrument analysis time. In order to improve cell counting throughput, methods to reduce sample analysis time with the Cedex cell counter and alternate counting methods were investigated.
The Cedex cell counting process was evaluated with three test cell lines used for the RMC program, including two CHO cell lines. Multiple steps of the process were considered to reduce the cell counting cycle time. The process was also optimized to address an issue of cell adhesion to plastic surfaces. Modifications in the process were compared to original method to ensure there was no loss in accuracy or precision. The results for the Cedex process optimization show that the cycle time per sample could be reduced by >20 % without negatively impacting the reliability of the method.
In order to find alternative methods to the Cedex cell counter and to achieve higher throughput, Guava cell counting technology was investigated. The Guava ViaCount system is capable of counting samples in a 96 well plate format. The Guava process was optimized to achieve counts comparable to the reference Cedex counting method using CHO-K1 as a model cell line. A cell dispersing reagent, optimization of incubation time and the sample handling were identified as key variables affecting cell counts with this method. The Guava method was refined to achieve a 2-fold reduction in cell counting process time.

P4.90. MEDIA AND FEED PLATFORMS: CONTROL OF CELL FUNCTION BY NUTRIENT AND PROCESS INTEGRATION

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Sustained growth of the biopharmaceutical market has created a need for high-titer production processes to meet increasing market demand and to reduce manufacturing costs. Advances in recombinant cell line engineering have resulted in high producing clones with high nutritional demands. Depletion of critical nutrients in a production process can limit the potential of these clones, causing reduced titers and resulting in inefficient, costly processes to compensate for media or feed deficiencies. Rational design approaches seek to provide high performance recombinant clones with nutritionally balanced basal media and feed supplements. Through integration of base and feed media development, we were able to address nutrient limitation issues in rCHO cultures and obtain substantial improvements in titer by sustaining specific productivity for an extended period of time. Such approaches are clearly effective, but are time consuming and are limited by the large number of possible component combinations and the need to integrate process parameters like pH, temperature, dissolved oxygen, agitation rate and time of feed addition with a balanced nutrient composition. Often, nutrient composition that is optimal for cell growth may be sub-optimal for product titer or product quality. We have applied high throughput (HT) tools that enable simultaneous evaluation of multiple nutrient and process variables using Design of Experiment (DoE) approaches. This approach has made possible fed batch process development experiments of up to 420 simultaneous conditions, greatly expanding the design space and number of replicates possible. The power of HT tools to evaluate broad design spaces has been demonstrated by successful elimination of proteins and undefined components in production processes. In a recent study, we successfully developed a chemically defined process which more than doubled peak viable cell density and increased titer by 150% over the original undefined process. Product quality was unchanged in the improved process. Finally, by using HT methods to integrate workflow steps, significant process development time savings can be realized. This was demonstrated in an integrated basal medium and feed development project for a rCHO cell line expressing erythropoietin (EPO). Sixteen media variants with different levels of nutrient supplementation were evaluated along with three diversified feeds. The integrated, HT approach made it possible to achieve an EPO titer 2.7 times that of the original fed batch control process in less than two months. In summary, fed batch process development efforts have been shown to be greatly facilitated by approaches that employ HT tools to rapidly evaluate broad design spaces and identify the integrated basal medium, feed supplement and process parameters necessary to achieve the desired endpoint.

P4.91. MEDIUM AND FEED OPTIMIZATION FOR FED-BATCH PRODUCTION OF A MONOCLONAL ANTIBODY IN CHO CELLS

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Mammalian cells are used extensively in the production of recombinant proteins, and of monoclonal antibodies (MAbs) in particular. The trend towards avoiding animal-derived components in biopharmaceutical production processes has led to the extensive use of non-animal origin hydrolysates such as plant hydrolysates or yeast hydrolysates. The source of hydrolysates affects cell growth and productivity and may also affect product quality. Accordingly, careful consideration should be given during process and cell culture media development, in order to determine the appropriate type and amount of hydrolysates to be added, for the cell and product at hand.
In this study we assessed the impact of several hydrolysate additives and chemically defined commercial feeds on MAb titers, average cell specific productivity (average Qp) and integral of viable cell concentration (IVCC) in suspension cultures of recombinant CHO cells in shake flasks and 2-L bioreactors. Experimental design and results have been statistically analyzed to guide further the selection of feeding strategies, in a high-performance and high-titer process. A combination of two hydrolysates added throughout the culture enabled the maintenance of high cell viability (above 80%) throughout the production process. In comparison to the initial process platform, a 100% increase in IVCC was obtained. Moreover, this medium and feed optimization strategy led to improvements in MAb titer and average Qp by 110% and 105%, respectively.

P4.92. DEVELOPMENT OF A CHEMICALLY DEFINED CHO MEDIUM BY ENGINEERING BASED ON A FEED SOLUTION

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The use of chemically defined culture media for production of biopharmaceuticals has become state of the art. In combination with a suitable feed solution chemically defined media are the most potent driving factor for yield improvement in fed-batch processes. Although various chemically defined media are available of-the-shelf and deliver good results, those media are developed with the aim to sustain growth for various host cell lines, thus are not optimal for all projects.
We describe here the development of a chemically defined medium for fed-batch culture of recombinant Chinese hamster ovary cells. The medium was developed by engineering based on an optimised proprietary feed medium, which was developed previously. In a first step of development, the components of the proprietary feed medium were categorized into five component groups: bulk salts, trace elements, vitamins, amino acids, and other components. Necessary components not present in the feed medium were considered while establishing a basic medium formulation based on theoretic considerations of cellular needs in culture (buffer system, adaptation of amino acid concentrations, glucose, growth factors, vitamins, etc.). This basic formulation was combined with various x-fold concentrates of the component groups of the feed medium. The resulting media were used for adaptation in the second step of development. At this stage cell densities up to 4×10^6 cells per ml in batch experiments were achieved without feeding. The most suitable formulation was selected for fine tuning of component groups (e.g. vitamins) and individual components as well as optimisation of the buffer system in the third phase of development.
The final medium formulation was tested with different CHO clones producing either IgG or fusion proteins and exceeded the performance of the best commercial available chemically defined medium tested in this experimental set-up. Cell densities of more than 10^7 cells per ml were achieved in fed-batch experiments; specific productivities could be significantly increased. Moreover, data of the third phase of medium development deliver a starting point for medium optimisation to clonal demands, since the effects of several key components were studied in detail.

P4.93. COMPLEX MEDIUM SUPPLEMENTS OPTIMIZED FOR PERFORMANCE ENHANCEMENT OF BIOPHARMACEUTICAL PRODUCTION CELL LINES

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A series of cell-line specific complex media supplements have been developed for enhancing performance of various biopharmaceutical production systems. Created using in-house media optimization methods, these supplements are manufactured using innovative process technology which allows for the formulation of complex and/or chemically defined animal-component free media additives into a single homogenized functional supplement. These supplements have been optimized for individual cell lines, and have proven to be suitable for use in a range of basal media. Data are presented which demonstrate the effectiveness of these optimized supplements as performance enhancers for application in CHO and SP2/0 batch culture, and as a vehicle for serum-reduction in MDCK and BHK media. While these particular supplements do contain some undefined components, preliminary research indicates that this same technology may be applied to chemically-defined, multi-component supplements. Work is underway to develop a comparable series of strictly defined supplements, similarly optimized for specific cell lines.

P4.94. ANIMAL-FREE CRYOPRESERVATION AND RECOVERY MEDIA

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Demand is increasing for cryopreservation of cell stocks to be devoid of serum or any animal-derived components especially when it precedes an animal-free biomanufacturing process.
In this presentation, we examine an animal -component free recombinant human albumin (rAlbumin) for its ability to replace serum in the cryopreservation of CHO and hybridoma (Sp2/0) cells. Other protein components of serum, largely responsible for maintaining cell viability and promoting cell growth, are known to be important in the recovery media of cryopreserved cells. Recombinant forms of these proteins were also assessed for their ability to support cell growth during the initial recovery period following thawing.
Results indicate rAlbumin successfully replaced foetal bovine serum (FBS) in the cryopreservation of both CHO and Sp2/0 cells and that addition of recombinant protein supplements also improved cell growth and productivity following thawing.

P4.95. IMPLEMENTING A NEW CHEMICALLY-DEFINED MEDIA FOR ANTIBODY PRODUCTION: CHALLENGES AND SOLUTIONS

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Genentech has developed a proprietary chemically-defined media (CDM) for the cell culture platform process used to manufacture monoclonal antibodies. When implementing this new CDM for clinical manufacturing, we encountered unexpected challenges. Using examples from several clinical products, we will discuss a media preparation challenge that affected product quality. Specifically, through case studies, we will describe (1) our initial discovery of the product quality issue, (2) investigations conducted to identify the cause, (3) cellular biochemical mechanisms to explain the observations, (4) measures taken to address the problem, and (5) subsequent successes in overcoming this challenge.

P4.96. ANALYTICAL TECHNIQUES FOR CHARACTERIZATION OF RAW MATERIALS IN CELL CULTURE MEDIA

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Raw materials are a critical part of any cell culture medium; therefore, it is of utmost importance to understand and characterize them for high quality product. The raw material characterization (RMC) program at SAFC focuses on individual screening of raw materials both analytically and biologically. The goal of the program is to develop a best-in-class knowledge base of the raw materials used in SAFC's media formulations and their impact on performance of the products.
A prioritized list of "high-risk" raw materials was developed based on a risk assessment performed within SAFC. This talk will focus on the analytical screening of the "high-risk" raw materials to identify any variability and critical contaminants present. In order to achieve that, orthogonal methods were used that include ultra-high performance liquid chromatography-mass spectrometry (U-HPLC/MS) for non-volatile polar components and gas chromatography-mass spectrometry (GC/MS) for volatile materials. Inductively coupled plasma-optical emission spectrometry (ICP-OES) was also used to identify any metal contamination present. The data generated indicates lot-to-lot and vendor-to-vendor variability for some critical raw materials that can affect cell culture performance or product quality. This led us to track back on the manufacturing processes used for the raw materials and thus aided in better understanding of raw materials. The data presented will demonstrate the significance of the RMC initiative and how it helps SAFC to better understand their raw materials.

P4.97. ISOLATION OF ACTIVE PEPTIDES FROM PLANT HYDROLYSATES THAT PROMOTE VERO CELLS GROWTH IN STIRRED CULTURES

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Vero cells are adherent cell lines commonly used for the production of viral vaccines. We had developed an animal component free medium that allows an optimal growth of this cell line in stirred bioreactor (Rourou et al. 2009a; 2009b). We had also showed that Vero cells grown in this medium (called IPT-AFM) sustained rabies virus replication, and resulted in an overall yield comparable to the level obtained in serum-supplemented medium.
IPT-AF medium contains plant hydrolysates, namely soy (Hypep 1510) and wheat gluten hydrolysates (Hypeps 4601 and 4605). These peptones were shown to promote cell attachment and growth. However, although these components are of non-animal origin, their use in vaccine production process has several drawbacks, mainly due variability between lots.
The aim of this work is to identify active peptides from these hydrolysates that show a positive effect on cell adhesion, attachment and growth. For this purpose, the hydrolysates were fractionated using chromatography and precipitation techniques. The effect of the isolated fractions on Vero cells growth were tested in 24 and 6-well cell culture plates using experimental design approach. Fractions that sustain cell growth, were further tested in stirred culture on Cytodex1 microcarriers, to confirm their positive effect on Vero cells growth.

P4.98. DEVELOPMENT OF A CHEMICALLY DEFINED SUPPLEMENT AS AN ALTERNATIVE TO YEAST-BASED PEPTONES

Omune D., Oliver J., Chaturvedi K., Barbacci D., Berdugo C., Hunt C., Conaway K., Spriesterbach A., **Dodson E**.
BD Advanced Bioprocessing, Maryland USA

Yeast-based peptones have been effectively used to promote cell growth and enhance production of recombinant protein in mammalian cell cultures. Yeast-based peptones are undefined complex mixtures of amino acids, polypeptides, nucleic acids, vitamins, and other nutrients. Its complexity may contribute to variations in performance due to the effects of unknown components in the mixture. In an effort to eliminate these variables and improve batch-to-batch consistency while maintaining performance equivalency, we have characterized yeast extract (YE) and developed a chemically defined (CD) animal-free formulation that serves as an alternative supplement in Bioproduction applications. The BD Recharge™ powder has demonstrated comparable cell growth characteristics and protein production compared to YE in CHO cell lines expressing monoclonal antibodies and recombinant proteins. The BD Recharge powder offers lot-to-lot consistency and stability that is attractive for pharmaceutical and bioproduction applications.

P4.99. RETHINKING CELL CULTURE SUPPLEMENTATION: FROM PEPTONES TO CHEMICALLY DEFINED

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Peptones have a long history of use in cell culture media. However, chemically defined alternatives are often desired for bioprocess applications. Chemical separation and analysis of peptones have led to a greater understanding of their functional components; this knowledge has inspired the formulation of a new chemically-defined (CD) cell culture supplement. Optimization of the components through Design of Experiments has resulted in a formulation which is functionally comparable to peptones. The composition of the CD supplement is fully controllable, a distinct advantage over peptones. In testing on mammalian cells, growth and protein yields are equivalent or superior to that seen with peptone supplementation. Moreover, an assay of mAb protein quality is also found to be equivalent. These attributes suggest that the CD supplement should be a positive substitution for peptones, affording greater lot-to-lot consistency while maintaining protein quality and production.

P4.100. INVESTIGATION OF THE ROLE OF A SOY PEPTONE, AS AN ADDITIVE FOR THE CULTIVATION OF CHO-320 CELLS

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Peptones are widely used to cultivate animal cells in serum-free media to increase either cell growth or protein production or both. However, this remains largely empirical and few convincing data have been obtained to explain the origin of this effect. The addition of a commercial soy peptone during batch cultivation of CHO-320 cells in a serum-free medium increased significantly the recombinant g-IFN (g-interferon) production, compared to the cultivation in the absence of the peptone (21 % increase). Some authors hypothesized that peptones contain a few biologically-active compounds that could explain their beneficial effects on cell productivity. The peptone was previously fractionated, in order to identify some putative active compounds. Our results, together with those of other fractionation works, indicated that although the existence of a couple active compounds can not be totally excluded, the global peptone composition may play a major role in the increased protein production phenomenon. Therefore, our aim was to investigate the molecular and cellular effects of peptones on CHO cells and to determine which main cellular functions are affected by the peptone presence. A global analysis of the steps of g-IFN production in the presence and absence of the soy peptone was performed. Firstly, g-IFN mRNA levels were not different between conditions. Secondly, the soy peptone increases global protein translation as it was observed that cellular protein content was increased by 14 % at day 4 in the presence as compared in the absence of the soy peptone. While the intracellular g-IFN content was increased by 87 % at day 4 in the presence of the soy peptone, its secretion efficiency was increased by 37 % between days 3 and 4. Since a proper glycosylation is needed for protein secretion, glycosylation status of g-IFN was evaluated. However, it was found to be not dependent to peptone presence. Additionally, endoplasmic reticulum(ER)-stress, which appears when incorrectly glycosylated and folded proteins accumulate in the ER, was not decreased in the peptone presence. Furthermore, the hypothesis that the soy peptone prevents the proteolytic degradation of g-IFN in the cultivation medium was investigated. However, no protective effect against degradation was observed. Hence, the soy peptone appears to increase both protein translation and secretion. Although further experiments are needed to precise the mechanisms related to the influence of the soy peptone on these cellular functions, the effects of soy peptone on cellular functions appear to be multiple.

P4.101. EFFECTS OF VITAMIN K ADDITION ON THE METABOLISM OF CHO CELLS PRODUCING A VKDP

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Vitamin K plays an important role as a co-factor of the enzyme carboxylase, which post-translationally converts glutamyl residues to γ-carboxylated glutamyl residues in vitamin K-dependent proteins (VKDPs) in the endoplasmic reticulum during their secretion. *In-vivo*, VKDPs play an important physiological role in many tissues and are relevant e.g. to hemostasis, bone mineralisation, arterial calcification, and signal transduction.
Recombinant VKDPs can be produced in animal cell culture processes to be used in replacement therapies and, in order to have a functional protein product, vitamin K must be added to the medium. However, there is no consensus in literature about ideal dosing, timing of addition and stability of vitamin K in cell cultures.
Therefore, in this work, the metabolic behavior of a suspension-adapted CHO cell line producing a VKDP was studied. Different strategies to feed vitamin K were evaluated: 10 µg/mL vitamin K addition upon inoculation, 10 µg/mL vitamin K addition every day, 10 µg/mL vitamin K addition when cells reached the stationary growth phase (24 h before culture termination), and no vitamin K addition as a control. Cell concentration and viability, glucose and lactate concentration, and product activity were monitored along culture time.
In the two situations, where vitamin K was added from the beginning of the culture, cell growth was enhanced, but a significant decrease in viability was observed at 96 h. Glucose consumption was similar in all cases, but lactate formation was more pronounced when vitamin K addition occurred already at culture beginning, and this may be related to the viability decrease observed.
Biological activity of the VKDP was similar in both cases where vitamin K was present from the beginning, and undetectable in the negative control and in the culture that received vitamin K after reaching the stationary phase. The latter fact indicates that VKDP formation is probably growth-associated, otherwise functional product would have been formed by the large amount of cells present in the last 24 h of cultivation. The fact that similar biological activities were found in both situations where vitamin K was added from the beginning indicates that it is rather stable under cell culture conditions and does not need to be supplemented regularly. However, in these two cultures a sharp decrease in biological activity was observed at 72 h, indicating that proteases released from dying cells might be degrading the VKDP product.

P4.102. EVALUATION OF DIFFERENT PROTEIN FREE MEDIA FOR NSO CELL LINE USING A STATISTICAL DESIGN AND METABOLIC FLUX ANALYSIS

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In this work a basal nutrient formulation of cell culture medium capable to sustain protein and lipid free proliferation and recombinant protein production in NSO cell line was obtained. Eight amino acids (Gln, Phe, Ser, Ile, Met, Thr, Val, Leu), the iron salt, four vitamins (Biotine, B_{12}, A, D_2) and ethanolamine were identified as important for cell growth and/or antibody production from the screening analysis of a set of different nutritional components (amino acids, vitamins, salts) employing a DOE approach, based on a Plackett-Burman design and this first formulation was named as basal MyeloCIM. The next step was to duplicate amino acids and vitamins concentration in the basal medium (named as fortified MyeloCIM). Kinetic studies were carried out in batch culture mode and specific consumption/production rates of all aminoacids, glucose, lactate, ammonium, oxygen and carbon dioxide were determined for both formulations. Only three aminoacids (Asp, Pro, Ser) had large differences between formulations in their specific rates and two of them (Asp, Pro) showed a different consumption/production pattern. The ammonium formation is increased too.
Obtained results allowed us to quantify the physiological state of recombinant NS0 cell line in both formulations, determining fluxes through the bioreaction´s network that represents the main pathways related with macromolecules and energy generation. In fortified MyeloCIM the glycolysis is more active than in basal MyeloCIM and cells contain a truncated TCA cycle in which glutamine is a major energy source and the citrate is not completely oxidized to CO_2. Instead the citrate leaves the mitochondria to provide a source of acetyl units for synthesis of cholesterol and lipids. Consequently, flux through the α-ketoglutarate-to-oxaloacetate sequence of the cycle is greater than flux from citrate to α-ketoglutarate. On the other hand serine was more utilized in the antibody and biomass synthesis. The results showed that flux metabolic analysis was a useful tool to describe cell metabolism as a function of changes in amounts supplements in the media.

P4.103. IMPLEMENTATION OF ANALYTICAL TOOLS AND METABOLIC MODELS FOR OPTIMIZING CELL CULTURE MEDIA DEVELOPMENT

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Successful bio-therapeutic production of monoclonal antibodies and recombinant proteins is highly dependent on utilizing effective cell culture media, as well as on optimized feeds and processes. Cellular metabolic requirements significantly impact cell culture performance including cell growth, titer and product quality. Metabolic profiles are cell line dependent and greatly affected by media, feed and processes. To that end, evaluation of effective cell culture media and feed formulations is reliant on implementing appropriate analytical tools and metabolic models. EMD Millipore recently initiated an in-house program to establish effective analytical tools, relevant cell culture processes and metabolic models to evaluate cell culture media, feed and process optimization activities. Through the use of both high throughput expression platforms and metabolic analyses, media formulations and feeds are being developed to maximize cell densities and protein titers in mammalian cell cultures, which also focus on ensuring biotherapeutic product quality and consistency. This presentation will describe progress that EMD Millipore has made in building their media development program with scientific understanding of cell metabolism and cell biology. Our approach will provide significant value to business partners seeking to optimize their biomanufacturing activities.

P4.104. CHARACTERIZATION OF YEAST EXTRACT CHROMATOGRAPHIC FRACTIONS DISPLAYING CHO CELL GROWTH IMPROVEMENT

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Many studies underlined the great benefits of yeast extracts (YE), used as supplements in animal free culture media, on cell growth and recombinant protein production. Nevertheless, their complex and undefined composition remains a constraint for industrial processes. In regards of this statement, we propose to deepen our knowledge of the YE molecules showing an effect on CHO cell growth by implementing various fractionation processes.
In a first time, the YE retentate, issued from a previous nanofiltration step, was fractionated by anion exchange, hydrophobic interaction or gel filtration chromatography. Buffer salts added during chromatography steps were further removed by nanofiltration. Analyses of total amino acids, carbohydrates and nucleic acids were performed on all fractions. The anion exchange chromatography allowed isolating nucleic acids, strongly bound to the gel, in one fraction, while peptides were distributed among all fractions depending on their electrical charges. In the fractions from the hydrophobic interaction chromatography, important quantities of buffer salts still remained despite the desalting process. Finally, the gel filtration chromatography led to three fractions containing various proportions of nucleic acids, polysaccharides and peptides. Then, all fractions and YE retentate were added in a reference culture medium and the CHO cell growth was monitored in 96-well microplates with the Cellscreen® cell analysis system. The fractions coming from gel filtration chromatography presented similar results than the YE retentate. The two fractions from hydrophobic interaction chromatography did not stimulate the cell growth, probably due to residual buffer salts. Furthermore, only one fraction from anion exchange chromatography, which was deleted from nucleic acids and enriched in positively charged peptides, presented a similar impact than the YE retentate.
In a second time, the YE retentate was divided in 18 fractions by a process successively including anion exchange, hydrophobic interaction and gel filtration chromatography. The final gel filtration step presented the advantage to remove the residual salts. The monitoring of the CHO cell growth in presence of the fractions pointed out two fractions, which led similar results than the YE retentate. These selected fractions contained peptides, mainly positively charged and hydrophilic, low quantities of polysaccharides and no nucleic acids. The peptides were analyzed by using reversed phase chromatography coupled to a mass spectrometer.

P4.105. NON-NUTRITIVE SMALL MOLECULES POSITIVELY IMPACT CULTURE PERFORMANCE

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Here, we present data demonstrating the application of novel, non-nutritive metabolic enhancers, which act on ubiquitous cellular pathways to increase antibody titers in CHO cells and enable reduction of animal-derived serum/components commonly employed in vaccine-producing cells such as BHK and MDCK. Effects of adaptation, passage number, and synergies with more traditional supplements are also illustrated. Data demonstrating compatibility with serum-containing, serum-free, hydrolysate-containing, and chemically defined media will also be presented. Antibody titer improvements in CHO cells averaged >30% in 6 different commercially available media. In MDCK cells, supplementation with the RS-Vax metabolic enhancer enabled reduction of FBS from 10% to 1%, with no drop in growth or viability characteristics. Future investigations may identify novel synergies among components and help to simplify media optimization strategies.

P4.106. PROCESS DEVELOPMENT AND RAW MATERIAL CHARACTERISATION: ESSENTIALS FOR RELIABLE MAMMALIAN CELL CULTURE MEDIA PRODUCTION

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Mammalian cell culture media have huge impact not only on cell growth and therapeutic protein yield, but also on quality and robustness of the biopharmaceutical molecules. The performance of any medium is closely linked to the formulation as well as the production process and the quality of raw materials used for the production. We will show how process parameters in the production of dry powder media influence the performance from cell growth to the quality of the final API produced by mammalian cells and its biological implications. Currently, we do not have sufficient insight into cellular signalling and so do not know exactly how cells behave in defined cell culture media and what their nutrition needs are and how they metabolize raw materials in large scale biopharmaceutical processes: Beside the classical approach to optimise the media composition based on the clone used, we elaborate to improve the medium performance by supplying cells with nutrients that are able to circumvent limitations caused by solubility, stability or cellular uptake mechanisms.
We as medium producers have lots of reliable models in our design space to understand the mandatory quality attributes of the large scale biopharmaceutical production process. We will show that reproducibility to deliver GMP cell culture media is not only a part of formulation optimisation, which is mainly driven in dedicated laboratories in Pharma R&D, but is also a case to case production optimisation process. We will show how we have a process up and running to deliver constant raw material and process quality and thus are able to deliver batch to batch consistent dry powder cell culture media scalable from kg to tons.

P4.107. UTILIZING ROCHE COBAS TECHNOLOGY AND EXPERTISE FOR IN PROCESS MONITORING IN BIOTECH PRODUCTION

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The production of recombinant proteins is often based on animal or bacterial cell culture. One task during the development and control of such bioprocesses is to deliver fast, accurate and reliable analytical process data. Monitoring of nutrients and metabolites is essential to avoid limitations or build up during fermentations while the measurement of product titer like IgG is needed to track productivity or yield during protein purification processes.

The aim of this evaluation and method validation was to compare the performance of this new Roche Bioprocess Analyzers to common analytical systems like membrane analyzers, 96-well based photometric assays and HPLC methods. For this study typical mammalian cell culture parameters like: glucose, lactate, glutamine, glutamate, ammonia, lactatedehydrogenase, sodium, potassium and IgG were analyzed.

Besides the possibility to combine three analyzers into one and to reduce manual operating steps as well as labor-intensive maintenance our studies show-better accuracy, precision, specificity, sensitivity, linearity and reliability of data using the Roche analyzers. Furthermore, the devices provide a new flexibility to implement additional parameters.

P4.108. ATF PERFUSION-BASED PRODUCTION PLATFORMS FOR BIOPHARMACEUTICALS AT GOOD GRIP - CONCEPTS FOR ROBUST CONTROL AND OPERATION

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Continuous perfusion is comparatively unpopular rather than considered as a first choice production platform for complex biopharmaceutical glycoproteins. Major concerns that have been addressed are mainly related to risk of process failure during long-term operation, complexity and scalability of cell retention devices, supply and definition of consecutive lots and, finally, handling of continuous harvests in downstream processing.
We have cleared-up these stereotypes and designed a robust modular perfusion-based production platform for biopharmaceuticals demanding highest quality at industrial scale.
Beyond using stable cell lines and well-defined process media, this platform is built on both knowledge- and risk-based principles. Main components comprise the identification of critical process parameters (CPPs) and consequent robustness testing, the use of well-characterized, scalable perfusion devices and sound solutions for on-line biomass and perfusion control as well as for continuous product capture.
Having regard to actual quality by design related issues, the main criteria for achieving predictable robustness with our continuous production platform will be disclosed and discussed.

P4.109. ON-LINE MONITORING OF THE LIVE CELL CONCENTRATION IN BIOREACTORS BASED ON A ROCKING PLATFORM

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The use of RF Impedance or capacitance to monitor cell culture processes is well established in biopharmaceutical applications. With the increasing trend toward using disposable bioreactors, there is now a need for single use biomass probes suitable for these systems. This will ultimately enhance the opportunity to use these bioreactors in both process development and ultimately in cGMP manufacturing.

Single-use biomass probes are now available for use with capacitance based instruments for measuring the live cell concentration and publications to date have been based on bioreactors using agitators. Adoption has been slower than expected but in many cases the initial evaluation of the disposable technology has been at the small to mid-scale where traditionally there has been a very low level of automation and instrumentation. In this paper, we show how a biomass probe has been optimized for use with a disposable bioreactor based on the rocking platform.

In order to demonstrate the performance of the disposable biomass probes, data will be presented on the growth of SF-9 insect cells in a 25L prototype Sartorius Stedim Biotech rocking platform bag. The study shows how the positioning of the probe within the vessel is critical for performance and the way the probe deals with varying levels of fluid due to the motion of the bag and increasing volumes during fed batch culture. The poster will also show how the disposable biomass probe is able to track the viable SF-9 cell density of the culture before the addition of a Baculouvirus and how it successfully detected the infection phase of the culture.

P4.110. AN ALTERNATIVE TO OFF-GAS ANALYSIS FOR REAL-TIME OXYGEN UPTAKE RATE MEASUREMENTS IN BENCH-SCALE BIOREACTORS

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Greater insight into the metabolic state of mammalian cell culture can be gained by determining the volumetric oxygen uptake rate (OUR) or the cell-specific oxygen uptake rate (SOUR). The use of off-gas analysis makes it possible to determine the OUR through direct measurement of oxygen concentration in the bioreactor exhaust. This approach is expensive and difficult to implement in bench scale reactors due to the need for dedicated instrumentation. The current work describes an alternative approach using a mathematical model of mass transfer coefficient (k_La), and continuous measurement of bioreactor operational parameters.
Initially, the k_La was measured using standard techniques, at three levels of agitation rate, air flow rate, temperature and working volumes. The resulting full factorial data-set was fit to the following empirical model:

$$k_La = A(P/V)^{\alpha}(Qg/S)^{\beta}$$

Where the (P/V) term represents the mixing power input per unit volume, and the (Q_g/S) term represents the superficial gas velocity. Values for A, α and β were determined from best fit of the data-set to the equation. Software-based automation was then developed that allowed for the real-time calculation of k_La based on instantaneous values of bioreactor operating parameters (including agitation, aeration, temperature and working volume), and the best-fit values for A, α and β. The calculated value for k_La was then used to calculate OUR using the steady-state assumption that $d[O_2]/dt$ at any given time is 0. The SOUR was estimated from the values for the current viable cell concentration measured daily as well as an estimate for the growth rate and peak viable cell concentration. OUR values are populated automatically into the bioreactor database and are available to end users immediately.
Examples of how OUR can be sensitive to changes in CHO cell culture conditions such as nutrient depletion will be shown; also, the feasibility of an automated method for feed delivery that is based on feed-back logic from the OUR results will be demonstrated.

P4.111. UTILIZATION OF MULTIFREQUENCY PERMITTIVITY MEASUREMENTS IN ADDITION TO BIOMASS MONITORING

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In the last couple of years dielectric spectroscopy is being used for online biomass monitoring. As an example, breweries use it as an established method for fermenter inoculation and bioprocess control. For animal cell cultivations the correlation between the permittivity and the offline determined cell densities varies over the course of the cultivation. Hence, several authors used the permittivity signal as a measure for oxygen and glutamine consumption as well as intracellular nucleotide phosphate concentrations. The latest generation of biomass monitoring devices allows multifrequency permittivity measurement that is supposed to improve the correlation between the biomass and the permittivity signal. Further information about the physiological state of the cells is expected to be deducible from the diverse parameter of the permittivity measurement.
In our study we reviewed the FOGALE nanotech online biomass monitoring system in 2 L benchtop bioreactor cultivations. The system was tested for human, chinese hamster ovary and hybridoma cell lines. A good correlation of the permittivity and the offline measured viable cell density for the growth phase was verified, while a pH-shift and increasing cell aggregation had a negative impact on the correlation. Subsequently the online biomass monitoring system was used to realize a closed-loop control of the cell bleed in a 2 L spin filter perfusion process, resulting in a constant viable cell density of about $1 \cdot 10^7$ cells/mL during the perfusion.
Additionally a linear relationship ($R^2 > 0.96$) between the online signal and the concentrations of a number of substrates, e.g. alanine, asparagine, serine and glycine, was found during the exponential growth phase of CHO-K1 cultivations. These correlations are currently under investigation for developing a closed-loop feeding strategy. Compared to a pre-defined feeding schedule or intermitting feeding this would have the advantage of avoiding nutrient limitations and substrate accumulation that might occur due to unexpected high or low cell growth.

P4.112. CREATING NEW OPPORTUNITIES IN PROCESS CONTROL THROUGH RADIO-FREQUENCY IMPEDANCE SPECTROSCOPY

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Radio frequency (RF) impedance spectroscopy is a measuring technique that allows on-line monitoring of cells in a variety of fermentations. Specifically, RF impedance spectroscopy measures the biomass volume of viable cells in suspension through its capacitive signature. To date, monitoring the impedance of cell suspensions usually involves measuring the capacitance at single or dual frequencies. While monitoring the total biomass volume of cells can be accomplished through single frequency readings, a full spectroscopic interrogation leads to a better understanding of the principle driving factors of changes observed in the biological process.
In this paper we show how impedance scans at multiple frequencies between 50 KHz and 20 MHz break the ambiguity inherent in biomass measurements between the cell number density (cells/mL), cell diameter, and internal conductivity. We present data from a new software package called Futura SCADA which allows frequency scanning and analysis simultaneously on multiple fermenters. The data highlights the importance of distinguishing the different physical properties of the cell, such that fermentations may focus on the cell characteristics that are most important to the desired product.

P4.113. EVALUATION OF AN ONLINE BIOMASS PROBE TO MONITOR CELL GROWTH AND CELL DEATH

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The estimation of cell density and cell viability of mammalian cell lines in cell culture has traditionally been performed using the exclusion dye trypan blue that stains "dead" cells when their cell membrane is damaged. In large scale cell cultures using bioreactors this estimation is performed off-line. The online biomass probe is based on the principle that under the influence of an electric field between two electrodes, ions in suspension migrate toward the electrodes. The cell plasma membrane is non-conductive so that the cells with intact plasma membranes are polarized and act as tiny capacitors and it has been shown that capacitance increases as the cell concentration does. The measurement is based on the linear relationship between the permittivity difference $\epsilon 1 - \epsilon 2$ and the viable biomass concentration.
This study compares the data obtained using the biomass probe against the cell counts and viability determined by trypan blue exclusion, and also with apoptosis determination measurements using rhodamine-123 and pan-caspase activation by flow cytometry for a number of mammalian cell lines.

P4.114. IN-SITU CELL DENSITY MONITORING AND APOPTOSIS DETECTION IN ADHERENT VERO CELL BIOREACTOR CULTURES

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Vero cells are one of the most employed cell platform for viral vaccine productions. Nevertheless, because of its adherent characteristics, very few techniques were developed for the in-line monitoring of their cell density in comparison to the methods proposed for suspension cells. However, viable cell concentration is a very important parameter in cell-based processes, as it is impacting several strategic events like feed additions, scale-up time or even the time of infection for processes dedicated to vaccine productions. Cell death and more specifically apoptosis, has also a great impact on cell-based process course, but at the moment, no in-line quantification or detection technique exist for mammalian cells.
This work proposes for the first time, in-line monitoring of Vero adherent cell density and apoptosis, based on multi-frequency permittivity measurements, using the Fogale Biomass System®. Cellular characteristics as cell size, cell membrane state or intracellular conductivity were proved to have a great impact on permittivity measurements, and so on cell density quantification. So, different operating conditions, impacting Vero cell physiology, were tested, such as medium feed-harvest or medium formulations.
Thus, with permittivity measurements, it was possible to monitor Vero cell density until 90×10^3 cell.cm^{-2} in our reference medium. A different medium formulation was observed to modify the Vero cell permittivity, while increasing the cell density monitoring limit to 110×10^3 cell.cm^{-2}. The characteristic frequency of the cell population, fc, determinated through permittivity frequency scanning, also provided additional indications on the cell physiology while, for all culture conditions tested, fc was a good indicator of the beginning of massive Vero cell apoptosis in the bioreactor.
These results demonstrated that permittivity measurement is a valuable tool for in-line monitoring of Vero cell growth and apoptosis, and that it has a great potential for PAT development in cell-based culture processes and for trouble shooting strategies in vaccine production processes.

P4.115. IMPROVED BIOPROCESS MONITORING THROUGH THE USE OF FLOW CYTOMETRY

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Production processes for the manufacturing of biotherapeutics rely predominantly on recombinant mammalian cells for the synthesis of drug substance. Despite the central importance of cells in manufacturing processes for biologics, the only cellular assays that are routinely performed are counts of cell number and crude viability measurements. Implementation of routine and comprehensive cellular monitoring during process development and manufacturing has the potential to enable improved process control and increased product yield and quality.
Flow cytometry is a cellular analysis tool that has found extensive use in academic, clinical, and industrial research settings. Traditionally, flow cytometers were expensive instruments that were difficult to use, which limited their adoption in bioprocess monitoring environments. The Guava flow cytometer from EMD Millipore was the first bench top instrument with reduced complexity, and it has helped make flow cytometry more accessible to a wider variety of users. Application of flow cytometry to bioprocess monitoring can provide more complete cell health information than currently used methods.
We have used flow cytometry to analyze typical samples taken from seed and production bioreactors. The flow cytometer demonstrated an improved ability to accurately count cells and measure fractional viability relative to trypan blue exclusion-based instrumentation. The improved viability assessment capabilities of flow cytometers are especially pronounced for low cell density samples such as those from early in a bioreactor run, and for samples with moderate amounts of apoptotic cell death such as those from late in a production run. Furthermore, flow cytometry is capable of providing insight into the cause of cell death if and when it does occur.
Additional cellular health assays are available that can be performed using flow cytometry. These include assays for mitochondrial membrane potential, intracellular pH, cell-surface antigens, several different apoptosis-specific markers, oxidative stress, and cell cycle stage. Of these more advanced assays, cell cycle analysis in particular has shown utility as a leading indicator for changes in cell number and fractional viability. As such, we have found cell cycle analysis to be especially useful in monitoring of seed train vessels.
Our results demonstrate that routine, comprehensive cellular analysis enables improved process control. More widespread use of flow cytometry to monitor the physiological state of cells during all stages of bioprocess development, from clone selection to manufacturing, will lead to advances in industrial process understanding that in turn will likely result in further improvements in process and product quality.

P4.116. CONTINUOUS OUR MEASUREMENT USING OFF-GAS ANALYSIS AND ITS APPLICATION FOR MONITORING AND IMPROVING CELL CULTURE PROCESSES

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Continuous OUR data can give real-time information on the metabolic state of the cells in the culture. With up to 10-fold increase in cell densities on newer processes (from ~3x10^6 cells/ml to ~30x10^6 cells/ml), continuous measurement of Oxygen uptake rate (OUR) and Carbon dioxide evolution rate (CER) and its use for process improvement is becoming more feasible. Measurement of OUR with exhaust gas analysis is truly non-invasive and compared to dynamic measurement does not need to impact the culture as dissolved oxygen levels do not have to be manipulated.

Proof of concept for continuous measurement of OUR has been demonstrated for several cell lines (CHO, HEK293) using a gas analyzer capable of quantifying oxygen and carbon dioxide in the off-gas from a cell culture bioreactor. We have explored different applications for continuous OUR measurement including the use as a PAT tool for real time process consistency monitoring, as an application for process optimization, specifically nutrient feed strategy, and better insight into scale-up and process transfer.
Results of these experiments and challenges overcome to implement technology will be discussed.

P4.117. USE OF FOCUSSED BEAM REFLECTANCE MEASUREMENT (FBRM) FOR MONITORING CHANGES IN BIOMASS CONCENTRATION.

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The development of cell culture technology underpins the success of the rapidly growing and lucrative biopharmaceutical industry. It is essential to maintain high levels of biomass in order to maximize the productivity of a bioprocess. At high levels of biomass, the culture environment can change rapidly due to the large metabolic load, making it difficult to maintain the optimal environment by feeding based on off-line measurements and the benefits of on-line measurement intensify. FBRM is a probe-based technology with the potential to provide non-destructive, non-invasive, continuous real-time monitoring of biomass concentration. FBRM utilises laser light backscatter technology to supply a chord length distribution (CLD) in real time by projecting a highly focused, monochromatic laser into the suspension through a set of rotating optics and robust sapphire window. When the beam intersects a particle, the laser light is backscattered in many directions, some of which reaches a discrimination circuit in the probe which converts the duration of the backscatter to a chord length. Typically, thousands of particles are measured during one measurement duration and the results are presented as a CLD. The CLD is dependent on the solids concentration and on the size, shape and nature of the particle.
The potential of focussed beam reflectance measurement (FBRM) as a tool to monitor changes in biomass concentration was investigated in a number of biological systems. The measurement technique was applied to two morphologically dissimilar plant cell suspension cultures, *Morinda citrifolia* and *Centaurea calcitrapa*, to a filamentous bacteria, *Streptomyces natalensis*, to high density cultures of *Escherichia coli* and to a murine Sp2/0 hybridoma suspension cell line, 3-2.19. In all cases, biomass concentration proved to be correlated with total FBRM counts. The nature of the correlation varied between systems and was influenced by the concentration, nature, size and morphology of the particle under investigation.
It was demonstrated that biomass concentrations in plant cell cultures of both *C. calcitrapa* and *M. citrifolia* correlated well with total counts measured. Biomass levels in *C. calcitrapa* cultures of 10 - 160 g FCW L^{-1} were linearly correlated with total counts. A linear correlation was observed for cultures of *M. citrifolia* in the range of 0 - 100 g FCW L^{-1} while at higher levels of 100 - 300 g FCW L^{-1}, a non-linear correlation was observed. FBRM tracked biomass levels of the filamentous bacteria *S. natalensis*, over the course of its growth in cultures with dry cell weights up to 10 g L^{-1} and containing mycelial aggregates of approximately 100 to 200 □m in size. The total counts measured were directly related to the dry cell weight. At-line monitoring of pilot scale high density E. coli cultures with FBRM demonstrated sensitivity to biomass levels up to 45 g DCW L^{-1}. FBRM proved to be sensitive to changes in both total cell density and viability of a hybridoma culture in both off-line and in-situ studies. However, due to the challenges associated with detecting a particle whose refractive index is similar to its environment, the response of the probe to changes in both the total cell density and viability was confounded in the total FBRM counts statistic. There was a positive linear correlation between total cell density and total FBRM counts and a negative linear correlation between viability and total FBRM counts when the effect of the other was removed.

P4.118. MOBUIS® SENSORREADY TECHNOLOGY: A NOVEL APPROACH TO MONITORING SINGLE-USE BIOREACTORS

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EMD Millipore has developed a novel bioreactor monitoring approach, SensorReady technology, which is used to monitor and control its Mobius CellReady 250L single-use bioreactor system. The SensorReady technology is an external circuit containing sampling ports and a configurable number of additional probe port options sterilely connected to the CellReady bioreactor at the time of use. The SensorReady options include redundant pH and dissolved oxygen (DO) probes, optical single-use sensor technology and supplementary probe ports as desired. This modular design provides the user with ultimate flexibility to monitor and control their bioreactor processes with a variety of both conventional and cutting edge sensor technologies. SensorReady technology also contains a "bearingless" pump with a low shear rate, which is based on magnetic levitation, and a non-invasive flow measurement device that ensures a continuous and efficient flow of well-mixed bioreactor contents to the sensors. Here we present data from several studies conducted to demonstrate the utility of the SensorReady technology for real time measurements used to monitor and control bioreactor conditions. Concerns around the potential impact of the pump on mammalian cell viability through the introduction of shear stress were mitigated through in-depth cell culture studies. Additionally, we investigated the potential effects of shear stress on cells using a contractional flow device with several cell lines. The results of these studies have demonstrated that the SensorReady technology can be used effectively to monitor bioreactor operation with negligible impact on cell viability.

P4.119. ON-LINE AND REAL TIME CELL COUNTING AND VIABILITY DETERMINATION FOR ANIMAL CELL PROCESS MONITORING

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Two of the key parameters to be monitored during cell cultivation processes are cell concentration and viability. Until today, this is very often done off-line by sterile sampling and subsequent counting using a hemocytometer or an electronic cell counter. Cell biology lacks a measurable quantity by which single cells in suspension can be non-invasively diagnosed as dead or alive. However, it would be of significance for process monitoring and in the light of initiatives like PAT if cell density as well as viability could be determined directly and on-line.

Optical measurement of cell density by *in situ* microscopy eliminates the need for sampling and allows for continuous monitoring of this key parameter; see e.g. Guez et al. 2004, Joeris et al. 2002; Guez et al describe an in situ microscope which does not use any moving mechanical parts within or outside the fermentation vessel. It transmits in real time images taken directly in the stirred suspension within the bioreactor. Image data is processed and evaluated to provide monitoring of cell-density and morphological parameters, e.g. cell size by means of assessing the obtained *in situ* cell-micrographs.

Previously, we have extended *in situ* microscopy towards viability assessment of suspended cells (Guez et al. 2010). Now, we present new findings on this topic and show that in cultures of suspended cells, cell-death corresponds to a transition between two levels of entropy of *in situ* cell-micrographs. By analysing the transient entropy-histograms over the course of hybridoma, Jurkat and CHO cultures we show that entropy states represent live and dead cells, respectively. For research and process applications, this work introduces non-invasive live/dead-classification of suspended mammalian cells in real time.

References:

Guez JS, et al (2010) Process Biochemistry 45:288-291; Guez JSet al (2004). Journal of Biotechnology 111:335-343.
Joeris K, et al (2002). Cytotechnology 38:129-34.

P4.120. MODELLING AND CONTROL OF A PAT ENABLED BIOPROCESS

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The biopharmaceutical industry is rapidly expanding and to date, a large proportion of the products are produced in mammalian cell cultures which demand a high level of process management. Mammalian cell culture processes are difficult to characterize and demonstrate large variability between batches, leading to up to 30 % of batches failing or requiring some degree of reprocessing. This has a significant impact on the cost of production. To reduce batch variations and improve process economics, many biopharmaceutical manufacturers are moving beyond today's "quality-by-inspection" methodology and adopting Quality by Design (QbD) methods under the FDA's PAT initiative. These methods centre on measuring critical quality and performance attributes during processing. Timely availability of such data also creates an opportunity to increase the sophistication of automation and control used in such processes.

The aim of the work presented here is to investigate the benefit of implementing a number of control strategies on a CHO320 fed-batch process producing interferon □. The process had a fixed set-point of either glucose or glutamine in order to limit their concentration under all control strategies investigated. The bioreactors used in the study were a 3 L bench-top and a 15 L pilot scale system with standard pH, DO and temperature control. The real-time glucose/glutamine concentrations were determined by FT-IR or Raman spectroscopy.

A number of closed loop control strategies were evaluated, mainly, traditional auto-tuned PID control and advanced linear and nonlinear model predictive control (MPC) for the control of glucose/glutamine concentration to a fixed set-point within the bioprocess. PID seeks to minimise the instantaneous error between the measured process value and the desired set-point. MPC takes a longer term view, the extent of which is determined by the control and prediction horizons specified. It aims to minimise the difference between the measured value and the set-point over the prediction horizon. The prediction is made by applying a model to the process. In this case, a finite step response (FSR) model was used for the linear MPC and a nonlinear unstructured unsegregated model describing the dynamic evolution of the main substrates (glucose, glutamine, ammonia, lactate) and biomass was developed for the nonlinear MPC.

Based on simulations conducted using Matlab and Simulink, the performance indices for PID, linear and nonlinear MPC were calculated. The ISE values were 0.0674, 0.4516 and 0.0232 respectively for glutamine fixed setpoint control. A similar trend was observed for glucose fixed setpoint control. It is evident that nonlinear MPC had the lowest performance index and thus was the optimum strategy for set-point tracking for the bioprocess under investigation. Experimental verification of the simulations is currently underway.

P4.121. ROLE OF INTERCONNECTIVITY IN ENABLING IMPROVED BIOPROCESS QUALITY

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An increasingly common discussion ongoing amongst bioprocess scientists and engineers alike is the idea that quality can not be tested in to a product but must instead be deployed throughout a process. As if to emphasize this point, regulatory agencies in the U.S. and Europe have tasked our industry with achieving that most elusive of objectives, the ability for real time release of final product. To accomplish this objective, the U.S. FDA has teamed with Europe's EMEA to provide bioprocess professionals with expanded guidelines in the form of the Process Analytical Technologies Initiative (PAT) and proven concepts such as Quality by Design (QbD). Both of which emphasize the need on the part of the bioprocess professional to understand that quality should be built into a product through a thorough understanding of the product and process by which it is developed and manufactured along with a knowledge of the risks involved in manufacturing the product and how best to mitigate those risks.
The purpose of this talk will be to introduce the role of interconnectivity in enabling translation of *Scientific Know Why* into *Technological Know How* and thereby improving bioprocess quality. To accomplish this we will define interconnectivity as it relates to bioprocess management in general, while utilizing a Case Study involving a bioreactor control system and bioprocess analytical instrument to illustrate a specific application. The content of which will focus on the role of interconnectivity in enabling achievement of the objectives set forth in the PAT initiative and subsequent Pharmaceutical Quality for the 21st Century: A Risk Based Approach.
Those attending this talk should expect to come away with an improved understanding of the practical role interconnectivity can play in their bioprocess management applications. Specifically, its ability to enable achievement of the quality objectives set forth in the PAT initiative and Risk Based Approach by the regulatory agencies which govern our industry.

P4.122. THE WAY TO A DESIGN SPACE FOR AN ANIMAL CELL CULTURE PROCESS ACCORDING TO QBD

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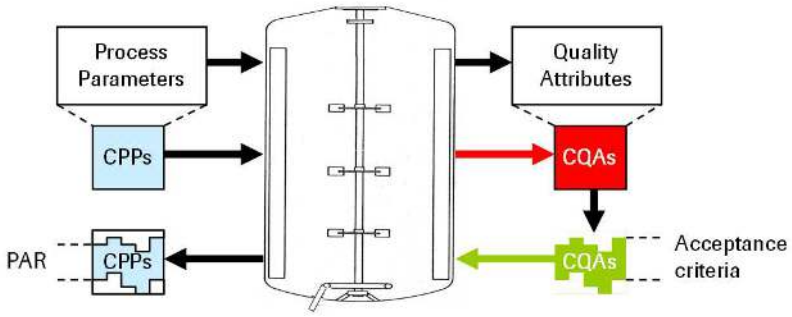
The presentation provides an overview of our strategy regarding the implementation of the QbD (Quality by design) approach in upstream processing of therapeutics proteins. This approach consists of the identification of critical process parameters (CPPs) that have a statistically significant influence on the critical quality attributes (CQAs) of a specific process. By applying the acceptance criteria to the CQAs, proven acceptable ranges (PARs) for the critical process parameters can be deduced from experimental data. The multidimensional combination of theses ranges form the design space and thus assures the quality of the product (Fig. 1).



The QbD approach according to Q8, Q9 and Q10 may be subdivided in scale down model qualification, risk analysis, process characterization and range studies. The foundation of the QbD approach is represented by the scale down model. Several different scale down criteria were applied and adapted until a satisfactory match of scale down to commercial scale data was achieved. The scale down model is then used to investigate cause effect relationships between process parameters and quality attributes of the production process. Since a standard cell culture process from thawing of the vial up to the final production fermenter can comprise up to 100 process parameters, a risk based approach is helpful to filter the most important ones. Those parameters are then experimentally investigated to verify their criticality for the quality attributes of the process. This approach relies on design of experiment (DoE) to reduce the number of required experiments to a manageable number while maintaining meaningful results. During the range studies, those critical parameters will be investigated with the help of a high resolution DoE matrix so as to be able to reveal possible interactions and higher order effects. Case study data will be shown and the benefit for future processes will be discussed.

P4.123. IMPROVING CELL CULTURE PROCESS ROBUSTNESS USING A QUALITY BY DESIGN APPROACH

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In an effort to develop a deeper understanding of cell culture processes, a methodical strategy has been employed in design space development for late stage biotherapeutic programs. Application of this strategy will be discussed in which qualification of scale-down cell culture models are developed using multivariate analysis techniques to enable process characterization studies. Experiments designed using a rational statistics-based approach are then executed, followed by generation of mathematical models describing the impact of input parameters on process responses. Experimental verification of the quantified relationships is then performed, and finally the design space based is defined based on limitations imposed by critical quality attributes. In the course of defining the design space, opportunities for process improvement have been identified and a greater understanding of the complex relationships within the process has been achieved.

P4.124. "QUALITY BY DESIGN" TO INCLUDE CELL CULTURE MATERIALS THROUGH STRONG PARTNERSHIP BETWEEN BIOTHERAPEUTIC PRODUCERS AND SUPPLIERS

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Mammalian cell cultures have become the main platforms to produce biotherapeutic proteins. Cell culture media and feeds have great impact on biotherapeutic product titer and qualities. Recently, most of biopharmaceutical companies have implemented programs on "quality by design" to develop and implement more robust biotherapeutic production systems that support reliable products and supplies. Historically, cell culture media and feeds include some undefined materials such as sera, hydrolysates and protein extracts, which make them difficult to be included in defined quality design space of biotherapeutic production. Chemically defined cell culture media and feeds as the new trend of the biopharmaceutical industry give the promise of implementing "quality by design" to include cell culture materials. However, analytical tools and methods are still limited for chemical raw materials and final cell culture media and feed analysis due to cost and technology constraint.

Recently, we have demonstrated that, with strong partnerships between biotherapeutic producers and cell culture suppliers, we can select the right advanced analytical tools and methods that allow us to narrow down the limited factors or contaminants in cell culture materials that have major impact on biotherapeutic productions. Through close partnerships and collaborations, we can leverage the analytical tools and methods, cell culture performance data and cell culture (media, feeds and process) development results from both biotherapeutic producers and cell culture suppliers. By working together, we can identify critical components or contaminants for your cell line and cell culture process and implement specific analytical methods to monitor the specific raw materials, contaminants or cell culture media and feeds.

By including cell culture material control and measurements into the "quality by design" systems of biotherapeutic production, we can define the design space for the whole biotherapeutic production process can be defined to thus secure the quality and consistent supplies of the biotherapeutic drugs. Working with regulatory agents using the specific cell culture material measurements combining with other quality parameters in upstream, downstream and formulation processes, biotherapeutics can be approved and launched faster. Although the concept of "quality by design" including cell culture materials is still in its early stages, we have already seen its promises and benefits. The successes require close partnerships and qualified cell culture suppliers who are very good on quality systems with in-depth understanding of regulatory demands and appropriate handling of raw materials, product development and manufacturing processes.

P4.125. DOE OF *FED-BATCH* PROCESSES - MODEL-BASED DESIGN AND EXPERIMENTAL EVALUATION

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Experimental process-development and optimization is expensive and time-consuming. Real optimization by means of design of experiments involves data generation before optimization can be aimed for. This can make the way from process development to process establishment even harder, since academia or start-up research facilities might not have the possibility to generate these data. Furthermore, bioprocesses involving mammalian cells deal with many critical variables; processes are not only carried out batchwise, but increasingly in fed-batch mode with desired feeding profiles.
The use of DoE tools in combination with an appropriate growth model might allow the experimenter to develop and to test *fed-batch* strategies *in silico*, before experiments are carried out in the laboratory.
In our work, an unstructured model for mammalian cell culture was used. After short shake-flask experiments for kinetic parameter determination of a human production cell line (AGE1.HN, ProBioGen AG), the model was tested for data generation on common *fed-batch* strategies. By means of design of experiments strategies, relevant conditions were selected and experimentally tested. In this way, suitable *fed-batch* strategies for mammalian cell lines are evaluated *in silico* before bioreactor experiments are to be performed. This results in a significant reduction in the number of experiments during process development for mammalian cell culture.

This work is a part of SysLogics: Systems biology of cell culture for biologics, a project founded by the German Ministry for Education and Research (BMBF).

P4.126. „BIOPROZESSTRAINER" AS TRAINING TOOL FOR DESIGN OF EXPERIMENTS

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Design and optimization of cell culture processes requires intensive studies based on "Design of experiments"-strategies. In academia teaching of DoE-concepts is often insufficient, as in most cases only simple culture strategies (batch) can be performed, as time and money are limited. More complex tasks such as feeding strategies for fed-batch culture can be discussed theoretically only. To close this gap the virtual "BioProzessTrainer" [1], a model based simulation tool, was developed. It supports biotechnological education with respect to process strategies, bioreactor control, kinetic analysis of experimental data and modeling. Along with a set of examples for different control and process strategies (*batch*, *fed-batch*, chemostat etc.) learners are prepared for real experiments. The "BioProzessTrainer" helps to improve the quality of education by using interactive learning forms and by transmitting additional knowledge and skills. Costs for practical experiments can be minimized by reducing plant operation costs. Finally the value of simulation techniques is shown.
Here a concept for teaching DoE-concepts for *batch*- (optimization of e.g. substrate concentrations and inoculation cell density) and *fed-batch*-processes (evaluation and optimization of feeding strategy) using the "BioProzessTrainer" is shown.

[1] Hass, V. und R. Pörtner: Praxis der Bioprozesstechnik. Spektrum Akademischer Verlag (2009)

P4.127. SCALE-DOWN MODEL ASSESSMENT FOR QBD CELL CULTURE CHARACTERIZATION: A CASE STUDY FOR A MONOCLONAL ANTIBODY PRODUCTION PROCESS

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The goal of using a Quality by Design (QbD) approach for cell culture process characterization is to gain overall process understanding, with the final outcomes being both univariate and multivariate acceptable ranges for each parameter tested, and a design space which ensures acceptable process performance and product quality. Due to the sheer number of studies required, the use of scale-down models makes such characterization effort possible. However, the applicability of the QbD characterization study results to the full scale depends on the validity of the scale-down models that are used. This case study will present the scale-down model comparison approach and the strategy that is used to allow meaningful translation of the scale-down QbD study results to the full scale.

P4.128. TRANSIENT TRANSFECTION OF HUMAN CAP-T CELLS - BASICS AND OPTIMIZATION

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As a contract manufacture of recombinant proteins and antibodies InVivo Biotech ever since employed transient expression technology for prompt production of small and medium size batches. Therefore we have strong interest in continuously optimising expression efficiency aiming on a reliable process capable for production of a broad variety of recombinant protein at maximum quality. For transient transfection HEK cells as well as variants thereof like HEK-EBNA and 293-F are the most commonly used cell lines. Recently a new cell line, CAP-T, developed by CEVEC Pharmaceuticals GmbH, Cologne, arose as new potent platform for transient gene expression. CAP-T is a human amniocyte cell line, derived from amniotic fluid cells and immortalised using adenoviral functions. In addition, CAP-T cells stable express SV40 large T antigen. The cell line is characterised by good transfection capabilities, easy cultivation in serum free suspension and high viability even in extensive expression processes. In addition these cells are specially promoted for most human like glycosylation pattern and therefore are a promising tool for production of complex mammalian proteins for all kinds of purposes. In combination with expression plasmids harboring the SV40 ori a high long-term plasmid yield after transfection is achieved subsequently resulting in surprisingly high titers of recombinant proteins.
Consequently we started to use CAP-T cells for transient gene expression. Here we report experiments leading to a solid process allowing cost effective and standardised production providing efficiencies beyond conventional expression systems. Cultivation of CAP-T cells was established in suspension with densities up to 2 x 10^7 cells per ml. Cultivation as well as transfection for analytical purpose was performed in a small scale of 4 - 8 ml culture volume in vented 50 ml tubes and preparative test batches were realised in up to 1,5 l culture volume in shaker flasks. We optimised the transfection process regarding multiple parameters, such as cell density, media, amount of plasmid DNA, PEI to DNA ratio, etc as well as comparing different suppliers of PEI (polyethyleneimine) and derivates. With the deduced transfection protocol we subsequently performed further optimisation of productivity. Supplementation of the culture medium in various ways was assayed to improve viability of cells and extension of the expression period. The optimised protocol for transient transfection based on those findings was verified by preparative production of different proteins: Among others an immunoglobulin (IgG), procalcitonin (PCT), a small sized prohormone, thrombomodulin (TM), a highly glycosylated complex recombinant protein, and the secreted alkaline phosphatase (SEAP) as reporter protein in general. In conclusion our optimised protocol for protein expression in CAP-T cells enables a significant improvement of process efficiency compared to other systems.
Furthermore in order to cut down costs and become independent from commercially available transfection reagents we are all about to develop new innovative polycationic transfection reagents in cooperation with emp Biotech GmbH (Berlin), which are also tested using CAP-T cells and already show very promising results in transient transfection (see poster: Möller, U. *et al*).

P4.129. OPTIMISATION OF CELL ENVIRONMENT FOR TRANSIENT RECOMBINANT PROTEIN PRODUCTION IN CHO CELLS USING DOE.

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With ever increasing need for cost effective and rapid production processes, it is essential to look for alternatives to stable expression to generate the quantities of therapeutic candidates needed for preclinical studies. Transient expression in CHO cells has not yet proved to be a viable generic alternative, because no one to date rationally developed methods of optimisation taking into consideration the complexity of the process, as well as the interdependency of the variables affecting its efficiency. In this study, DOE response surface and factorial designs were adapted to the optimisation of a PEI-mediated transient transfection process. A computer program specifically written to analyse response surface designs proved to be successful at selecting accurate mathematical models for the identification of optimal conditions that maximised process efficiency. Methods presented here may become a standard for the rapid and low cost optimisation of transient transfection processes.

P4.130. CHARACTERIZATION OF ANTI-DIGOXIN MONOCLONAL ANTIBODY CLONES OBTAINED BY PHAGE DISPLAY TECHNOLOGY

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Introduction: Digoxin is a pharmaceutical used in the control of cardiac disfunction. Its therapeutic window is very narrow, with therapeutic dosage very close to the toxic dosage. To counteract the toxic effect, a polyclonal anti-digoxin Fab fragment is injected by intravenous route. This work aims to obtain anti-digoxin Fab fragments selected by phage display technology. The monoclonal nature of this product would account for a specific potency and more precise dosage for the detoxicating of patients under digoxin treatment. The phage display technology allows the selection of high affinity and specificity antibody sequences to a determined antigen and its production in unlimited amounts. This technology makes use of filamentous phages able to incorporate fragments of exogenous DNA and expose the synthesized protein on its surface, like antibody fragments, that can be selected by the appropriate antigen.
Objective: To obtain variants by phage display and characterize anti-digoxin Fab fragments by its affinity.
Methodology: An anti-digoxin mAb was generated at the Heart Institute (Sao Paulo, Brasil). This work started with the total RNA extraction for the cDNA synthesis. Specific primers were used for the light chain and Fd amplifications, then cloned sequencially in a phagemid vector (pComb3XTT) for the combinatorial Fab library construction. Clones displayed on the surface of phages were selected by the binding to the antigen (digoxin-BSA conjugate). Random selected clones were evaluated for the presence of light and heavy chains and the positive clones were sequenced. The clones displaying variation in the variable regions were induced to produce soluble Fabs.
Results and Discussion: The constructed library was analyzed for anti-digoxin expression. Out of 10 clones randomly chosen, 6 resulted positive. The sequence showed 2 identical clones and one presented a pseudogene in the light chain. Four clones presenting variations in the framework 1 were induced to express soluble Fabs, which were positive for anti-digoxin binding in ELISA assays. The clones will be further analyzed by its production capacity and kinetic analyses by BIAcore technology to evaluate affinity association and dissociation constants.
Financial support: CNPq, Brasil

P4.131. SIMPLIFY YOUR 'MINICIRCLE' PRODUCTION: A NEW SEMI-SYNTHETIC PROCESS FOR MANUFACTURING MINICIRCLES

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MiniCircles (MCs), nonviral replicating circular S/MAR-episomes completely free of prokaryotic sequences, are fascinating new vector tools in the field of mammalian gene expression. These extra-chromsomal DNA circles utilize the replication apparatus of the host cell to divide and segregate synchronously with the cell cycle (hitchhiking principle). The absence of prokaryotic vector parts overcomes cellular and immune defense mechanisms that would otherwise lead to silencing. The more common nondividing MiniCircles have been used for years in preclinical gene transfer research as alternative to regular plasmids because of their 10- to 1,000-fold enhanced, long-term expression capacity in quiescent tissues. This is explained by the absence of gene silencing triggered by plasmid backbone including increased contents of non-methylated CpGs. Traditionally, MCs are generated from a parental plasmid (PP) by site-specific recombination in bacteria. This process yields two circular products: a miniplasmid (MP) carrying the undesired backbone sequences, and the MiniCircle (MC) containing the eukaryotic gene-of interest. MCs of this origin have to be purified, i.e. separated from the MP counterpart and from non-reacted educt (PP). The necessary efforts present a substantial disadvantage of bacteria-based methods, the yields are low and the purity is sometimes inadequate.
To circumvent complications of this type, a novel, semi-synthetic route for manufacturing MiniCircles has been developed, which requires neither recombination nor complex and cost-intensive separation steps such as affinity chromatography. Our routine enables the generation of supercoiled (ccc-)circles at high concentration and excellent purity for a wide range of purposes. Common, time-consuming cloning steps such as the appropriate placement of two recombinase target sites are no longer necessary and the procedure lends itself for processing plasmids of any origin. Of particular relevance are the facts that i - contaminations by bacterial sequences are safely prevented, that ii - this approach enables an adjustable highly defined topoisomer distribution and iii - that it reduces the vector´s propensity to integrate into the genome of the target cell. Overall, this new semi-synthetic process provides a straightforward, cost-effective and time-saving alternative for generating fully characterized, high-purity minicircles for use in the fields of gene therapy, cell line development and iPS generation, DNA vaccination and RNA-interference experiments. Application of the advanced replicating (S/MAR-containing) MiniCircle variant enables the generation of cell lines, which share properties both of transient and stable expression systems as stable cell lines can be established following the initial transcriptional burst.

P4.132. IMPLEMENTATION OF RECOMBINASE MEDIATED CASSETTE EXCHANGE SYSTEMS IN SF9 CELLS FOR EXPRESSION OF MULTIPLE RECOMBINANT PROTEINS

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Baculovirus-mediated expression in insect cells has been widely employed as production platform for recombinant proteins, mainly because of the high cell densities reached in serum-free suspension cultures and the relative short timelines required to establish a new protein production process. However, inherent drawbacks of this system include the effort to maintain the baculovirus stock, the lytic infection mode of baculoviruses which can compromise the quality of the product due to cellular proteases release, and the recognized difficulties to separate the recombinant protein from virus proteins during the purification process.
Stable expression in insect cells using recombinase-mediated cassette exchange systems potentially represents a valuable alternative to the BEVS. This technology enables the repeated use of pre-characterized genomic sites with good expression rates to produce multiple recombinant proteins. Therefore, comparable development timelines can be achieved with the advantages of a stable expression system.
There are not many studies addressing stable protein expression in insect cells, thus the list of promoters which have proven to work for this purpose is rather short. Five of those promoters were used to drive the expression of reporter proteins in Sf9 cells, employing optimized transfection protocols developed at our lab (either cellfectin- or electroporation-based); the promoters which allowed the best expression levels were selected for the design of the tagging/target cassette constructs. We will present results on the feasibility of using the FLP/*FRT* site-specific recombination system in Sf9 insect cells for consistent production of recombinant proteins.
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P4.133. IMPROVING THE TRANSFECTION EFFICIENCY OF EIAV VECTOR COMPONENTS IN SUSPENSION HEK293T CELL LINES

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Large-scale production of gene therapeutics comprising Equine Infectious Anaemia Virus (EIAV)-based lentiviral vectors would benefit from a suspension manufacturing methodology, facilitating a more flexible large scale process compared with adherent culture. ProSavin® is Oxford BioMedica's EIAV-based therapy for Parkinson's disease. ProSavin® manufacture is carried out by the triple transfection of adherent HEK293T cells. Utilising suspension HEK293T based cell lines would be a more preferable production method as it allows for greater ease of scale-up. However the efficiency of transfection in suspension cells is low and variable.

A suspension packaging cell line containing regulated stable copies of EIAV Gag/Pol and VSV-G envelope vector components has been generated and on transient transfection of EIAV genome, vector is produced. It is thought that improving the transfection efficiency in this suspension packaging cell line will further improve titres. Methods to improve the transfection efficiency in these packaging suspension cells will be described, which will aid the advancement of the development of a suspension manufacturing process for the large scale production of ProSavin®.

P4.134. TRANSIENT GENE EXPRESSION WITH CHO CELLS IN CONDITIONED MEDIUM: STUDY APPROACH USING TUBESPIN® BIOREACTORS

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Transient gene expression (TGE) allows rapid protein production in mammalian cells and has become a very important tool in the pharmaceutical product development pipeline. Polyethylenimine (PEI)-mediated, high-density transfections have allowed for volumetric yields up to processes exceeding 1 g/L within 1-2 weeks of transfection. The challenge for TGE, however, is in its volumetric scale-up. One major issue, at least for most high-yielding TGE methods, is the need to perform the transfection in fresh rather than conditioned medium. In CHO cells we observe up to a 100-fold decrease in protein production for transfections in conditioned medium as compared to those in fresh medium. The reason for such a negative effect of conditioned medium on transfectability and/or protein production expression in general is not known. To study this problem we transfected CHO cells at small-scale in TubeSpin® bioreactor 50 tubes using 41 different serum-free media formulations in combination with different transfection parameters and culture conditions. By comparing the transient production of a recombinant IgG antibody among the different media, we observed variation of up to 400-fold when transfecting in fresh media and up to 20-fold when using conditioned medium. For transfection in conditioned medium, the cell density to which cells are allowed to grow prior to transfection in combination with optimizazion of the PEI:DNA ratio allowed the improvement of yields by about 10-fold. Through this approach we hope to identify critical parameters for overcoming the negative effects of conditioned medium on TGE yields in the presence of partially or fully conditioned medium.

P4.135. NEW APPLICATIONS FOR STAR-SHAPED CATIONIC POLYMERS WITH MAGNETIC CORE RESPECTIVE TO PURIFICATION AND NON-VIRAL GENE DELIVERY

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The controlled and precise delivery of genes is an important step towards efficient gene therapy and genetic modifications of eukaryotic cells. Besides viruses, cationic polymers have been widely explored for that purpose. Gathering information about the intracellular fate of transfection complexes, notably identifying macromolecules (e.g., proteins) which interact with these complexes, will help to understand the intracellular transfection mechanisms.
By using modern polymer chemistry, a precise tailoring of functional groups and topology of the polymers are available. Here, we present magnetic core-shell nanoparticles based on poly(2-(dimethylamino)ethyl methacrylate) (PDMAEMA). Due to the high stability of the particles an unbiased performance of the polymer during transfection can be expected. The magnetic separation is only observed with polymers complexed with DNA (polyplexes), allowing a fast and easy separation from the free polymers without using expensive SEC (size exclusion chromatography) columns. Furthermore, cells containing the magnetic PDMAEMA can be separated via MACS (magnetic activated cell sorting) technique. This system was used to study the uptake of polymers in CHO-K1 cells. One observed effect is that the amount of polymer, taken up by the cells, is not always associated with an efficient delivery of DNA to the nucleus as only a fraction of the "magnetic cells" expresses the transgene. Therefore, we conclude a strong influence of cytoplasmic events involved in the breakdown of the complex and playing a crucial role in efficient gene delivery. The magnetic PDMAEMA used in this study can also be applied for the identification of interacting proteins with the polyplexes. Based on this, a purification process of these proteins was established and will be presented here.
Given the fact that the effects involved in transfection are not yet understood, the new tools and obtained results with magnetic PDMAEMA provide important information for the understanding and optimisation of efficient mammalian cell based production processes.

P4.136. THE USE OF FILLER DNA FOR IMPROVED TRANSFECTION AND REDUCED DNA NEEDS IN TRANSIENT GENE EXPRESSION WITH HEK 293 AND CHO CELLS

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Transient gene expression (TGE) is a rapid method for the production of recombinant proteins. While protein productivity in TGE has improved significantly over the past decade, the amount of plasmid DNA needed for transfection remains relatively high. In order to reduce the amount of plasmid DNA per transfection, we examined the possibility of replacing some of it with herring sperm DNA (non-coding "filler" DNA) for TGE in HEK 293 and CHO cells. Furthermore, we investigated the mechanism of action of the filler DNA by quantifying the intracellular plasmid DNA copy number and transgene mRNA levels and by studying DNA-PEI complex strength. We found that the amount of the expression vector could be reduced considerably in the presence of herring sperm (filler) DNA without a major loss in recombinant protein productivity. The use of filler DNA did not affect delivery of the expression vector or its stability in the cell. However, we observed that its presence led to a significant increase in transgene mRNA levels and recombinant protein titers compared to the control transfection with plasmid DNA alone. Reducing the plasmid DNA amount while keeping the amount of PEI constant resulted in a tighter DNA-PEI complex as measured *in vitro* by the exclusion of ethidium bromide from the complex in the presence of increasing amounts of dextran sulfate. The addition of increasing amounts of filler DNA, however, relaxed the complex. Based on these results, we speculate that the presence of filler DNA results in a more efficient intracellular release of the plasmid DNA from the DNA-PEI complex and in an improved access of the transgene for transcription.

P4.137. OPTIMIZATION OF A SCALABLE SERUM-FREE TRANSIENT GENE EXPRESSION PROCESS IN CHO CELLS

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NovImmune SA, a drug development company focused on immune-related disorders and inflammation, has to set-up and optimize MAb production processes at different levels (from lab-scale to large production). Transfection is the process of introducing foreign DNA into eukaryotic host cell nucleus. The transfection may be stable if DNA is integrated into the host cell genome or transient if it does not occur. At NovImmune, major amounts of r-MAbs are produced by stable transfection during which DNA is linearized in order to integrate into the host genome. However, this method is not readily amenable to high-throughput mode because it requires considerable investment in time (about 3 months), resource and equipment. Furthermore, it may frequently be necessary to rapidly perform *in vitro / in vivo* assays to evaluate the therapeutic potential of each candidate antibody (clinical and preclinical trials, activity testing and formulation assessments). NovImmune SA is optimizing a scalable transient transfection protocol by electroporation in Chinese Hamster Ovary (CHO) for the production of milligram-to-gram amounts of MAbs in a fast, cost-effective and reproducible process.

P4.138. 'MINICIRCLES' - NEW HORIZONS FOR STABLE, RECOMBINANT GENE EXPRESSION

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MiniCircles (MCs) are plasmid derived circular, supercoiled DNA expression vectors which are free of any impairing bacterial backbone. Besides the regulatory and immunological advantages this privileged property leads to an improved expression stability of the MiniCircle vectors especially after in vivo application compared to common plasmid vector systems. Thus, MCs are a main subject of interest in non-viral gene transfer for gene therapy approaches. Enhanced types of MiniCircles, which additionally contain S/MAR elements were shown to be able to replicate episomally (hitch-hiking principle) in proliferating cells in vitro following MiniCircle transfection. This allows an even prolonged if not stable, extrachromosomal transgene expression. The highlighted criteria of MiniCircles are the comparably easy techniques for their production, the simple transduction of host cells, and finally the fast establishment of stably transduced production cells without the need for applying a selection pressure.
Based on the beneficial characteristics of MiniCircles known from gene therapy we proceeded to establish MiniCircles for an application as DNA vectors for the production of recombinant therapeutic proteins in biotechnology. We dominantly focused on industrial-oriented aspects of the development of recombinant producer cell lines and their general process capability such as feasibility, scalability and reliability. In this context results on an improved supercoiled-directed generation of MiniCircles will be presented. The data are based on the application of a completely new semi-synthetic process for manufacturing MiniCircles of a defined quality. The attention is also rivet on the ratio of episomal MC replication to a spontaneous genome integration and on the long-term stability of clones generated by MiniCircle transfection, as well as on the performance and speed of a competitive cell line development using MiniCircles.
The data provide a profound insight into the use of episomally replicating MiniCircles as an alternative to the host cell genome integration of classical plasmid expression vectors for the industrial protein production.

P4.139. RECOMBINASE MEDIATED CASSETTE EXCHANGE (RMCE) FOR ESTABLISHMENT OF POTENT CHO PRODUCER CELL LINES

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The generation of production cell lines for industrial applications is limited by time and costs required for establishment of production cell lines. State of the art production of recombinant proteins involves random integration of transgenes in non-defined chromosomal loci of CHO cell lines previously optimized for growth in suspension and defined media. Since both level and long term stability of transgene expression is highly influenced by the chromosomal elements flanking the integration site, the generated cells differ significantly in these features. Accordingly, intensive screening of cell clones is necessary to identify those cells that meet the requirements. Furthermore, to increase the expression level, amplification of transgene copy numbers with compounds such as MSX or MTX is frequently necessary. Together, the whole process of cell line generation normally takes around 18 months and has to be started for each and any transgene of interest.
Recombinase mediated cassette exchange (RMCE) is a highly efficient method to target expression cassettes of choice to previously identified chromosomal loci in various cell lines. Thereby, the features of a given integration site can be exploited for expression of transgenes of interest. The advantage of this strategy is that time-consuming screening for high expressing chromosomal loci has to be performed just once, while the re-use of this predefined locus can be achieved in 2-6 weeks. Thereby, the tagged cells represent a platform for integration of genes of choice for rapid generation of producer cells with predictable expression.
Here, we describe the identification of high and stable expressing loci in CHO genome by tagging and screening CHO cells with an RMCE compatible tagging plasmid. Several single copy tagged cell clones were targeted with various expression cassettes including a construct coding for an IgG2a antibody and monitored for over three months for productivity and stability. Our results show that this approach allows the rapid generation of high, stable and predictable protein expression from single copy integration sites. This platform provides generation of CHO producer cell lines with high yields of therapeutic proteins in reduced time and effort.

P4.140. DEVELOPMENT OF A POLYCATIONIC REAGENT FOR TRANSIENT TRANSFECTION OF MAMMALIAN CELLS

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For rapid recombinant protein production in small to medium size batches, transient transfection of mammalian cells is still the method of choice in biotechnology. However, the transfection reagent most notably remains a cost intensive bottleneck. As a consequence, there is a strong and continued interest for novel transfection reagents that exhibit higher DNA-transfer efficiencies, with lower toxicity, than the widely used polyethylenimine (PEI), all without the high cost associated with lipofectamines. The project was initiated as a joint effort with emp Biotech GmbH (Berlin), a manufacturer of complex chemicals and various tools for molecular biology and biotechnology. The task at hand for emp Biotech was the design and synthesis of various functionalized polycationic moieties that can mediate between polyanionic plasmid DNA and the negatively charged cell surface, thereby facilitating uptake of DNA into the cell. Two improvements for transfection reagents were of primary interest, namely that of increased solubility and lower cytotoxicity. Synthetic strategies focused on the introduction of hydrophilic functional groups into the polymer structure, such as polyglycols, to enhance solubility, as well as cleavable ester groups to allow rapid intracellular degradation and decrease overall cytotoxicity.
Several different candidates were chemically synthesized and provided to InVivo Biotech. Cytotoxicity was then tested over a broad range of potential handling concentrations. Appropriate candidates were subsequently chosen for evaluation of transfection activity, using a selected range of DNA-to-polymer ratios. Results demonstrate several novel synthetic polymers exhibiting transfection efficiencies similar to or even higher than common PEIs (25 kDa linear polyethyleneimine) after optimized ratios of DNA-to-polymer were applied. Further experiments using secreted alkaline phosphatase (SEAP) as a reporter for transient protein expression have indicated that these substances are at least equal to PEI, and notably one which actually enables higher expression levels when compared to transient transfections using commercially available PEI.
In conclusion, our results using HEK 293, CHO and CAP-T cells demonstrate the development of a promising new alternative transfection reagent for transient protein expression.

P4.141 A VERSATILE EXPRESSION VECTOR SYSTEM FOR MAMMALIAN CELL CULTURE

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To allow for an efficient and high through put method to engineer mammalian cells, we are developing a versatile expression vector system for Chinese hamster ovary (CHO) and other mammalian cells. The vector system is based on traditional vector parts to obtain amplification in bacteria and selection and expression in mammalian cells. However, the USER (uracil-specific excision reagent) cloning technique is applied to obtain maximal flexibility. With this system, vector parts are synthesized separately and can be put together as preferred in a very fast and easy way compared to traditional cloning. This allows fast and dynamic gene, promoter or selection marker swaps. Furthermore, the technique allows easy preparation of fusion proteins in particular. In this way, fluorescence, purification and localization tags can easily be inserted. Upon cloning, the constructed vectors are purified and verified by sequencing and the functionality of the vectors tested in CHO and human embryonic kidney (HEK) cell lines using eGFP as model protein. Initially, the expression vectors will be tested for transient and stable protein expression by non-specific integration. However, with the upcoming release of the CHO genome sequence, the system is planned to be expanded to include site-specific integration and knockout cassettes. In addition to CHO and HEK cell lines, the vector system is planned to be introduced to other cell lines applied in research as well as industry.

P4.142. ANALYSIS OF INNATE IMMUNITY SIGNALING PATHWAYS IN MDCK CELLS IN AN INFLUENZA VIRUS VACCINE PRODUCTION PROCESS

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Despite tremendous progress in basic research concerning virus-host cell interaction over the past decade, only little efforts have been undertaken to characterize cell culture-based influenza vaccine production. Since manufacturers have to operate within limited time frames to provide sufficient amounts of vaccine, fast, reliable and robust production processes are of crucial importance. This requires a detailed knowledge of cellular events relevant for influenza A virus replication. For instance, it has for a long time remained unclear what role the activation of host cellular innate immunity plays in influenza vaccine production and what impact signaling has on virus yields.

In this work, the activation of a number of signaling cascades crucial for influenza A virus replication (NF-κB, IRF-3, PI3K-Akt, Jak-Stat, Raf/MEK/ERK, PKR/eIF2α) was monitored using phospho-specific antibodies. For two variants of influenza virus A/PuertoRico/8/34 H1N1 that replicate to different final titers it could be shown that these pathways are induced stronger by the variant that replicates less well. These results obtained in high multiplicity of infection experiments could be confirmed under bioprocess conditions and in part also at a varying multiplicity of infection. Current efforts are aimed at clarifying the actual significance of the monitored pathways for virus replication by RNAi technology and by the use of small molecule inhibitors. In the long run, these efforts facilitate the understanding of virus replication in cell culture-based vaccine production and might contribute to the design and optimization of production cell lines. [1, 2]

[1] Heynisch B, Frensing T, Heinze K, Seitz C, Genzel Y, Reichl U. Differential activation of host cell signalling pathways through infection with two variants of influenza A/Puerto Rico/8/34 (H1N1) in MDCK cells. Vaccine 2010 Nov 29;28(51):8210-8.
[2] Seitz C, Frensing T, Hoper D, Kochs G, Reichl U. High yields of influenza A virus in Madin-Darby canine kidney cells are promoted by an insufficient interferon-induced antiviral state. J Gen Virol 2009 Jul;91(Pt 7):1754-63.

P4.143. SECRETORY PRODUCTION OF VIRUS-LIKE PARTICLES BY RECOMBINANT INSECT CELLS

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Vaccination is one of the most effective ways of controlling virus infections. Most vaccines for viral diseases are manufactured using infectious pathogens, making them costly and dangerous to produce. Recombinant DNA technology can address these problems by synthesizing viral immunogens and antigens *in vitro*. Expression of viral surface proteins in heterologous systems sometimes leads to spontaneous assembly of the surface proteins into virus-like particles (VLPs), which completely lack the DNA and RNA genome of the virus. VLPs offer several advantages as potential vaccines because they are safe and structurally similar to authentic viruses. In the present study, the production of a secreted form of Japanese encephalitis (JE) VLPs in stably transformed insect cells was investigated. The genes encoding the JE virus envelope glycoprotein (E) and a precursor (prM) of the viral membrane protein (M) were cloned into the plasmid vector pIHAbla. pIHAbla contains the *Bombyx mori* nucleopolyhedrovirus (BmNPV) IE-1 transactivator, the BmNPV HR3 enhancer, and the *B. mori* actin promoter for high-level expression, together with a blasticidin resistance gene for use as a selectable marker [1]. The major surface protein E contains many protective epitopes. During virion maturation, prM is cleaved to M protein by a cellular protease. The cleavage from prM to M is essential to reveal toxic cell-fusion activity of E protein. To minimize the toxicity of the prM/E gene product, a cDNA encoding a mutated pr/M cleavage site [2], as well as the cDNA encoding the native form of prM, was used. When *Trichoplusia ni* BTI-TN-5B1-4 (High Five) cells were transfected with the native or mutated JE virus prM/E gene, enzyme-linked immunosorbent assay (ELISA) detected E antigen in each culture supernatant, but a higher E antigen yield was obtained with the mutated prM/E gene. After transfection with the mutated prM/E gene, High Five cells were incubated with blasticidin and cells resistant to the antibiotic were obtained. Analysis of the culture supernatant via sucrose density-gradient centrifugation suggested that secreted E antigen molecules were in a particulate form. While the yield of E antigen from recombinant CHO cells was 0.1 μg/ml in a previous study [2], a significantly higher E antigen yield (> several □g/ml) was achieved in shake-flask culture of recombinant High Five cells. Recombinant cells also showed a greater E antigen yield than the baculovirus-insect cell system. These results indicate that recombinant insect cells may offer a novel approach for efficient production of VLPs for vaccine applications.

[1] Yamaji, H., Manabe, T., Watakabe, K., Muraoka, M., Fujii, I., Fukuda, H.: Production of functional antibody Fab fragment by recombinant insect cells, *Biochem. Eng. J.*, **41**, 203-209 (2008).
[2] Konishi, E., Fujii, A., Mason, P. W.: Generation and characterization of a mammalian cell line continuously expressing Japanese encephalitis virus subviral particles, *J. Virol.*, **75**, 2204-2212 (2001).

P4.144. INFLUENCE OF HOST CELL DEFENCE DURING INFLUENZA VACCINE PRODUCTION IN CELL CULTURE

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For cell culture-based influenza vaccine production few efforts have been undertaken to characterise virus-host cell interactions to optimize manufacturing. In vivo influenza replication is limited by the immune system, but for production cell lines the impact of cellular defence mechanisms on virus yield is largely unknown. Therefore, stimulation and inhibition of the host cell defence were performed analysing its impact on virus titres in adherent Madin-Darby canine kidney (MDCK) cells used in vaccine manufacturing.
The stimulation of the antiviral state by conditioned medium led to enhanced IFN-signalling, which initially slowed down influenza A and B virus replication but had only minor effects on final virus titres. In addition, over-expression of viral antagonists could reduce IFN-signalling up to 90%. However, maximum virus yield was not enhanced significantly. Interestingly, minireplicon assays revealed that canine Mx proteins are lacking an antiviral activity against influenza A and B viruses.
In summary, for MDCK cell culture-based influenza vaccine production host cell defence mechanisms seem to play only a minor role for final virus yields. Antiviral mechanisms of these epithelial cells may slow down influenza replication, which in vivo gains time for the immune system to be activated, but do not reduce maximum virus titres obtained in the bioprocess. The lack of inhibitory potential of canine Mx proteins against influenza virus replication makes MDCK cells an ideal host for high yield vaccine production.

P4.145. PRODUCTION OF GFP EXPRESSING MODIFIED VACCINIA VIRUS ANKARA IN AVIAN CELL CULTURES: MONITORING INFECTION AND CELL PHYSIOLOGY BY FLOW CYTOMETRY

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Recently, it was shown that the duck designer cell line AGE1.CR.pIX (provided by ProBioGen AG, Berlin, Germany) can produce modified vaccinia virus Ankara (MVA) and influenza virus in cell culture [1, 2]. This first evaluation demonstrated that good virus yields can be obtained using chemically defined media. However, more insights into cell culture conditions and virus propagation should be helpful to understand and further improve the production phase and the virus yield.
A central aspect of vaccine production processes is the physiology of host cells during growth and virus replication which is assumed to have a strong impact on virus propagation and cell-specific yield [3]. Flow cytometry can assess the physiological status on the single cell level. Using this technique, changes in cell cycle distribution, induction of apoptotic status, and progress of infection can be monitored for representative samples of the cell population in a cultivation vessel.
For MVA studies, a recombinant GFP expressing MVA (kindly provided by Prof. Sutter, LMU München) was used that allows a fast determination of the infection status of cells [4]. Moreover, analysis time and costs are greatly reduced as no antibodies are required. In addition to infection state, virus-induced apoptosis was measured via annexin V staining and cell cycle distribution was determined using propidium iodide. Together with $TCID_{50}$ values, this allowed a detailed investigation of the impact of the physiological status on virus yield. Furthermore, experimental data can be used for mathematical modeling of cell culture-based MVA production in the future.

References

[1] Jordan I., Vos A., Beilfuss S., Neubert A., Breul S., Sandig V. "An avian cell line designed for production of highly attenuated viruses". Vaccine 2009;27(5):748-56.
[2] Lohr V., Rath A., Genzel Y., Jordan I., Sandig V., Reichl U. "New avian suspension cell lines provide production of influenza virus and MVA in serum-free media: studies on growth, metabolism and virus propagation". Vaccine 2009;27(36):4975-82.
[3] Sandhu K.S., Al-Rubeai M. "Monitoring of the adenovirus production process by flow cytometry". Biotechnol Prog 2008;24(1):250-61.
[4] Cosma A., Buhler S., Nagaraj R., Staib C., Hammarin A.L., Wahren B., Goebel F.D., Erfle V., Sutter G. "Neutralization assay using a modified vaccinia virus Ankara vector expressing the green fluorescent protein is a high-throughput method to monitor the humoral immune response against vaccinia virus". Clin Diagn Lab Immunol 2004;11(2):406-10.

P4.146. ANALYSIS OF GENE-SPECIFIC TRANSCRIPTION & REPLICATION DYNAMICS DURING HUMAN INFLUENZA A VIRUS PRODUCTION

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To better understand influenza virus replication dynamics in vaccine production using adherent mammalian MDCK cells a structured mathematical model has been developed by Sidorenko & Reichl (*Structured model of influenza virus replication in MDCK cells,* Biotechnology and Bioengineering, 2004, 88(1), 1-14). However, detailed modeling of gene-specific transcription and replication for each class of intracellular viral RNA was only partially possible due to a lack of experimental data. Therefore, a quantitative realtime-PCR assay was developed to follow transcription and replication of viral gene segments coding for structural or membrane proteins. Differentiation between vRNA, cRNA and vmRNA was possible due to the use of polarity specific primers.
Subsequent application of this assay for the determination of intracellular RNA concentrations in time series infection experiments with MDCK cells showed gene- and RNA-specific dynamics of transcription and replication during virus production. The comparison of the infection of MDCK cells with influenza A/PR/8/34 from two different providers (RKI, NIBSC) also showed differences in replication dynamics. After infection of MDCK cells with influenza A/PR/8/34 from RKI (MOI of 6), the cRNA of gene segment 5 (NP) and 8 (NS) was detected after 30 min, segment 4 (HA) was found after 60 min, 6 (NA) was found after 210 min and segment 7 (M) after 240 minutes. The infection of MDCK cells with influenza A/PR/8/34 from NIBSC (MOI of 6) showed a faster replication dynamic. The cRNA for all 5 gene segments was detected after 30 min. When comparing the detection time points of the different RNA classes, viral mRNA was found clearly before vRNA for all tested gene segments.
Overall, experimental data resulted in a detailed characterization of viral RNA synthesis in mammalian cells supporting validation of structured mathematical models describing virus propagation in mammalian cell cultures.

P4.147. INSECT CELL LINES AND BACULOVIRUSES AS EFFECTIVE BIOCONTROL AGENTS OF FOREST PESTS

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Canada is a nation of trees and as such, a nation to a variety of forest insects that may limit the economic use of certain tree species and/or render areas of forests unsuitable for commercial, recreation, wildlife habitat, or other uses. The Investigations of *in vitro* Pathogens Study at the Great Lakes Forestry Centre, (GLFC) conducts research involving the initiation of insect cell lines, determining nutritional requirements of healthy and infected cells, and developing low cost insect tissue culture media for the large scale propagation of insect pathogenic viruses as an ecologically sound alternative to chemical pesticides. In addition, insect cell lines are used for the bioassay and strain selection of viruses and bacterial toxins, for the production of foreign gene products using baculovirus- and entomopoxvirus expression vectors, and offer a cleaner, viable alternative to insect larvae for producing viral pesticides. Our research focuses on Baculoviruses (family Baculoviridae), the largest known group of naturally occurring insect viruses. Their unique characteristics of being species-specific and safe for humans, plants, mammals, birds, fish, or even on non-target insects make them ideal candidates as part of an integrated pest management (IPM) program for forest pests.

Since 1969, over 150 continuous cell lines have been produced for forest insect pest research at GLFC. This collection represents approximately 25% of insect cell lines in existence. Continuous cell lines have been developed from tissues of the eastern spruce budworm (*Choristoneura fumiferana*), western spruce budworm (*Choristoneura occidentalis*), forest tent caterpillar (*Malacosoma disstria*), tobacco hornworm (*Manduca sexta*), white-marked tussock moth (*Orgyia leucostigma*), red-headed pine sawfly (*Neodiprion lecontei*), gypsy moth (*Lymantria dispar*), white pine weevel (*Pissodes strobi*), the tarnished plant bug (*Lygus lineolaris*) and the ash and privet borer (*Tylonotus bimaculatus*). These cell lines represent six tissues of origin, namely neonate larvae, pre-pupae, embryos, ovaries, hemocytes, and midgut and four Insect Orders namely Lepidoptera, Hymenoptera, Coleoptera and Hemiptera.

Presently, cell lines developed at GLFC are being used by 41 researchers in 8 Canadian provinces, 42 researchers in 21 US States and 28 researchers in 12 countries worldwide.

P4.148. HUMAN AMNIOCYTE-DERIVED CELLS FOR PRODUCTION OF ADENOVIRUS VECTORS FOR GENE THERAPY

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Adenovirus vectors (AdV) have been extensively used as vectors for vaccines, protein production and gene therapy. Strategies that will effectively respond to the increasing demands of such vectors are thus urgently required. Primary human amniocytes can be efficiently transformed by adenoviral E1 functions allowing the production of AdV with, compared to 293 cells, potentially reduced generation of replication competent adenovirus (RCA), opening a new host alternative.
The aim of this work was to evaluate the growth behavior of a newly developed human amniocyte-derived cell line and to investigate its ability to produce AdV. Transformed human amniocyte cells were adapted to grow in suspension in serum-free media. The pO_2 content and pH of the medium were monitored accurately and non-invasively during cell growth and AdV production in shake flask, allowing identifying critical points in a small scale. The effects of multiplicity of infection, harvesting time, and medium formulation on AdV production were evaluated in shake flask. The effect of cell concentration at infection was also evaluated in shake flask and bioreactor. The production yields were determined by Flow Cytometry and PCR; 293 cells were used as control. Data on the metabolic characterization during cell growth and infection will be presented. The cells in bioreactor with serum-free medium reached cell densities up to $9x10^6$ cells/ml. Using these cells it was possible to perform infections at concentrations higher than $3x10^6$ cells/mL without a critical loss in the cell specific productivity (>1000 infectious particles (ip) per cell) thus increasing the volumetric productivity to more than $3x10^9$ ip/ml. Our data indicate that this cell line suffered less from the cell density effect than normally occurs in 293 cells leading to AdV volumetric productivities values below $1x10^9$ ip/mL.
These results constitute valuable information for the development of cell culture process for AdV production using a potentially safer human cell line ensuring simultaneously the product quality, potency and safety necessary for clinical applications.
This research was supported by the EU Network of Excellence, the project CLINIGENE.

P4.149. IMPACT OF DIFFERENT INFLUENZA CULTIVATION CONDITIONS ON HA *N*-GLYCOSYLATION

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Influenza virus is a highly contagious human pathogen causing infections of the respiratory track. Especially regarding threatening pandemics cell-culture-based vaccine production gained more and more importance over the last years. The glycoprotein hemagglutinin (HA) represents the major component in influenza vaccines. It is highly abundant in the virus particle and able to induce a strong and protective immune response. Variations in *N*-glycosylation of glycoproteins such as HA, can alter quality characteristics of antigens and influence immunogenicity of influenza vaccines. In order to analyze and further investigate HA *N*-glycosylation, we established the in-house glyXtool® (1).
In this study we present a detailed *N*-glycan analysis of Influenza A Uruguay/716/2007 (H3N2) HA. Furthermore, we characterize the impact of different cultivation conditions and host cells in vaccine production on the HA *N*-glycosylation pattern of Influenza A Uruguay/716/2007 (H3N2). We show that harvest time point and production scale have only a minor impact on HA *N*-glycosylation mainly resulting in differences in relative peak heights (relative quantities of *N*-glycan structures). In contrast, changing the production cell line can completely change the *N*-glycan pattern of HA and totally alters the repertoire of attached *N*-glycan structures.

1. Schwarzer, J., E. Rapp, and U. Reichl. 2008. N-glycan analysis by CGE-LIF: profiling influenza A virus hemagglutinin N-glycosylation during vaccine production. Electrophoresis 29:4203-14.

P4.150 USE OF PROTEIN HYDROLYSATES TO REDUCE SERUM REQUIREMENT IN THE PRODUCTION OF VETERINARY VACCINES BY BHK CELLS

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Despite the benefits of animal serum for cell growth, its use in cell culture media shows many shortcomings, such as the potential risk of introduction of animal-derived contaminants, the lot-to-lot variability and the high costs. The replacement of animal serum can be achieved by supplementing culture media with protein hydrolysates, which are obtained by hydrolysis of proteins derived from plants, milk or meat.
In this work three different protein hydrolysates (HyPep™ 1512, HyPep™ 7504 and Pepticase™) were individually added at different concentrations to Glasgow Minimum Essential Medium (GMEM) containing 3% adult bovine serum (ABS) and compared to a medium used in veterinary vaccine industries, composed of GMEM supplemented with 8% ABS. HyPep™ 1512 and HyPep™ 7504 are derived from soy and cotton seed, respectively, whereas Pepticase™ is derived from milk casein. BHK-21 cells, previously adapted to grow in suspension, were inoculated at a density of 0.5×10^6 cells/mL in shake flasks with 25-mL working volume. Samples were taken every 24h for determination of viable cell density, cell viability and glucose, lactate and aminoacids concentration.
The results obtained showed that protein hydrolysates can effectively be used to replace animal serum in the veterinary vaccine industry, corroborating to decrease the use of animal-derived components and to reduce costs.

P4.151. VIRAL VACCINE PRODUCTION AT MANUFACTURING SCALE (1000 M² SURFACE) INTO ICELLIS™ DISPOSABLE FIXED-BED REACTOR

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Viral vaccines are usually produced by anchorage-dependent cell lines. At industrial scale, these cells are either cultivated in static mode on multitray systems (Cell Factories, Cell Cube, etc.), in roller bottles or on micro-carriers (porous or non-porous) in suspension in bioreactors. Multitray systems and roller bottles are bulky and require a lot of handling operations, whereas microcarrier cultures require numerous operations (sterilization and rehydration of carriers, etc.) and lots of steps from precultures to final process with complex operations (i.e. bead-to-bead transfers).
However most of the currently available disposable reactors (available at different scales) are not well adapted to cultivate animal cells immobilized on microcarriers as perfusion is the technology of choice for these productions.
Fixed-bed bioreactors are known to retain and perfuse cells at high cell densities in small reactors with low shear stress. In addition, cells in packed-bed remain much longer viable during cultivation in an easy operation manner. The disposable iCELLis™ fixed-bed bioreactor is based on an agitation system powered by a proprietary centrifugal-based flow impeller, a waterfall oxygenation of the culture medium and a packed-bed made up of macroporous carriers. In such a bioreactor, the following parameters are measured (and controlled): pH, DO, biomass (using an impedance-based biomass probe), temperature, perfusion rate, etc. The iCELLis has the advantages of fixed-bed reactors and the advantages of disposable technologies, in particular the reduction of capital investment and time needed for development and production capacity increase.
Here we present the concept validation: the culture of adherent cells at high cell densities and the multiplication of viruses in iCELLisTM fixed-bed bioreactors at different scales – from small scale to manufacturing scale (25L fixed-bed reactor – with 1000 m2 of surface area accessible to cells). At such a scale, we obtained a cell biomass of 1.75 x1012 Vero cells and a viral bulk production of 600 L in a disposable reactor with a foot-print of 1.5 m2 (device and skid).

P4.152. REAL-TIME MONITORING OF VIRAL VECTOR AND VACCINE PRODUCTION PROCESSES

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Real-time monitoring of cell culture operations is critical to accelerate process development and optimization. In recent years, a renewed interest has been generated in in-line monitoring tools and strategies from the release of FDA's PAT initiative.
An increasing number of virus-based products generated in cell cultures are evaluated for gene and cell therapy applications; the development of large scale vaccine production also recently gained momentum due to the urgent need to develop strategies with shorter response times. Real-time monitoring tools that either directly quantify viruses or provide a measure of the production kinetics are thus required to support these processes.
This presentation will summarize and review recent advances concerning the real-time monitoring of viral vector and vaccine production processes. In particular when analyzed in combination with complementary techniques, permittivity signals convey significant real-time information on virus production independent of whether production is induced by infection or transfection. The replication and the release of progeny virus is generally associated with significant physiological changes in the producer cells. These include variations in dielectric properties and biovolume that are both reflected in the permittivity and which are determined by the underlying virus biology.
Permittivity monitoring allows the determination of the success of transfection/infection, the detection of viral release and the identification of characteristic process phases to follow the progress of production. We also provide evidence supporting that process performance and productivity can be assessed in real-time. We thus conclude that permittivity measurements are a valuable tool for process characterization and supervision of viral vector and vaccine production processes.

P4.153. SERUM-FREE PRODUCTION OF GAG-BASED VIRUS-LIKE PARTICLES BY POLYETHYLENEIMINE-MEDIATED PLASMID TRANSIENT TRANSFECTION IN MAMMALIAN SUSPENSION CELLS.

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Virus-like particles (VLPs) are empty viral shells that lack genetic material. Since they resemble viruses but are non-infectious by nature they represent an attractive and safer means for vaccination. VLPs are composed of one or more recombinantly produced viral proteins, which upon expression self-assemble into particles. For instance, VLPs for HIV-1 can be generated by simple co-expression of Gag (the main HIV-1 structural protein that forms the capsid shell) and Env (the HIV-1 surface antigen). The aim of this study is to develop a production method for HIV-1 VLPs in HEK 293 serum-free adapted suspension cells. These mammalian cells have been selected due of their capacity to perform appropriate post-translational modifications and authentic assembly of viral particles. The technique used for the production of VLPs is transient transfection with PEI, as it gives flexibility to exchange Env-protein candidates facilitating the selection of an optimal VLP structure. We have analyzed the kinetics of HEK 293 cell growth in serum free medium HyQ SFM4 Transfx293, HyClone. The cells grow to a maximum concentration of ~ 3×10^6 cells/ml with viability over 90% after 100 h in culture, showing an average doubling time of 24h. In addition, we have evaluated cell growth in two other commercial serum-free culture media (ExCell 293, Sigma and Freestyle, Invitrogen), that are also compatible with PEI mediated transient transfection, observing similar cell growth compared to those obtained in HyQ SFM4 Transfx293, HyClone medium. We have evaluated the effect of increasing concentrations of foetal bovine sera (FBS) in this serum-free culture medium. Cells can triplicate their maximum cell densities ($9,3\times10^6$ cells/ml) in the presence of 10% FBS. Due to the important effect of serum on HEK 293 cell density, we decided to evaluate the effect of non-animal serum derivates on cell growth in attempt to improve cell densities while keeping animal-free production conditions. For these studies, we have selected 3 recombinant proteins (albumin, transferring and insulin) and an in-house lipid mixture composed of synthetic cholesterol, fatty acids, tocopherol and emulsifying agents. The optimal combination of these proteins in the final formulation has been determined by using design of experiments (DoE). Results have shown that by adding a mixture of animal free supplements to the cell culture medium, it is possible to reach cell densities comparable to those attained in the presence of 10% FBS while avoiding the problems derived from its use.

P4.154. PLATFORM TECHNOLOGY FOR FUTURE VIRAL VACCINE PRODUCTION

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Viral vaccine production processes are often tailor-made, or use established (e.g. egg-based) technologies. Such techniques and approaches have lower fitness for future applications due to labour intensive procedures and troublesome scale-up. A promising development is the use of platform technologies such as currently used in therapeutic protein production processes. When the blue-prints are available, such an approach can accelerate vaccine development. In the current study, an example of such an approach is given by using inactivated poliovirus vaccine (IPV) production technology as a model for future viral vaccine production processes.
Cell-culture based IPV production in Bilthoven dates back to the 1950s. Later, in the 1960s, an industrial-scale process was developed based on micro-carrier technology and primary monkey kidney cells. More recently, the Vero cell-line was introduced. This knowledge was shared, and RIVM participated in free transfer of technology. Even today, at various locations, comparable processes based on this classical example of a platform technology are in use for the production of polio and other viral vaccines.
Currently, opportunities to implement process improvements and optimization are being explored. To achieve these objectives, a scale-down - scale-up strategy was followed using historical manufacturing data. Based on this, a 3-L scale-down model of the current 750-L bioreactors has been setup. At this moment, in this lab-scale process, both USP (cell and virus culture) and DSP (clarification, concentration, purification and inactivation) unit-operations approximate the large-scale. Subsequently, using this lab-scale model, a new process using attenuated Sabin poliovirus strains, was developed up to production-scale (i.e. approximately 2 × 350-L bioreactor working volume) and used to generate phase I clinical trial materials. Clinical studies are scheduled to start early 2011.
In parallel to the above, a research program was initiated to further modernize and optimize the process, and reduce the cost per dose. In these process improvement studies the scale-down model will be used, and the performance will be compared to that in the standard configuration. Finally, technology transfer to vaccine manufacturers in low and middle-income countries is foreseen.

P4.155. DEVELOPMENT OF LIVE CULTURAL PANDEMIC INFLUENZA VACCINE "VECTOR-FLU"

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In 2009 we prepared and submitted to the Ministry of health and social development of Russia the project targeting both the pilot technology of live cultural influenza vaccine production and the vaccine production fermentation site set-up.
To date we have obtained the following results: Ever in Russia, based on MDCK cell line, we have developed 2 seed cell banks and 2 operating cell banks of MDCK cells with these banks stored under liquid nitrogen temperature. MDCK cell banks are certified in accordance with WHO recommendations. In June, 2009 we received the vaccine strain A/17/California/2009/38 (H1N1) delivered from Institute of Experimental Medicine of the NorthWest Branch of the Russian Academy of Medical Sciences, St.-Petersburg. Based on influenza A/H1N1 virus strain obtained, we have developed live cultural influenza vaccine production technology with the following steps involved:

- microcarrier cultivation of MDCK cells in fermenters with serum-free medium;
- virus growth in the cell culture;
- virus-containing fluid collection and purification;
- introduction of stabilizers and biodegradable polymers for designing the microcapsulated vaccine formulation;
- freeze-drying;
- ready-to-use vaccine formulation production.
- 3 laboratory-tested batches of vaccine "Vector-flu" have been produced and certified; they have specific activity index as 7,33-7,5 lg EID50/ml.
- The vaccine has passed the preclinical tests which had included the following controls:
  - acute toxicity of three batches in two types of animals;
  - chronic toxicity for tenfold daily intranasal administration in mice and Guinea pigs;
  - influence on the hematological indexes in mice and Guinea pigs;
  - local irritant effect for double intranasal administration in mice and Guinea pigs;
  - influence on central nervous system function;
  - influence on the liver detoxication function;
  - allergenic properties;
  - immunotoxic properties;
  - embryotoxic properties;
  - immunogenicity in laboratory animals;
  - specific vaccine strain safety in animals.

The preclinical trials showed the laboratory animal vaccine safety. The immunogenicity monitoring in ferrets showed explicit immune response development. The ferret serum titer tested for hemagglutination-inhibition reaction for single administration was 1/1280, for double administration it was 1/1636, in microneutralization test it was 1/1138 and 1/2133 accordingly. Vaccine strain A/17/California/2009/38 (H1N1) induced a high level of neutralizing antibodies in ferrets against the highly pathogenic influenza A/H1N1 strains collected in the territory of Russia, as well as against highly pathogenic A/California/7/2009 strain. In intranasal immunization of mice we observed virus neutralizing antibodies at the level of 1/160 - 1/320 against the unadapted highly pathogenic influenza A/H1N1 strains. This level happened to be sufficient for protecting mice from the aerosol contamination by a dose of 100 mouse aerosol infectious doses (AID50). The obtained data allow to estimate protection coefficient of mice after the double vaccination; and this coefficient exceeds 2,0.
We have developed the vaccine production pilot schedule and have prepared the set of specification documents for vaccine registration application submission to Ministry of health and social development of Russia. We are planning to do the clinical trials Phase I in Russia.
The technology used for pandemic influenza vaccine "Vector-flu" production has a versatile use mode, and it could be used for growing live cultural vaccines based on the other influenza virus subtypes. The use of microcapsulation concept in the production technology allows to produce the vaccine with the improved immunogenic properties. We have obtained the patent of Russia #2330885 for the developed technology, the patent application for a new patent has been submitted.

P4.156. PURIFICATION OF BACULOVIRUS AND VLP BY MONOLITHIC COLUMNS

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The structure and size of the porous network of monolithic columns is optimal suited for virus purification and is a tailored application. Viruses and virus-like particles are used as vaccines as well as delivery vehicles for gene therapy. A scheme for virus purification includes three steps: a capture step, an intermediate step, and a polishing step. Modern vaccines and viral delivery vehicles are produced in cell culture, while traditional vaccines are produced in the amnion fluid of eggs. Baculoviruses are produced in SF9 cells and Hi5 cells are an important vehicle for production of virus like particles by overexpressing respective virus antigens at the surface of the baculovirus as previously shown for influenza virus. General strategies for separation of virus with polymethacrylate monoliths so-called CIM-disks and CIM tubes have been developed with an overall yield of 70%. Virus is loaded at intermediate salt concentration and eluted by a linear salt gradient. Thereby it is possible to separate virus from VLP and DNA. The viruses and the VLP can be purified to homogeneity with a high yield of infectious virus as measured by plague assays. Purity is assed by chemical and biophysical methods such as electrophoresis Westernblots, TEM and Nanosight. A full purification cycle can be accomplished within a day.

P4.157. PRODUCTION OF CANINE ADENOVIRUS VECTORS IN SERUM-FREE SUSPENSION CULTURES OF MDCK CELLS

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The potential of Madine Darby Canine Kidney (MDCK) cells for the production of influenza vaccines have been greatly explored in the past decades. Recently, a new MDCK cell line (MDCKsus) that was able to grow in suspension in a fully defined system was established (Lohr, V., *et al.*, 2010. Vaccine. 28:6256-64). This new cell line is suitable for the development of robust industrial manufacture of viral based products. Adenovirus vectors are widely used in Gene Therapy trials, Canine Adenovirus Vectors (CAV) have been developed as an alternative to human adenovirus vectors since they present lower immunogenicity. We have validated the use of MDCK cells for the amplification of CAV-2 vectors in monolayer cultures (Santiago *et al.*, 2010 ASGCT).
In this work we investigated whether the MDCKsus cell line was suitable for the amplification of CAV-2 vectors in serum-free suspension cultures, which facilitate GMP compliance. We tested four different serum-free media: two formulations of SMIF8, AEM, ExCELL MDCK and OptiPRO SFM. The maximal cell densities achieved varied from 2 x 10^6 cells/ml (SMIF8 and OptiPRO SFM) to 5 x 10^6 cells/ml (AEM and ExCELL MDCK). The initial screening of CAV-2 production with an multiplicity of infection (MOI) of 5 in the four media resulted in low amplification of the vector (30 IP out/IP in) and high cell death. The decrease of the MOI resulted in an increase of the amplification yields to values up to 250 IP out/IP in (and cell specific productivities up to 750 IP/cell). Although, further optimization is still ongoing, our data shows that canine adenovirus vectors can be efficiently produced in serum-free suspension cultures of MDCKsus cells. It is anticipated that the CAV-2 production in MDCKsus described here can be successfully used to obtain the high vector titers required in gene therapy trials.

P4.158. CANINE ADENOVIRUS TYPE 2 (CAV-2) AS NEW VECTORS FOR HUMAN GENE THERAPY: SCALING-UP THE PRODUCTION PROCESS

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Viral systems provide the most efficient tools for gene therapy; adenoviral vectors (AdV) representing one of the best candidates for therapeutic gene delivery in humans due to a number of advantages including its wide cell tropism in quiescent and non-quiescent cells, inability to integrate into the host genome and high production titers obtained. However, the gene transfer efficacy and the clinical use of human AdV can be hampered by the preexisting immunity and increased cellular immune responses and chronic toxicity in repeated dosages found in most humans.
Canine Adenovirus type 2 (CAV-2) vectors are the most well characterized nonhuman adenoviral vectors. The inexistence of neutralizing antibodies in humans and appropriate gene delivery obtained when first generation CAV-2 vectors are used, make these viruses promising tools for therapy purposes. Nevertheless, the current lab-scale production system constrains the process reproducibility and scalability, making such vectors availability for further clinical studies a challenge.
This work describes the development of a scalable bioprocess for the production of ΔE1 CAV-2 vectors in stirred tank bioreactors compliant with the FDA recommendations for biopharmaceutical production, thereby using MDCK E1 producer cell-line and serum-free culture medium.
The impact of pH and pO_2 control in ΔE1 CAV-2 production yields was evaluated; a 10-fold improvement was obtained for stirred tank bioreactors with controlled pH of 7.2 and pO_2 of 40%, when compared with stirred culture systems without these parameters monitoring and control. Although the specific rates of main metabolites generally increased for bioreactor cultures, the lactate/glucose and ammonia/glutamine yields were lower. These results show that cells cultivated in controlled conditions have an active central carbon metabolism, which results in a higher cellular energetic state, therefore enabling higher productivities. A detailed understanding of the infection impact on the intracellular metabolism would provide important clues to manipulate the key metabolic pathways and maximize productivity.
These features demonstrate that this production process can be up-scaled and that CAV-2 production yields can attain productivities comparable with those observed for human adenoviruses. Future work aims at unraveling the MDCK cells metabolic fluxes and identifying potential metabolic targets for the development of an adequate re-feeding strategy.

P4.159. EFFICACY OF AN INACTIVATED AND ADJUVANTED "ZULVAC® 8 OVIS" VACCINE PRODUCED USING SINGLE-USE BIOREACTORS

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Bluetongue virus (BTV) first emerged in the European Union (EU) in 2006, peaking at 45,000 cases in 2008. The EU spent million of euros in 2008 and 2009 on eradicating and monitoring programs, co-financed with member states. The number of cases in 2009 was 1118, with only 120 reported across the EU so far last year. Vaccination has proven itself as the most effective tool to control and prevent the disease and to facilitate the safe trade of live animals.
Mammalian cells are the substrate for production of most of the vaccines. BHK-21 cells are commonly used to produce bluetongue vaccines. Most companies produce vaccines using roller bottles and some in conventional bioreactors.
One important issue to be taken into account in the development of vaccines is their cost, especially in veterinary use. The possibility of using Single-Use Bioreactor (SUB) technology as an alternative to roller bottles or conventional bioreactors was studied.
Preliminary results prove that by using SUB the yields obtained in roller bottles can increase more than three times. Based on this, the potency of antigens produced using the said technology has been verified in target species. The results show that the vaccine is effective to prevent viraemia.

As a conclusion, SUB technology can be an alternative to conventional production methods and also offers some advantages to other areas, such as cleaning, sterilization, validation and setting-up.

P4.160. INSECT CELL-BASED INFLUENZA VACCINE BIOPROCESS DEVELOPMENT

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The Insect Cell-Baculovirus Vector Expression System (IC-BEVS) has proven to be a valuable tool for the production of biopharmaceuticals including heterologous proteins, vaccines, virus like particles (VLPs) and, more recently, vectors for gene therapy. We have previously studied the impact of baculovirus infection on the central metabolism of Sf9 cells through the combined analysis of cellular cofactors (ATP and $NAD(P)^+$/NAD(P)H), enzyme activities and metabolic fluxes. The results highlighted baculovirus-mediated manipulation of the cellular metabolism as a major target for improved productivity and process performance. These findings are now being used to guide us through the bioprocess development of Sf9 cells producing recombinant neuraminidase (rNA), enzymatically active, for an Influenza vaccine. In a first stage, culture parameters (cellular concentration at the moment of infection, multiplicity of infection and time of harvest) were analysed. The best condition were selected for the production of recombinant neuraminidase in a 2 L stirred tank bioreactor. In parallel, a membrane-based purification process based on anion exchange and affinity is under implementation. The analytical methodologies that will allow to monitor final product quality, including protein integrity/assembly and enzymatic activity but also to assess the intracellular build-up of the protein are under development.
Ultimately we hope to establish a high-producing, cost-effective robust and scalable bioprocess for the production of an Influenza vaccine candidate using baculovirus technology.

P4.161. DROSOPHILA S2 CELLS AND BY SEMLIKI FOREST VIRUS FOR THE EXPRESSION OF RABIES VIRUS GLYCOPROTEIN (RVGP)

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Here we report the utilization of *Drosophila melanogaster* Schneider 2 (S2) cells and the Semliki Forest Virus (SFV) expression system to produce the rabies virus glycoprotein (RVGP). Although they represent different expression systems, they offer similar advantages in generating high-level expression of functional membrane proteins. They are both relatively easy and safe to handle and are scalable. RVGP synthesized by S2 cells and SFV carrying an mRNA coding for RVGP (SFV-RVGP) were assayed in "*in vivo*" mouse protection studies against experimental rabies virus challenges. Regarding the S2 system, we have constructed gene vectors with the hygromycin selection gene (H) in which the RVGP gene is inserted under the control of the metalothionein (MT) promoter. After transfection a S2MTRVGP-H cell population was selected and the expression of RVGP was evaluated by qPCR, flow cytometry, ELISA and western-blotting. Cell cultures in scalable bioreactor were performed and batches of RVGP were produced for further functional studies. The data showed a high RVGP expression level (52 % of positive cells, 4 g/10^7 cells). RVGP *mRNA* analysis enlightened the relationship between cell growth and specific productivity. Parameters for storage, lysis and concentration of cells bearing the RVGP were studied for productivity evaluation and purification. A protocol for cell preparation including cell freezing as dry pellet, cell thawing at 4°C with Tris, NaCl, $MgCl_2$, PMSF and cell lysis with the buffer containing NP-40 was developed fulfilling requirements of high level RVGP detection, as well as easier, faster and more cost saving lysis buffer formulation. Regarding the SFV expression system, the RVGP gene was cloned into a modified pSFV2genC expression vector. BHK-21 cells were electroporated with expression and helper RNA vectors and SFV-RVGP particles were obtained. These were titrated by qPCR and used to infect BHK-21 cells. RVGP expression was confirmed by qPCR, western blotting, ELISA and cell immunofluorescence. The ability of S2 cells derived RVGP and SFV-RVGP particles to produce both humoral and cellular immune response and protect mice against an experimental rabies virus challenge were investigated. High levels of antibodies against RVGP were found in both groups of immunized mice. A higher cellular immune response, as measured by the $CD4^+/CD8^+$ cell quantification was observed in SFV-RVGP immunized mice. Preliminary data show that immunization protocols were capable of inducing a high degree of protection against rabies experimental challenge. Taken together our data show several optimization steps for high-level RVGP expression in stably transfected S2 cells, which were shown to be biologically active by protecting mice against a rabies experimental infection. On the other hand, the promising SFV system constituted by recombinant viral particles carrying the mRNA coding for RVGP was shown to elicit a cellular immune response and protect mice against an experimental infection
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P4.162. INFLUENZA PRODUCTION IN HEK293 CELL CULTURES

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Over the last few years, the pharmaceutical companies involved in influenza vaccine manufacturing considered, among other expression systems, the use of mammalian cell cultures for mass production of influenza viral strains. Consequently, different cell lines including MDCK, Vero and PER-C6 cells, have been evaluated for industrial manufacturing of pandemic or seasonal influenza vaccines.
In the present work, HEK293 cells, already described as an efficient platform for the large-scale production of other viral vectors, were evaluated for the production of various influenza strains (H1N1, H3N2 and B subtypes), in serum-free and suspension culture conditions which are amenable to large scale productions. After producing the proof of concept that HEK293 cells are able to replicate and produce influenza viruses, process development was performed with the reference A/PR/8/34 H1N1 strain. This strain was produced by infecting HEK293 cell cultures at 4 x 10^6 cell/ml in a 3l bioreactor operated in batch mode allowing to achieve titers of 10^9 infectious viral particles (IVP)/ml and 4.01 logHA/ml. Further significant improvements of the production yield was obtained using a perfusion feeding strategy and a cell density of infection of 6 x 10^6 cell/ml, while we reached maximal titers of 3.2 x 10^{11} IVP/ml and 4.3 logHA/ml, corresponding to a total production of 1.0 x 10^{15} infectious particles and 7.8 logHA. Indeed, with the perfusion system, the potential lack of nutrients or accumulation of toxic products limiting the viral productivity and observed beyond 4 x 10^6 cell/ml was alleviated. These results are in the high range of productivity data reported to date for cell culture-based influenza production yields. Overall, these results, demonstrate that HEK293 cell is an attractive platform for industrial manufacturing of seasonal or pandemic influenza vaccines.

P4.163. AVIAN CELL LINE TECHNOLOGY FOR LARGE SCALE VACCINE PRODUCTION

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Important vaccines and viral vectors are still produced in embryonated chicken eggs or primary chicken embryo fibroblasts. Primary avian tissue for virus replication is provided by SPF (specific pathogen free) production plants. SPF-derived tissues are expensive and the quality of the supply material is often hard to control or restricted (e.g. pandemic situation). Therefore, inconsistency and shortage of supply are the most predominant disadvantages of technologies based on SPF eggs. The same is true for approaches where primary fibroblast monolayer cultures are used. Avian cell lines have become a modern option for vaccine manufacturing and will definitely replace egg and primary fibroblast technology.

We have generated a continuous avian cell line by specific UV-treatment of primary quail tissue followed by adaption to chemically defined growth medium. The non-genetically modified continuous cell line was adapted to grow in suspension culture and is thus suitable for large scale fermentation. Extended characterization showed that the cells are free of adventitious agents. Furthermore the cell line is F-Pert free and non tumorigenic and accordingly fulfills all the critical regulatory requirements. High product titers can be achieved with a broad range of viruses such as wild-type MVA, rMVA strains, Influenza and Flavi viruses. A scalable production technology for the production of live and attenuated vaccines has been established.

P4.164. INSIDE RETROVIRUS PRODUCING FACTORIES: TRANSCRIPTIONAL PROFILING OF HUMAN PACKAGING CELLS

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Enveloped virus production in mammalian cells, particularly retro and lentiviral vectors, faces challenges in several fronts. The low-yields, high non-functional particles content and the animal sera required in to sustain long-term productivity are particularly challenging. Our limited knowledge on the biochemical pathways related to virus production has impeded cellular and process engineering efforts to enhance production performances. In this work we examined transcriptome and metabolome profiles in retrovirus production using two human packaging cell lines, 293 FLEX and Te Fly and took a systems approach to integrate the data and gain new insights on the process.

Transcriptome profiling revealed that retrovirus production induces an "apoptotic -cell survival" balance, a typical feature of virus infection scenario. Fatty acid (FA) metabolism was among the most prominently up regulated pathways, indicating increased membrane recycling dynamics for viral replication. Additionally, several biosynthetic pathways related with the production of lipid rafts components were found to be up regulated, including sphingolipid and phospholipid metabolism. Cholesterol biosynthesis was extensively enriched as a functional class, but only under reduced serum conditions. Amino acid degradation was also found to be prominently up-regulated. Virus production appears to result in higher energy demands, evidenced by the strong up-regulation of the oxidative phosphorylation and electron transport chain. Te Fly cells, a packaging system presenting higher titers than 293 FLEX, showed to be more resistant to apoptosis and virus-induced oxidative stress, revealed higher FA and cholesterol biosynthesis capacity, and improved energy generation metabolism, suggesting these to be potential pathways for metabolic engineering towards enhanced productivity performances. The integration of microarray with metabolite profiling data (on-going) should provide further validation or additional insights on manipulation targets. These results have a direct application to the field of retro, lenti or other enveloped virus manufacture, contributing to improved vector design and novel cell line development strategies.

P4.165. EXPRESSION OF AN ANTIVIRAL RECOMBINANT PROTEIN, OBTAINED FROM *LONOMIA OBLIQUA* HEMOLYMPH, IN A BACULOVIRUS/CELLS SF-9 SYSTEM

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The control of viruses, especially those induced by influenza virus is of great interest to the public health area. Several studies have been conducted that show the presence of pharmacologically active substances in the hemolymph. Recently we have demonstrated the existence of a potent antiviral in hemolymph of *Lonomia obliqua* caterpillar. This purified protein reduced virus production ($TCID_{50}$ ml^{-1}) by more than 157 fold (from $3.3\pm1.25x10^7$ to $2.1\pm1.5x10^5$) to measles virus, 61 fold to polio virus ($2.8\pm1,08x10^9$ to $4.58\pm1.42x10^7$) and 64 fold to H1N1 inluenza virus (Antiviral Research 84, 84-90, 2009). Thus, this study aims to build recombinants bacmids containing sequences encoding this antiviral protein in baculovirus/SF-9 cell system. To synthesize cDNA, RNA of *L. obliqua* was extracted with Trizol reagent and used in polymerase chain reactions using reverse transcriptase polymerase (RT-PCR) with primers specific for the antiviral protein, based on the sequence deposited in GenBank database. Restriction sites were inserted in the cDNA for connection to the donor plasmid pFastBac1™ (Invitrogen). The recombinant plasmid was selected in *Escherichia coli* Top 10 and subsequently used in the transformation of DH10Bac *E. coli*, to obtain the recombinant bacmids. The bacmid recombinant containing the sequence of the antiviral protein was subjected to recombination in *E. coli* DH10Bac for construction of recombinant bacmids. This bacmid, containing the sequence of a protein with antiviral activity will be use for expression of this protein in baculovirus/SF-9 cells system. At the moment, we are expressing the bacmid recombinant containing the sequence of the protein with antiviral activity in baculovirus/SF-9 cell system.

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P4.166. NEW TECHNOLOGY FOR RAPID VIRAL STOCK QUANTIFICATION

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Traditional methods for virus quantification such as plaque assay are time and labor intensive, and can often be a significant bottleneck in the research and development process. We present the results from several case studies comparing a new instrument for virus quantification, the ViroCyt 2100 Virus Counter, to standard methods including viral plaque titer assay and quantitative PCR. The ViroCyt 2100 is a specialized bench top flow cytometer that has been designed to quickly and accurately determine volumetric concentration in real-time for intact virus particles in solution using fluorescence.

In a first case study, serial dilutions of a baculovirus viral stock were provided in a blinded fashion to three different laboratories. Plaque assays were conducted in triplicate at two sites, and several replicate ViroCyt 2100 measurements were made on each dilution by InDevR. The results indicate a linear correlation between the two methods ($R^2=0.86$), with the ViroCyt 2100 providing greater accuracy and precision in significantly less time. In a second case study using serial dilutions of a 2009 pandemic H1N1 influenza virus strain, ViroCyt 2100 measurements were compared to quantitative PCR measurements. A strong linear correlation between the threshold cycle (C_t) value and the ViroCyt 2100 measurements ($R^2=0.96$) was observed. The ViroCyt 2100 Virus Counter represents a new, rapid method for virus quantification that can be utilized to monitor bioprocesses in real-time with high accuracy.

P4.167. CAP TECHNOLOGY: PRODUCTION OF INFLUENZA VACCINE IN HUMAN AMNIOCYTES

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The pressure to innovate in cell-based production systems is weighting particularly strong on the manufacturers of vaccines, as the currently used manufacturing systems do encounter severe problems. In the case of currently used egg-based vaccine manufacturing technology an average production campaign of e.g. influenza vaccines takes up to 6 months. In contrast to the egg-based production technology, cell culture-based systems show the potential to drastically shorten production cycles and improve quality of the vaccine regarding e.g. glycosylation of immunogenic antigens and risk of animal contaminants.
CEVEC's Amniocyte Production (CAP) cell technology is a versatile production platform to generate difficult-to-express and manufacture therapeutic proteins. The generation and development of the CAP human cell system has been fully documented according to all relevant regulatory standards. During the past years CAP cells have been broadly used for production of complex proteins and antibodies and thus have proved its strong potential as new technology platform for proteins in high yields and with authentic human glycosylation pattern.
Addressing the needs of vaccine manufacturers, we have tested the suitability of CAP cells for the production of influenza vaccines. CAP cells growing in suspension in serum-free medium were evaluated for their potential as host cells for different human and animal influenza strains. The studies included extracellular metabolite concentrations during growth and virus production in different cultivation systems, test of different commercial serum-free media and evaluation of process conditions (trypsin concentration, multiplicity of infection, media feeding). In addition, time-course of infection and virus adaptation was characterized, and virus yields obtained with CAP cells were compared to those with MDCK cells.
Favorable robust process parameters and high virus yields obtained with different influenza strains demonstrated that CAP cells are very promising candidates for large-scale manufacturing of vaccines in serum-free medium.

P4.168. OPTIMIZATION STUDY ON PROPAGATION OF INFLUENZA A VIRUS IN VERO CELLS BY USING CYTODEX™ MICROCARRIERS IN WAVE BIOREACTOR™ SYSTEMS

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As the threat of an influenza pandemic has become more apparent, there is an increasing need for responding quickly to mass produce influenza vaccine. The shift from egg-based to cell culture-based vaccine production is seen as one way to secure a more rapid response. Furthermore, one additional obstacle is to overcome the need to greatly reduce time for clinical trials & approval i.e. to reduce time to market, where cleaning validation is of major importance.

Here, we present a way for eliminating cleaning validation by using a disposable bioreactor alternative. We have performed an optimization study on production of live Influenza A/Solomon Islands/3/2006 in Vero cells by using Cytodex™ microcarriers in WAVE Bioreactor™ systems. Parameters such as inoculation conditions, additives and composition of media etc. have been considered. Thus, the data shows that production of the influenza virus in WAVE Bioreactor™ system is a fast and convenient alternative to conventional systems.

P4.169. FLOW CYTOMETRIC EVALUATION OF INFLUENZA VIRUS REPLICATION IN ADHERENT MDCK CELLS: VARIATION OF VIRUS STRAIN AND MULTIPLICITY OF INFECTION

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With the ongoing threat of pandemic and seasonal influenza infections, the establishment and optimization of safe vaccines is of growing importance. Relatively new cell culture-based processes begin to replace the traditional vaccine process which uses embryonated hen's eggs. Adaptation of influenza strains from egg to cell culture systems reveals significant differences in achieved titers between various influenza strains. The molecular reasons for the differences between high and low yielding strains remain to be elucidated and are subject of this work.
Here, we present a study performed in adherent Madin Darby Canine Kidney (MDCK) cells on influenza replication using four different influenza strains at three multiplicities of infections (moi). Analyzed were two influenza A/PR/8/34 wild type strains and two A/PR/8/34-based high growth reassortants. Accumulation of the viral nucleo protein and virus-induced apoptosis were monitored by flow cytometry. Furthermore, total virus particle and infectious virion concentrations were determined with a hemagglutination assay and a TCID50 assay, respectively.
The higher the moi chosen for the infection, the earlier an increase of infected cells was observable. Apoptosis was induced faster and stronger in infections with higher mois. However, some strains reacted stronger to the change in moi than others. Virus end titers did not seem to depend on the moi, interestingly enough, the fraction of infectious virions did.
The obtained data give new insights in differences between high and low yielding influenza strains. For the virus strains investigated the moi has no impact on influenza vaccine production, as the resulting titers do not differ significantly and the fraction of infectious virions decreases. A fast progress in infection combined with a late onset of apoptosis results in higher titers as it prolongs the production phase. The gained knowledge can help to improve the vaccine production process efficiency.

P4.170. DOWN-REGULATION OF CD81 IN PACKAGING CELLS PRODUCING HCV-E1/E2 RETROVLPS: TAILORING RETROVIRAL VECTOR IMMUNOTOXICITY

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Retroviral derived vectors (RV) have found valuable therapeutic applications including gene therapy and VLP based vaccinology. Yet, they incorporate significant amounts of proteins derived from the producer cell, capable of inducing strong immune response. In this work we investigated the possibility of altering the composition of host immunogens incorporated in retroVLPs produced by an HEK 293 derived cell line stably expressing Murine Leukemia Virus (MLV) gag-pol. Tetraspanin CD81 down-regulation was targeted as it is a host protein largely integrated in RV membrane, conferring a strong immunogenic character to the vector. RNAi by shRNA lentiviral vector transduction was efficiently used to silence CD81 expression (up to 99%); the retroVLPs produced by knocked-down cells were shown to be absent in CD81. Silenced clones (shCD81 rVLP) were analyzed for cell proliferation, morphological changes, susceptibility to oxidative stress conditions and rVLP productivities. Although the results advised on careful attention when choosing a manipulation target and close monitoring for possible side effects on cellular production performance, they validate the manipulation of cellular protein content as tool for altering the composition of host derived immunogens in RV membrane. shCD81 rVLP cells are now being used as a production platform of retroVLPs displaying E1/E2 Hepatitis C Virus glycoproteins for further immunogenicity/immunotoxicity studies in animal models.

This work highlights cellular protein manipulation as a potential tool to improve the pharmacokinetic and pharmacodynamic properties of RV based vaccines. We envisage it will be useful for reducing the immunogenicity and enhancing in vivo half-life of gene therapy vectors, or to exploit potential adjuvant effects of host proteins in vaccine development. Moreover, it has the potential to be expanded to lentiviral and other enveloped virus based vectors.

P4.171. STABLE MDCK CELL LINE WITH INDUCIBLE NS1 EXPRESSION SUPPORTS PROPAGATION OF INFLUENZA VIRUS LACKING THE NS1 GENE TO HIGH TITRES IN AN INTERFERON INDEPENDENT MANNER

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Influenza A virus lacking the non-structural NS1 protein (delNS1) have potential use as live attenuated vaccines. Furthermore, they could be used for veterinary vaccines that allow differentiation between naturally infected and vaccinated-only animals. However, propagation of delNS1 in cell cultures yields low amounts of virus, prohibiting commercial vaccine production. We therefore established two stable MDCK cell lines that show inducible expression of the NS1 allele B protein.

Both cell lines expressed NS1 to about 1000-fold lower levels than in influenza virus infected cells. Nevertheless, expression of NS1 increased delNS1 virus titres to comparably high levels found after infection with an isogenic virus strain containing an intact NS1 gene. NS1 expression increased the infectious virus titres 244 to 544-fold and inhibited virus induced apoptosis.
However, virus-induced IFN-beta production was not significantly affected by NS1 expression. Thus low amounts of heterologous NS1 are sufficient to restore the replication of dNS1 in cell culture and this occurs in an interferon independent manner. In contrast to previous findings, heterologous NS1 expression itself did not induce apoptosis, nor did it affect cell growth. These cell lines thus show potential to improve the yield of delNS1 virus for vaccine production.

P4.172. APPLICATION OF ANIMAL-FREE RECOMBINANT BIOACTIVE PROTEIN SUPPLEMENTS TO IMPROVE THE PERFORMANCE OF CELL-BASED VIRAL VACCINE PRODUCTION.

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The development and regulatory approval of continuous cell lines for manufacturing viral vaccines has brought numerous benefits to production processes. We and others have contributed to upstream advances by improving cell culture media with the development of animal-free and chemically-defined recombinant protein supplements. The supplements developed include recombinant insulin-like growth factor-I (LONG®R3IGF-I), epidermal growth factor (LONG®EGF), transforming growth factor-α (LONG®TGF-α), transferrin, and albumin. Extensive literature on the action of these bioactive proteins on the cell types relevant to viral vaccine production, including Vero, MDCK, PerC6®, and HEK293 cells, supports their use in media design for these cell lines. In this presentation we present our initial results evaluating the effect of protein supplements on cell growth in several of these cell types. Recombinant supplements were added either alone or in various combinations and growth measured. Results indicated that individual supplements enhanced the growth of some cell types and various combinations of the supplements stimulated growth to a greater extent. The conclusion is that these animal-free recombinant bioactive protein supplements have the potential to improve growth performance of cell culture media in the absence of serum for cell types important in viral vaccine production.

P4.173 IN-SITU MICROSCOPY AND 2D FLUORESCENCE SPECTROSCOPY AS ONLINE METHODS FOR MONITORING CHO CELLS DURING CULTIVATION

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Chinese hamster ovary (CHO) cells have become the host cell line of choice for the biopharmaceutical industry. Because of rapid cell growth and high protein production rates they are often used to produce recombinant proteins.

For this reason it is important to cultivate these cells in large scale volumes and to monitor the cultivation process in order to keep a high product quality. Typical methods for this are offline analysis like cell counting, the measurement of various substances consumed or produced by the cells as well as the online monitoring of several process parameters (temperature, pH-value, concentration of dissolved oxygen).

For this work the CHO cell line CHO-K1 was cultivated in a 2.5 L stainless steel bioreactor with a work-volume of 2 L in a chemical defined medium. To monitor the cultivation process two different online methods were used: In-situ microscopy and 2D fluorescence spectroscopy.

The In-situ microscope (ISM) was immersed directly in the culture liquid and allowed an online observation of the cells during fermentation. Afterwards the obtained images were evaluated according to cell size, cell volume and cell concentration by automatic image analysis and compared with standard offline analysis methods, respectively.

The 2D fluorescence sensor was also directly connected to the bioreactor, thus enabling in situ measurements. The collected spectral data was analyzed and correlated with reference data determined through offline analysis. With the aid of chemometric models further cultivation processes can be predicted and monitored online.

P4.174 STEADY STATE AND DYNAMIC CONTROL PERFORMANCE OF THE AMBR™ AUTOMATED MICRO BIOREACTOR SYSTEM IN A CHO CELL BATCH CULTURE.

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Implementation of high throughput automated systems is recognised as a valuable approach in the field of bioprocess development, with broad acceptance that an efficient multi-parallel microscale bioreactor will become an important enabling technology. A standardised micro bioreactor system can unlock development bottlenecks in a variety of common bioprocess applications, such as cell line screening, media development, feed and process optimisation, QbD and DoE studies.

In this study we investigate the process control performance of the ambr™ micro bioreactor system, using 24 disposable micro bioreactors in parallel for a combination of steady state and dynamic control tests. We examine process control performance for three key process parameters (seeding cell density, pH, DO) and review cell count and glucose concentration profiles. Dynamic control tests challenge the capability of the ambr system to deliver common industrial process requirements such as pH and temperature shifts.

The results demonstrate the capability of the ambr system to support a range of typical steady state and dynamic control requirements for mammalian cell culture processes. The accurate and precise automated liquid handling and process control systems result in very low culture variation between replicate bioreactor conditions. Clear resolution in culture response (cell count, metabolism) is observed between different test conditions. The combination of tight process control performance, low replicate variation and high resolution between test results, illustrates the suitability of the ambr micro bioreactor system for large numbers of parallel bioreactor tests in a wide range of cell culture applications.

P5.01. EFFECT OF MITOCHONDRIAL AND ER-TARGETED Bcl-2 OVEREXPRESSION ON APOPTOSIS IN RECOMBINANT CHINESE HAMSTER OVARY CELLS UNDER SODIUM BUTYRATE-TREATED CONDITION

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Maintenance of mitochondria integrity by overexpressing Bcl-2, a typical anti-apoptotic protein, is a critical point in recombinant Chinese hamster ovary (rCHO) cell culture treated with sodium butyrate (NaBu) known as a typical specific productivity-enhancing factor. Bcl-2 distributes and functions in multiple intracellular organelles such as nucleus, mitochondria, and ER. To evaluate the effect of organelle-specific overexpression of Bcl-2 on NaBu-induced apoptosis in rCHO cells, Bcl-2 expression was restricted at mitochondria or ER by employing mitochondrial insertion sequence of ActA or ER-specific sequence of cytochrome b5 to its sequence. The rCHO cell lines overexpressing mitochondria-targeted Bcl-2 (MT-Bcl-2) or ER-targeted Bcl-2 (ER-Bcl-2) were established. Overexpression of MT-Bcl-2 or ER-Bcl-2 could increase cell viability and decrease LDH release under NaBu treatment condition. Also, it could suppress NaBu-induced apoptosis as evidenced from DNA fragmentation assay. Mitochondria membrane potential assay revealed that ER-Bcl-2 overexpression can maintain the mitochondria membrane integrity without helping MT-Bcl-2 overexpression, indicating that ER play an important role in alleviating NaBu-induced apoptosis by genetic modulation strategy. Taken together, it was found that restricted Bcl-2 overexpression at ER could inhibit the NaBu-induced apoptosis by the maintenance of mitochondria integrity in rCHO cells.

P5.02. A NOVEL MICRORNA REGULATES APOPTOSIS IN CHINESE HAMSTER OVARY CELLS

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Chinese hamster ovary (CHO) cells were induced to undergo apoptosis by exposing the cells to nutrient-depleted media. The apoptosis onset was confirmed by reduced cell viability and caspase-3/7 activation. A microarray comparison of known microRNA's in CHO cells exposed to fresh or depleted media revealed up-regulation of the mouse miR-297-669 cluster in CHO cells when subjected to depleted media. Mmu-miR-466h was chosen for further analysis as the member of this cluster with the highest overexpression and its up-regulation was confirmed with qRT-PCR. Since microRNAs suppress mRNA translation, we hypothesized that up-regulated mmu-miR-466h inhibits anti-apoptotic genes and induces apoptosis. A combination of bioinformatics and experimental tools predicted 38 mmu-miR-466h anti-apoptotic targets. Several genes were selected from this anti-apoptotic subset based on nucleotide pairing complimentarity between the mmu-miR-466h seed region and 3' UTR of the target mRNAs. qRT-PCR analysis revealed reduced mRNA levels of bcl2l2, dad1, birc6, stat5a and smo genes in CHO cells exposed to depleted media. Furthermore, the inhibition of the mmu-miR-466h increased the expression levels of these genes and resulted in increased cell viability and decreased caspase-3/7 activation. The inhibition of multiple anti-apoptotic genes suggests a pro-apoptotic role of mmu-miR-466h and its capability to modulate the apoptotic pathway in mammalian cell cultures.

P5.03. DIFFERENTIAL IN-GEL ELECTROPHORESIS (DIGE) ANALYSIS OF CHO CELLS UNDER HYPEROSMOTIC PRESSURE: OSMOPROTECTIVE EFFECT OF GLYCINE BETAINE ADDITION

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The use of glycine betaine combined with hyperosmolality is known to be an efficient means for achieving high protein production in recombinant Chinese hamster ovary (rCHO) cells. In order to understand the intracellular events and identify the key factors in rCHO cells cultivated with glycine betaine under hyperosmotic conditions, two-dimensional differential in-gel electrophoresis (2D-DIGE) followed by mass spectrometric analysis was applied. Differentially expressed 19 protein spots were selected and 16 different kinds of proteins were successfully identified. The identified proteins were associated with cellular metabolism (PEPCK, GAPDH, and PK), cellular architecture (□-tubulin and□ □-actin), protein folding (GRP78 and OSP94), mRNA processing (Rbm34, ACF, and IPMK), and protein secretion (□-COP). 2D-Western blot analysis of □-tubulin, GAPDH, Peroxidoxin-1, and GRP78 confirmed the proteomic findings. The proteins identified from this study, which are related to cell growth and antibody production, can be applied to cell engineering for maximizing the efficacy in the use of glycine betaine combined with hyperosmolality in rCHO cells.

P5.04. ENGINEERING CHO CELLS FOR IMPROVED CENTRAL CARBON AND ENERGY METABOLISM

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Studies with mammalian cells have shown that the presence and specific rate of lactate production reduces recombinant protein formation in culture. In a previous work we determined that lactate production was associated to a fast consumption of sugar leading to a high pyruvate synthesis rate. Only a small fraction is able to enter the TCA cycle due to saturation of the enzymes that metabolize this molecule and the rest is converted into lactate.
An improvement in specific productivity has been observed in mammalian cells after cell engineering. Expression of the yeast pyruvate carboxylase enzyme, provides and alternative pathway for pyruvate to enter the TCA [1]. Over expression of the enzyme malate dehydrogenase II also improved the TCA cycle´s flux. Manipulation of sugar transport leaded to a reduced carbon source consumption [2]. Knocking down of the lactate dehydrogenase gene reduced the specific rate of lactate production [3].
In this work we tackle the issue of engineering CHO cell metabolism for increased productivity by targeting multiple enzymes in order to reduce the rate at which the carbon source is consumed and decrease the rate of pyruvate accumulation. The objective is to obtain an engineered cell line with an improved energy metabolism and decreased lactate production. We propose to control both carbon uptake and its distribution in the pyruvate node by slowing the rate of transport and metabolism of the carbon source, and either limiting lactate production and/or increasing pyruvate flux towards TCA cycle. These changes were achieved by transfecting CHO DP-12 cells producing Human IgG with different genes involved in the central carbon metabolism that were selected based on the empirical evidence . By transfecting the cells with the and alternative sugar transporter we were able to observe in the selected clones a reduced carbon flux towards pyruvate production which lead towards less lactate synthesis. By transfecting the cells with the PYC or MDHII gene we expect to see further improvement in the metabolism by reducing further more lactate production [4] . Metabolic flux redistribution will be studied in all cases through metabolic flux analysis to compare carbon and amino acid utilization in the engineered cells with respect to the wildtype under normal culture conditions.
We expect that after the overall engineering process cells will have higher fluxes in the TCA cycle, and exhibit a redistribution in their metabolism. Cells with improved metabolic capabilities should lead to higher cell densities cultures with an extended lifespan and show an increase in the recombinant protein production.

[1] Irani, N., Wirth, M., van Den Heuvel, J., Wagner, R., Biotechnol Bioeng, 66(4):238-46 (1999)
[2] Wlaschin, K.F., Hu, W-S., J Biotechnol, 131(2):168-76 (2007)
[3] Chen, K., Liu, Q., Xie, L., Sharp, P.A., Wang, D.I., Biotechnol Bioeng, 72(1):55-61 (2001)
[4] Chong, W.P., Reddy, S.G., Yusufi, F.N., Lee, D.Y., Wong, N.S., Heng, C.K., Yap, M.G., Ho, Y.S., J Biotechnol, 147(2):116-21 (2010)

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P5.05. ENGINEERING CHO CELL METABOLISM FOR GROWTH IN GALACTOSE

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Chinese hamster ovary (CHO) cells are one of the main hosts for industrial production of therapeutic proteins, owing to well-characterized technologies for gene transfection, amplification, and selection of high-producer clones. This has motivated the search for different strategies for the improvement of their specific productivity being one of the key points for this approaches the reduction of metabolic end-products like lactate and ammonia. We are focusing on the use of different carbon sources as an alternative for enhancing the cultures' specific productivity, as they are metabolized more slowly than glucose leading to lower production of metabolic end-products. Particularly, we have observed that cultures in presence of glucose and galactose undergo a metabolic shift in which they are capable of remetabolize lactate. However, in this cultures, the specific growth rate is lower than the one in glucose, due to the a slower metabolism associated to the incorporation of galactose. Additionally, cultures were unable to survive in galactose as their unique carbon source due to lack of enzymes required for its metabolization.
We have targeted key genes of galactose metabolism: the galactokinase (GALK1) and a galactose transporter (GLUT8) as these enzymes may be the ones limiting cell growth. t-PA producing CHO TF 70R cells were transfected with galactokinase. Selected clones show reduced lactate production, undergo a metabolic shift where they remetabolize lactate, are capable of growing in the presence of galactose at a higher specific rate than control cells.
The positive results obtained motivate the identification of other points of optimization for the galactose metabolism previously mentioned, such as sugar transport. The transfection with this gene could enhance the effect of the galactokinase, due to the increase of galactose available as a substrate. This allows higher cell concentrations to be achieved in fed-batch processes. These results suggest that the development of pharmaceutical production processes based on cells with engineered metabolism is a promising alternative for achieving higher productivity cultures.
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P5.06. DESIGN AND SIMULATION OF A CONTROLLER SYSTEM FOR METABOLIC SHIFT REGULATION IN MAMMALIAN CELLS

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The existence of multiple steady states in mammalian cell culture with distinct cellular metabolism is of great importance in bioprocess design [1]. Different metabolic states are represented by different lactate to glucose stoichiometric ratios ($\Delta L/\Delta G$). Experimental evidence suggests that the existence of multiple steady states involves the interaction between metabolic and gene networks and their regulation [2]. The problem of providing an optimized strategy for glucose feeding in order to achieve a specific metabolic state is yet to be studied. We propose a model based strategy for designing a control system for metabolic state regulation that considers the biological complexity of the regulation of the cellular system.
We formulated a detailed metabolic model for mammalian cell metabolism, based on a system of differential equations for the main metabolic variables, involving a large number of variables and parameters. The model's parameters were obtained from literature and through a fitting process to experimental data. The existence of only one stable attractor supports the idea that metabolic regulation alone cannot explain the metabolic shift. Therefore, gene regulation is a crucial element that must be considered in a model that correctly describes this phenomenon. A discrete gene network model based on a potential model, which depends on the concentration of residual glucose, satisfies this need. A metabolic model, which is capable of representing the metabolic shift of mammalian cells in low glucose cultures, has been obtained. The finality of said model is to design a model based controller capable of maintaining a low metabolic state ($\Delta L/\Delta G$ ratio under 0.5) under continuous operation. The tentative input variables are the glucose and lactate concentrations as well as the $\Delta L/\Delta G$ ratio. The controller will modify the response of the system by manipulating the dilution rate and the glucose feed concentration of the culture.
[1] A. Europa, A. Gambhir, P-F Fu, W-S. Hu, Biotechnol. bioeng., 67, 25-34 (2000)
[2] R. Korke, M. Gatti, A. Lau, J. Lim, T. Seow, M. Chung, W-S. Hu, J. Biotech., 107, 1-17 (2004)
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P5.07. COMBINATORIAL ENGINEERING OF *BCL-2* AND *LDH-A* FOR IMPROVING CELL GROWTH AND REDUCING LACTATE PRODUCTION IN DIHYDROFOLATE REDUCTASE-DEFICIENT CHINESE HAMSTER OVARY CELLS

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Chinese hamster ovary (CHO) cells, as other continuously cultured cell lines, normally consume large amount of glucose, nearly 90% of which is converted to lactate even if oxygen is plentiful. The continuous production of lactate throughout a culture causes cell death due to acidification of the culture medium and elevated osmolality. Since the accumulation of lactate in the culture medium often exhibited a negative effect on cell growth and/or on valuable product formation, there were several attempts to reduce lactate production such as limitation of glucose supply, downregulation of lactate dehydrogenase-A (LDH-A), and enhancement of the flow of glucose carbon into tricarboxylic acid (TCA) cycle. These approaches could reduce lactate production and thereby increased maximum cell density and viability. However, cell culture was eventually terminated with the same reason in the normal late phase of culture, mostly apoptosis. These approaches could not block lactate accumulation but delay lactate accumulation to a certain point. Therefore, we developed less lactate-producing, apoptosis-resistant *dhfr*- CHO cell line (CHO-Bcl2-LDHAsi) by downregulating LDH-A and overexpressing Bcl-2, one of the most well-known anti-apoptotic proteins, in *dhfr*- CHO cell line. When the *dhfr*- CHO-Bcl2-LDHAsi cell line was used as a host cell line for the development of a recombinant CHO (rCHO) cell producing an Fc-fusion protein, culture longevity was extended without detrimental effect of genetic engineering on specific protein productivity (q_p). Furthermore, specific lactate production rate (q_{Lac}) and apparent yield of lactate from glucose ($Y_{Lac/Glc}$) was simultaneously reduced to 21-65% and 37-78% of the control level, respectively during rCHO cell development. Taken together, these results show that the use of a less lactate-producing, apoptosis-resistant *dhfr*- CHO cell line as the host cell line saves the effort of establishing a less lactate-producing, apoptosis-resistant rCHO cell line and expedites the development process of less lactate-producing, apoptosis-resistant rCHO cells producing therapeutic proteins.

Keywords: CHO cells; specific lactate production rate; apoptosis; LDH-A; Bcl-2

P5.08. A SYNTHETIC MAMMALIAN GENE CIRCUIT WITH TUNABLE BAND-PASS DETECTION CHARACTERISTICS

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Genetic signal processing circuits that can be configured to detect various chemical concentrations of ligand molecules have a pivotal impact on biological processes such as embryonic pattern formation in the development of higher eukaryotes. In order to understand these naturally occurring phenomena better, we designed a synthetic gene network which showed band-pass detection characteristics similar to e.g. some gap genes in the *Drosophila* embryo, by combining multiple transcriptional control mechanisms including autoregulation as well as state-of-the-art ligand dependent protein degradation. An accompanied computer model allowed us to search for parameter sets compatible with the preffered patterning. Engineered cells harboring the entire system are capable to sense and process environmental information (like a chemical ligand gradient) and integrate it into a reporter gene expression profile dependent on their positional-specification. Thereby only intermediate concentrations of the stimulus molecule result in evidenced and sharp reporter gene expression, whereas high and low stimuli molecule levels silenced the system. Moreover by exchanging transcriptional control components, the band-detect maximum can be tuned in the desired concentration range. With this system responding to one linear ligand gradient, we were able to generate simple patterns under controlled conditions. By logical interconnecting two different, independent ligand gradients more sophisticated pattern formation with specific output processing were feasible allowing the anticipation that this system will be applicable to test general design principles of biological networks with a spatial component. Furthermore engineered matured synthetic gene circuits, like this band-detect filter, with a particular, reliable and predictable behavior will ultimately foster advances in future tissue engineering, next- generation "programmed" therapeutics, tailor-made gene therapy scenarios and bioprocessing applications.

P5.09. EFFECTS OF KNOCKDOWN OF TWO CELL CYCLE MODULATING MICRORNAS IN RECOMBINANT HUMAN IGG PRODUCING CHINESE HAMSTER OVARY CELLS

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MicroRNAs (miRNAs) play important roles in gene regulation and are proposed biomarkers and cell engineering targets, but remain understudied in CHO Cells. We used a Locked Nucleic Acid (LNA) array designed for human, mouse and rat to screen highly conserved miRNAs in CHO. Based on the screen results, differential miR expression studies of 16 miRNAs using qRT were carried out in the following CHO lines: four DG44-derived lines producing a recombinant human IgG, parental CHO DG44 and parental CHO-K1 cells. miR-221 and miR-222 were significantly down-regulated in all IgG-producing lines compared to parental DG44. We also studied two of the DG44-derived lines amplified by step-wise increase of methotrexate, and found that let-7b and miR-221 were significantly down-regulated in the amplified lines. In parental CHO K1, let-7b, miR-15b and miR-17 were significantly down-regulated compared to DG44. Cdkn1b, a gene targeted by both miR-221 and miR-222, demonstrated up-regulation in all IgG producing DG44 lines. Two miR-17 regulatory targets, Cdkn1a and E2f1, were up-regulated as well. To explore modulating cell cycle to benefit IgG production, we transiently transfected two DG44 IgG lines with miRNA inhibitors for miR-221/222 and assessed IgG productivity. 96 hrs after transfection, we observed over 90% knock down in miR-221/222 and slight increase in IgG production in miR-221/222 inhibitor transfected cultures. Cdkn1b, Cdkn1a and E2f1 expression levels were all evaluated after miR221/222 knockdown. Following stable knockdown of both miR221 and miR222, cell growth, IgG productivity and cell cycle analysis were performed to evaluate the effect of long-term knockdown of these miRs. The results are our first steps towards utilizing highly conserved miRNAs in cell engineering.

P5.10. IDENTIFICATION OF THE PUTATIVE ROSA26 LOCUS IN CHINESE HAMSTER OVARY (CHO) CELLS AND TARGETED GENE INTEGRATION BY ZINC-FINGER NUCLEASE (ZFN) TECHNOLOGY

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The Gene Trap ROSA26 [Gt(ROSA)26Sor] locus has been identified within the mouse, rat and human genomes, and has been utilized for transgene integration in various applications, particularly in transgenic rodent models. This locus often exhibits relatively high efficiency when targeted by homologous recombination. Also, this locus is attractive due to strong, ubiquitous expression of integrated genes with minimal gene silencing. Here we report the first sequence identification and targeted gene integration study in CHO cells, as the first step towards using this locus for therapeutic protein expression. Initially, we identified a 1.3-kb segment from our proprietary CHO cDNA sequence database that was highly homologous to the mouse ROSA26 transcripts. Two microarray probes designed based on this sequence demonstrated that this putative ROSA26 transcript was present in five recombinant CHO lines. Further sequencing of parental CHO K1 genomic DNA revealed homologous sequences to mouse ROSA26 promoter as well as Intron 1. We designed ZFNs targeting Intron 1, and validated ZFN activity using Cel1 assay. Subsequently, we employed a promoter-less donor Green Fluorescent Protein (GFP) construct containing a splice acceptor and a 2A self-cleavage peptide flanked by homology arms. The ZFN expression vectors as well as the donor vector were co-transfected to parental CHO K1 cells. FACS enrichment based on GFP fluorescence was performed 10 days post transfection. Junction PCR yielded correct size products covering both homology arms, and the PCR products were sequence confirmed to support TI events. The enriched population was single-cell cloned using FACS, and junction PCR was repeated in selected GFP positive clones to calculate efficiency of integration. Lastly, RT-PCR and sequencing was performed of the transcript. These results are the first effort to employ ROSA26 in CHO cells for therapeutic protein production.

P5.11. ENGINEERING CHO CELL GROWTH BY STABLE MANIPULATION OF MIRNA EXPRESSION

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MicroRNAs are small non-coding RNAs involved in many biological functions such as cell proliferation, apoptosis and cell cycle. They are capable of regulating hundreds of genes in a post-transcriptional manner by translation repression and/or mRNA degradation. These characteristics make miRNAs attractive tools for CHO cell engineering as multiple genes, and possibly entire biological pathways, may be targeted simultaneously. After temperature-shifting CHO cell culture from 37°C to 31°C, several miRNAs were found differentially regulated of which miR-7 was found downregulated.
Our laboratory has previously demonstrated that transient overexpression of miR-7 leads to a significant decrease in cell growth. On the other hand, transient inhibition provoked increased cell growth, though to a lesser extent.
"Sponge" technology is an effective tool to stably knockdown mature miRNAs and can be used to understand post-transcriptional regulation in CHO cells and to identify cellular pathways related to cell growth, cell viability and cell productivity – these parameters being key targets for cell bioprocess improvement.
To achieve more complete knockdown of miR-7 in cells, stable CHO cell lines were engineered to express a miR-7 sponge consisting of a destabilised enhanced GFP with four tandemly repeated miR-7 binding sites downstream to increase efficacy of endogenous miRNA sequestration. These pools were found to have reduced GFP expression and improved growth rate compared to control pools.
Thus we demonstrate that stable miRNA knockdown may be used to manipulate as well as study the molecular mechanisms involved in CHO cell growth and productivity.

P5.12. ABOUT MAKING A CHO PRODUCTION CELL LINE "RESEARCH-FRIENDLY" BY GENETIC ENGINEERING

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The use of Chinese Hamster Ovary (CHO) cells for transient gene expression is gaining importance steadily, as it has been shown that both quality and quantity of proteins derived from CHO cells can be superior to material derived from Human Embryonic Kidney (HEK293) cells.
In order to augment yields HEK cell lines are frequently genetically modified, by e.g. stable integration of an expression cassette coding for Epstein-Barr Virus Nuclear Antigen 1 (EBNA-1) used in conjunction with expression plasmid vectors containing the EBNA-1 interaction site oriP.
Here we describe the generation of a CHO cell line stably expressing the EBNA-1 gene to enhance yields after transient transfection with polyethylenimine (PEI). Data on stable CHO pool generation, single cell cloning and characterization of single clones by Western Blot including the difficult identification of a suitable anti-EBNA antibody are shown. In a first approach 133 cell clones were characterized via a functional assay by transient transfection of a reporter gene. Due to unexpected instability of the EBNA-1 protein no candidate exhibiting enhanced production capabilities as a consequence of a functional EBNA-1/oriP interaction was identified. In a second approach, however, employing flow cytometry to identify and enrich for positive cell populations, EBNA-positive clones could be isolated. These clones show increased levels of reporter protein production in dependence on the presence of the oriP element on the expression plasmid. Characterization and expression data, also in comparison to a commercially available engineered CHO cell system will be presented.

P5.13. STRATEGIES FOR AN MAB-PRODUCING CHO STRAIN THAT GIVES A HIGH YIELD IN A SHORT CULTURE PERIOD

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Innovation in monoclonal antibody (MAb) production continues to be driven by cell engineering strategies that increase yield and improve product quality. To investigate the effectiveness of transporter overexpression strategies, we prepared a TAUT overexpressing host cell line that produced a higher proportion of high-MAb-titre strains than the parent host cell line, and selected a single TAUT/MAb strain. This TAUT/MAb strain remained viable for longer (up to 1 month) under common fed-batch culture conditions, and the improvement in viability can be attributed to improvement in metabolic properties. It also had higher productivity (up to >100 pg/cell/day) and MAb yield (up to 8.1 g/L/31 days), and the MAb it produced was comparable in quality to that produced by the parent cells. Those results suggested that this host cell engineering strategy has unique potential for the improvement of MAb-producing CHO cells; for example, it may be appropriate for high-density culture.
Our next challenge was to achieve a high titre in a short culture period by applying a strategy for modulating the metabolism using a by-product that is rapidly accumulated when the TAUT molecule is overexpressed and/or by enhancing the transcriptional machinery of the CHO cell. To accomplish this, we genetically modified the TAUT-MAb strain mentioned above to overexpress another gene. The resulting co-overexpression strain gave increased yield in a shorter culture period under common fed-batch culture conditions.

P5.14. INTEGRATIVE ANALYSIS OF METABOLISM AND METABOLIC BURDEN UPON GLYCOPROTEIN PRODUCTION IN HUMAN CELL LINE AGE1.HN

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The human designer cell line AGE1.HN represents a new and promising production system for biopharmaceuticals, particularly for those needing human-type post-translational modifications. For further rational improvement of the cell line and the cultivation process a detailed understanding of the metabolism and metabolic changes during glycoprotein production is desirable. Metabolism and metabolic burden upon production of α_1-antitrypsin were analyzed by comparing parental cells and a derived producer cell line. Exometabolome and biomass constituents were measured during batch cultivation of the cells. Metabolic fluxes were calculated and compared between both cell lines applying time resolved and stationary flux analysis methods. Interesting differences in the metabolism were found, e.g. increased glycine and glutamate production as well as serine consumption in the producer. The data indicate an influence on energy metabolism, C1 metabolism, nucleotide metabolism and cellular lipid content. This improved understanding of the metabolism and cellular changes during glycoprotein production supports the identification of targets for further improvement of (i) cell line, e.g. by genetic modifications and (ii) cultivation process, e.g. by improved feeding strategies.

P5.15. EFFECTS OF DIFFERENT GROWTH CONDITIONS ON THE CATALYTIC ACTIVITIES OF CENTRAL METABOLIC ENZYMES IN MDCK CELLS

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It is of common knowledge that Madin-Darby canine kidney (MDCK) cells are very suitable for the propagation of different influenza strains and, therefore, for the production of cell culture-based vaccines [1]. However, during growth in glutamine-containing media, the glycolytic and glutaminolytic fluxes of most cell lines are up-regulated and large amounts of toxic by-products, such as lactate and ammonia, are secreted into the medium. This metabolic imbalance often not only affects cell viability and productivity but also can prevent growth to high cell densities [2,3]. A promising approach to reduce waste-products is the substitution of one or several components in the culture medium [4,5]. In glutamine-free medium with pyruvate as carbon source, MDCK cells not only released no ammonia during cell growth but glucose consumption and lactate production was also reduced significantly [4]. In previous work with MDCK cells, several assays were developed, to determine the extra- and intracellular metabolite concentrations [6]. Furthermore, mathematical models were established to analyze the switch from glutamine-containing to glutamine-free (pyruvate) medium [7]. However, concerning the interpretation of experimental data and corresponding flux distributions, still some open questions remain.
The objective of this study was to further elucidate the impact of media changes on metabolism by establishing a high-throughput platform for enzyme activity measurements of mammalian cells [8]. The method established uses four sensitive enzymatic cycling assays, and allows the determination of 28 key enzyme activities of central carbon metabolism in extracts of MDCK cells. Adherent MDCK cells were grown to stationary and exponential phases in 6-well plates in serum-containing GMEM supplemented with glutamine or pyruvate as well as in serum-free EPISERF medium, and key metabolic enzyme activities of cell extracts were analyzed.
Significant differences were found in maximal enzyme activities from cells grown with pyruvate-containing medium compared to glutamine-containing medium. In particular, the overall activity of the pentose phosphate pathway was up-regulated during exponential cell growth in pyruvate-containing medium, which suggests that more glucose 6-phosphate was channeled into the oxidative branch and therefore more NADPH was required. Furthermore, the anaplerotic enzymes pyruvate carboxylase and pyruvate dehydrogenase showed higher cell specific activities with pyruvate, indicating an increased flux into the TCA cycle. An increase was also found for NAD^+-dependent isocitrate dehydrogenase, glutamate dehydrogenase and glutamine synthetase, which is a strong indicator for an increased flux through the right part of the citrate cycle in MDCK cells grown with pyruvate. It can be assumed that extracellular pyruvate was directly shunted into the TCA cycle, and that the increase in enzyme activities was most likely required to compensate for the energy demand and to replenish the glutamine pool. On the other hand, the activities of the glutaminolytic enzymes aspartate transaminase, alanine transaminase, malic enzyme and phosphoenolpyruvate carboxykinase were decreased in cells grown with pyruvate, which seems to be related to a decreased glutamine metabolism. Based on the established enzyme assays metabolic states of production cell lines can now be further characterized. This can then be used to validate mathematical models of cellular metabolism and to improve our understanding of intracellular metabolic interactions relevant for process characterization and optimization.

[1] Genzel, Y., Reichl, U., (2009). Expert Rev Vaccines 8, 1681
[2] Glacken, M.W., (1988). Bio-Technol 6, 1041-1050.
[3] Ozturk, S.S et al., (1992). Biotechnol and Bioeng 39, 418-431.
[4] Genzel, Y., et al. (2005). Biotechnol Prog 21, 58-69.
[5] Butler, M., Christie, A., (1994). Cytotechnology 15, 87-94.
[6] Ritter, J.B., Genzel, Y., Reichl, U., (2008). Anal Bioch 373, 349
[7] Sidorenko, Y., Wahl, A., Dauner, M., Genzel, Y., Reichl, U., (2008). Biotechnol Progr 24, 311-320.
[8] Janke, R., Genzel, Y., Wahl, A., Reichl, U., (2010). Biotechnol and Bioeng 107, 566-581.

P5.16. SELECTING FOR DESIRABLE BIOPROCESS PHENOTYPES IN CHO BY DIRECTED EVOLUTION

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This study utilised a directed evolution approach in an attempt to improve CHO cell bioprocess-relevant phenotypes by first stably transfecting a population of cells with a mutant library of a selected gene. In this case, Tata-Binding Protein (TBP) was selected for mutation due to its involvement in the transcription of a large number of mammalian genes, as well as its interaction with a wide range of transcription factors. Theoretically, non-deleterious mutations to this gene could generate a wide range of perturbations in metabolic pathways within the cell, allowing for a diverse pool of CHO clones. Once a stable population had been established, these were then subjected to an environmental stress during continuous passages in an attempt to select for cells that could better withstand this pressure and outperform their counterparts. From this 'evolved' pool single cell clones were then isolated and their characteristics compared to those of a wild type-TBP transfected control population that had undergone the same environmental stress in order to determine if any mutant TBP copies conferred observable advantage over those overexpressing the wild type gene.

P5.17. METABOLITE CHANNELLING AND COMPARTMENTATION IN THE HUMAN CELL LINE AGE1.HN DETERMINED BY ^{13}C LABELLING EXPERIMENTS AND ^{13}C METABOLIC FLUX ANALYSIS

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A thorough knowledge of the metabolism and its compartmentation in mammalian cells is desirable to enable rational design and optimization of producing cell lines and production processes for biopharmaceuticals. ^{13}C-Labelling experiments were conducted using glucose, glutamine, alanine and lactate tracers to identify active pathways in the central metabolism of AGE1.HN. It was observed, that alanine and lactate are produced mainly from one cytosolic pyruvate pool and that consumed alanine is mainly directly metabolized and secreted as lactate. Further important fluxes could be identified, e.g. backflux from TCA cycle. Using the obtained information, a carbon atom transition network of the central metabolism in AGE1.HN was set up. Special emphasis was put on the compartmentation of the metabolism distinguishing cytosolic and mitochondrial reactions. Metabolic flux distribution was analyzed by incorporating data from parallel labelling experiments feeding [1,2-$^{13}C_2$] glucose, [U-$^{13}C_6$] glucose and [U-$^{13}C_5$] glutamine. Several compartmented fluxes around the pyruvate/oxaloacetate node could be resolved accurately. It was found, that TCA cycle was mainly fed via oxaloacetate and 2-oxoglutarate during this phase. Mitochondrial pyruvate transport was low. Additionally, it was observed that pentose phosphate pathway activity was just about 2.3 % of the glycolytic flux. The ^{13}C flux analysis method that was applied in this study allows a detailed and accurate determination of metabolic fluxes in the central metabolism of mammalian cells and can thus be applied for studying related biological questions. The presented data is an important step for an improved system level understanding of the AGE1.HN cell line.

P5.18. LABEL FREE LC-MS CHARACTERISATION OF HOST CELL PROTEINS IN LATE STAGE CULTURE OF HYBRIDOMA CELLS.

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Separation of Host Cell Proteins (HCPs) from the mAb product is of significant interest due to their potential to negatively impact on both product safety and efficacy. Removal of HCPs requires multiple orthogonal separation methods as they are comprised of proteins with a diverse range of physiochemical properties. As a result, downstream processing now accounts for a significant portion of overall manufacturing costs. As mAb titres continue to achieve consistently high yields this places an increasing demand on downstream purification capabilities. Information on the composition and properties of HCPs could be useful for designing new mAb purification strategies.
Here we describe the use of label free LC/MS to characterise HCP components of conditioned media from mAb-producing hybridoma cell lines. Conditioned media samples were collected at various time points from late stage batch cultures. Following three hour reverse phase chromatography and mass spectrometry using an Orbitrap XL, data was analysed on Progenesis LC-MS software. Using this approach we have identified over 700 proteins present in the media. This includes a number of proteases which are known to be detrimental to product efficacy. Through studying the secretome of bioprocess cell lines we have also identified proteins that impact on cell culture viability. Information gained from this investigation could also be useful in devising engineering strategies to extend cell viability.

P5.19. INTRACELLULAR METABOLITE POOLS AND ENZYME ACTIVITIES OF AGE1.HN.AAT CELLS DURING CONTINUOUS CULTIVATIONS

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For the optimization of industrial processes for the production of biopharmaceuticals the metabolism of cultured mammalian cells is of crucial importance. Besides well established "omics" techniques like proteomics and transcriptomics, the application of a metabolomics approach for the quantification of intracellular metabolite pools in mammalian cells is still in its infancy. Continuous cultivations provide a constant environment for characterization of cell growth and product formation, and high-quality datasets can be obtained for detailed metabolic studies.
In this work an example is presented combining a metabolomics approach for the quantification of intracellular concentrations of metabolites [1,2] together with maximum enzyme activities [3] of various enzymes from energy metabolism of the human suspension cell line AGE1.HN.AAT (ProBioGen AG, Berlin, Germany). The cells were grown in continuous cultivation mode in serum-free medium at constant pH and dissolved oxygen concentrations. Data on cell growth, extra- and intracellular metabolites and maximum enzyme activities, as well as on product concentrations (alpha1-antitrypsin) were collected for different culture conditions from bioreactor cultivations with a working volume of 0.5 L. From these datasets the calculated rate constants for growth, substrate consumption and product formation for different steady states will be shown and discussed together with measured intracellular metabolite concentrations and maximum enzyme activities.
[1] Ritter, J. B.; Genzel, Y. & Reichl, U. (2006), *Journal of Chromatography* **843**(2), 216-226.
[2] Ritter, J. B.; Genzel, Y. & Reichl, U. (2008), *Analytical Biochemistry* **373**, 349-369.
[3] Janke, R.; Genzel, Y.; Wahl, A. & Reichl, U. (2010), *Biotechnology & Bioengineering* **107**(3), 566-581.

P5.20. THE REGULATION MECHANISM OF GLUCOSE TRANSPORTATION BY HYDROGEN AND PLATINUM NANOPARTICLES

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The regulation of glucose uptake into muscle and adipocyte cells is important for the control of blood sugar levels. Protein tyrosine phosphatase (PTP) is a redox-sensitive enzyme and reactive oxygen species (ROS) activate PTP to suppress the phosphorylation of insulin receptor and inhibited the insulin signal transduction pathway, inducing the blood sugar tolelance in type 2 diabetes patient. We found that electrolyzed reduced water (ERW) containing molecular hydrogen and a small amount of platinum nanoparticles (Pt nps) scavenge ROS [1] and improve the blood sugar tolerance in diabetic model mice. Pt nps are a new type of ROS scavenger and exhibit multiple activities like SOD, catalase and hydroxyl radical scavenger [2]. ERW and Pt nps elongated the lifespan of *C. elegans* by scavenging ROS in the nematodes [3]. Recently, molecular hydrogen has been reported to be a new scavenger of ROS and suppresses the symptons of various oxidative stress-related disease model animals [4]. However, the action mechanism of molecular hydrogen has not throughly been calarified yet. Molecular hydrogen can be activated to active hydrogen (atomic hydrogen) by Pt nps. Here we investigated the action mechanims of hydrogen and Pt nps on the glucose transportation in cultured animal cells.
Pt nps of 2-3 nm sizes were synthesized by the citrate reduction method. L6 rat myotube cells were treated with Pt nps in DMEM supplemented with fetal bovine serum for 2 h. Molecular hydrogen treatment was performed by cultivating cells under an atmosphere of 75%H_2/20%O_2/5%CO_2 for 14 h. The translocation of sugar transporter GLUT4 and the activation of AMPK were examined by western blotting. Intracellular glutathione levels were determined to reveal the effects of molecular hdyrogen and Pt nps. Pt nps and hydrogen stimulted the translocation of GLUT4 and phosphorylation of upstream AMPK, suggesting the enhancing effects of Pt nps and molecular hydogen on sugar transportation into muscle cells. Furthermore, Pt nps and molecular hydrogen regulated the balance of intracellular glutathion. In conclusion, it was suggested that Pt nps and molecular hydrogen are new types of signal transduction factors to regulate intracellular redox balance and stimulate glucose transport into muscle cells.
1. Shirahata S, Kabayama S *et al.*: Electro-reduced water scavenges active oxygen species and protects DNA. *Bioph Bioch Res Com* **234**, 269-274 (1997).
2. Hamasaki T, Kashiwagi T *et al.*: Kinetic analysis of radical-scavenging activities of platinum nanoparticles. *Langmuir*, **24**, 7354-7364 (2008).
3. Yan H, Tian H *et al.*: Extension of the lifespan of *C. elegans* by the use of electrolyzed reduced water. *Biosci Biotech Biochem* **74**, 2011-2015 (2010).
4. Ohsawa I, Ishikawa M *et al.*: Hydrogen gas as a therapeutic antioxidant by selectively cytotoxic oxygen radials. Nature Med **13**, 688-694 (2007).

P5.21. LDH-C CAN BE DIFFERENTIALLY EXPRESSED DURING FERMENTATION OF CHO CELLS

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Expression of CHO mRNA was measured with a special microarray from the CHO-Consortium leaded by WS Hu et.al. (http://hugroup.cems.umn.edu/CHO/cho_index.html). Cultivation experiments were performed in small scale 2L stirred tank bioreactors. During fermentation a temperature shift was performed. This was accompanied by a reduction of the cell specific lactate production rate.
The analysis of transcriptome samples before and after the temperature shift with microarrays showed several changes in the expression of available gene markers. LDH-C expression raised about 2 fold after temperature shift. LDH A and B did not change. As LDH-C is known to be a specialized isoenzyme in testis cells for consuming lactate in a lactate containing milieu, LDH-C could be proposed for a target of genetic engineering, facilitating lactate consumption in the late phase of high cell density cultures and prolonging longevity of CHO production cultures by reducing lactate concentration and consumption of base additions.

P5.22. A COMPREHENSIVE METABOLIC PROFILE OF CULTURED ASTROCYTES USING ISOTOPIC TRANSIENT METABOLIC FLUX ANALYSIS AND ^{13}C-LABELED GLUCOSE

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Metabolic modeling approaches have been useful to elucidate important aspects of brain metabolism, in recent years, and have contributed to a better understanding of the biochemistry underlying several neurological diseases. This work originally applies the concept of isotopic transient ^{13}C metabolic flux analysis (MFA) to estimate intracellular fluxes of cultured astrocytes, the most abundant cell type in the brain. This methodology explores the information provided by labeling dynamics time-courses in intracellular metabolites after administration of a ^{13}C labeled substrate. Metabolic fluxes were estimated by fitting a carbon labeling network model to isotopomer profiles experimentally determined by mass spectrometry during 24 h incubation with medium containing [1-^{13}C]glucose. Both the fast isotopic equilibrium of glycolytic metabolite pools and the slow labeling dynamics of TCA cycle intermediates could be well described by the model; the latter appears to be related with the large pools of glutamate and aspartate that are linked to the TCA cycle through reversible aminotransferase reactions. It was estimated that 11% of the glucose taken up by astrocytes was diverted to the pentose phosphate pathway. In addition, estimated fluxes through pyruvate carboxylase (PC) (PC/pyruvate dehydrogenase (PDH) ratio = 0.5), malic enzyme (representing 5% of the total pyruvate production) and catabolism of branched-chained amino acids (contributing with ~40% to total acetyl-CoA produced) confirmed these pathways as important contributors to the observed labeling dynamics. Consistent with the cellular need of maintaining cytosolic redox potential, the fluxes through malate-aspartate shuttle and PDH were similar. Finally, the estimated glutamate/α-ketoglutarate exchange rate (~0.7 μmol.mg prot^{-1}.h^{-1}) was about 2-fold the TCA cycle fluxes. In conclusion, this work demonstrates the potential of isotopic transient MFA to investigate brain cell metabolism at a global level. Furthermore, it represents a valuable approach to be used in the investigation and development of new therapies targeting specific metabolic pathways in the brain.

P5.23. INTRACELLULAR SIGNALING PATHWAYS IN HIGH DEFINITION

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Intracellular signaling pathways are a convenient construct with which to visualize and annotate information flow in cell signaling. Pathways incorporating a variety of generic signaling events (e.g., JAK–STAT, integrin binding, MAPK, and receptor tyrosine kinase signaling) have been described, usually at a high level, providing a view of participant molecules, reactions and interactions. More detailed representations of pathways have also been created for specific analyses, for example dissecting the effect of a pathway on a given disease. Unfortunately, most pathway resources are only conceptual in their representation of components and information flow (e.g., A leads to B which pairs with D, and BD leads to E) and often fail to identify the specific biochemical components, or the reactions necessary for the transmission of information.
More detailed and biochemically accurate models of signaling pathways give greater insight into the nature of intracellular signal transmission. For example, Palssøn's group at USCD constructed a stoichiometric model of JAK–STAT signaling. Highlighting the dearth of information available in existing pathway resources, this model comprised 297 reactions compared to only 25 in the publicly available Reactome database and 233 in the NCI Pathway Interaction Database.
We have developed a database for high resolution curation of intracellular signaling networks. As the precise identity of any given entity can alter its ability to participate in signaling reactions, this database is deigned to capture protein isoforms, sequence variation, subcellular localization, and reaction sites (i.e., reactive residues of a protein). By curating data at this level of resolution we can readily identify knowledge gaps in our understanding of fundamental biological process, identify new molecular entities and interactions as potential therapeutic targets, and gain deeper insights into the behavior of interconnected signaling pathways sharing common elements.

P5.24. A METHOD FOR METABOLOMIC SAMPLING OF SUSPENDED ANIMAL CELLS USING FAST FILTRATION

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Here we describe a sophisticated metabolomic sampling method for suspended animal cells using a fast filtration protocol. The main requirements for the fast filtration method were to reduce the time for quenching and cell–medium separation while keeping cell disruption to a minimum.
Additionally, the fast filtration method is compared to other sampling methods described in the literature.
The cell disruption using fast filtration and centrifugation was low at 5 % and 2.5 %, respectively. No influence of the cell disruption on the measured intracellular metabolite levels was observed. The intracellular amino acid content per cell was constant for filters loaded with up to $6x10^7$ cells. Thus, indicating a good extraction efficiency with the tested cell numbers.
The adenylate energy charge was used as an indicator of quenching efficiency for the comparison of the proposed method with other methods from literature. A significant advantage over sampling using a microstructure heat exchanger or a simple centrifugation protocol was observed. Comparison to quenching in cold methanol with ammonium bicarbonate and cold saline solution exhibited AEC values similar to quenching with fast filtration. Furthermore, experiments with isotope labeled glucose exhibited only small amounts of labeled glycolysis intermediates compared to saline solution quenching and centrifugation. An effect that is most likely due to the shorter overall sampling time of the fast filtration method.
This leads to the conclusion that the fast filtration method has a higher quenching efficiency than the other methods tested.

P5.25. EVALUATION OF SAMPLING AND QUENCHING PROCEDURES FOR THE ANALYSIS OF INTRACELLULAR METABOLITES IN CHO SUSPENSION CELLS

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Metabolomics, aiming at the quantification of all extracellular and intracellular metabolites, is a valuable tool for characterizing, understanding and manipulating the physiology of mammalian cells. While extracellular metabolite analysis is well established, required quenching and extraction procedures for intracellular metabolite analysis are not yet routinely available. Hofmann et al. (2008) successfully applied hot air for surface grown hepatic cells to analyze metabolites of glycolysis and pentose phosphate pathway. The analysis of intracellular metabolites in mammalian suspension cells is much more challenging particularly of those also present in the supernatant in substantial amounts, e.g. organic acids and amino acids. In this study a simple sampling and quenching strategy using ice-cold 0.9% saline as quenching solution (Dietmair et al., 2010) was tested on CHO cells. Quenching efficiency, preservation of cell integrity and avoidance of leakage, as well as cell separation and the use and necessity of washing steps were evaluated and possible sources of error are discussed. It was found that the cells are able to maintain cell viability and integrity in ice-cold 0.9% saline for at least 30 min. By using a tenfold excess of the quenching solution compared to the sample volume rapid cooling of the sample could be achieved and contamination with residues of the culture medium was found relatively low even without any washing steps. In quenched cells enzyme activity was efficiently halted and no significant change in intracellular metabolite concentrations was observed within the first 5 min of incubation at 0°C indicating complete quenching of the metabolic activity. However, by analyzing the quenching supernatant significant amounts of intracellular metabolites were found suggesting a cold-shock-effect as was observed in microorganisms (Wittmann et al., 2004; Bolten et al., 2008). The data indicates that reliable analysis of intracellular metabolite concentrations in mammalian suspension cells requires further refinement.

References:
Hofmann, U. et al. (2008), Biotechnol Bioeng 100, 344-54
Dietmair, S. et al. (2010), Anal Biochem 404, 155-164
Wittmann, C. et al. (2004), Anal Biochem 327, 135-9.
Bolten, C. J. and Wittmann, C. (2008), Biotechnol Lett 30, 1993-2000.

P5.26. METABOLIC FLUX ANALYSIS FOR QUANTIFYING CHO CELL RESPONSE TO CHANGING BIOREACTOR CONDITIONS DURING PERFUSION CULTIVATION

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We present metabolic flux data for CHO cells cultivated in perfusion culture at a steady-state cell density of 20 x 10^6 cells/mL. Bioreactor conditions such as pH, temperature and dissolved oxygen were varied over the course of the experiment and cellular physiological response to these changes was quantified using metabolic flux analysis. Cell specific nutrient consumption and metabolite production rates along with specific rates of amino acid metabolism were used to compute fluxes in a typical mammalian cell bioreaction network using metabolite balancing. Both oxygen uptake and carbon dioxide production rates were also measured to ensure an over-determined system that allowed comprehensive error checking during flux calculations. Multiple steady-states under varying bioreactor conditions were examined and metabolic fluxes and the consistency index, *h*, were computed for each condition. In addition to providing valuable information on cell physiology, our results suggest that design space studies for cell culture processes can be augmented with metabolic flux data. Flux information, when used in conjunction with productivity and product quality data, could substantially enhance process understanding and can lead to the design of more efficient and robust processes with high yields and consistent product quality.

P5.27. METABOLOMICS – A USEFUL TOOL FOR PREDICTION OF PROTEIN PRODUCTION AND PROCESSING?

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Although CHO cells are widely used as hosts for recombinant protein production, still only little is known regarding the interaction of metabolic alterations and protein production and processing. Therefore, a more sophisticated look into the cellular metabolism might lead to a more efficient use of the production medium resulting in high quantities of the protein with the desired product quality.
For intracellular metabolite quantification the sample preparation including quenching of cells is a very critical step strongly affecting subsequent results. A simple and straightforward protocol, not needing special equipment, was investigated focusing on the efficiency of stopping metabolic activities and the potential metabolic leakage due to losses in membrane integrity.
In a next attempt, the impact of a modified process on the metabolism and subsequently on the product quality of a recombinant glycoprotein was characterized using targeted mass spectrometry approaches for intra- and extracellular metabolite quantification. In order to find a link between the determined output parameters, detailed data evaluation including multivariate data analysis tools was implemented. Moreover, a comparison between the producer cell line and the corresponding mock cell line was performed. The influence of the increased protein production capacity and the need of a complex glycosylation machinery on metabolite profiles was assessed.

P5.28. TARGETED METABOLOMICS FOR BIOPROCESSING

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Bioprocesses like the cell-based production of biologicals, i.e. mainly recombinant proteins and monoclonal antibodies, require optimal culture conditions to obtain a high yield of quality products. The performance of a bioreactor highly depends on the cell characteristics as well as on the composition of the cell culture medium and the process conditions. As the metabolic activity of the cells is very high during fermentation, the external and internal metabolite compositions vary tremendously throughout the process. The quantification of a wide range of metabolic substrates and products is a prerequisite to understand and optimize the underlying cell-based activities. Furthermore, metabolite quantification reveals the composition of biologically derived cell culture supplements, thus serving as a tool to monitor supplement quality or providing the base for the formulation of a chemically defined medium supplement.
Targeted metabolomics was carried out using a mass spectrometry-based platform. Analyses were performed with commercially available KIT plates that allow the simultaneous quantification of 180 metabolites. The fully automated assay is based on PITC (phenylisothiocyanate)-derivatization in the presence of internal standards followed by FIA-MS/MS (for acylcarnitines, lipids, hexoses) as well as LC-MS/MS (amino acids and biogenic amines) using a SCIEX API 4000 QTrap mass spectrometer in the multiple reaction monitoring detection mode with electrospray ionization. On the same instrument, a validated HPLC-MS/MS quantification method is used for the analysis of energy metabolism intermediates. The concentration of individual fatty acids are determined as their corresponding methyl ester derivatives (FAME's) using GC-MS on an Agilent 7890 GC/5795 MSD instrument after derivatization. Vitamins were determined using LC-ESI-MS-MS technique after their pre-separation into two fractions (water- and fat-soluble vitamins).
The metabolomics approach to bioprocess monitoring made it possible to determine the concentration of a multitude of analytes from several metabolite classes in a sample- and time-efficient way by using small sample volumes and a multi-assay strategy. The analyte portfolio went beyond routinely determined culture media components, as it covered proteinogenic and non-proteinogenic amino acids, e.g. ornithine and citrulline, and intermediates of the energy metabolism, e.g. lactate, hexoses, succinate, as well as acylcarnitines, biogenic amines, glycerophospholipids, sphingomyelins, fatty acids, and vitamins. As an example of application, we will present case studies from mammalian cell fermentation to point out the feasibility and significance of the metabolomics technology for optimization and monitoring of cell-based protein production.

P5.29. EXPERIMENTAL DESIGN OF DYNAMIC ^{13}C LABELLING EXPERIMENTS FOR METABOLISM COMPARTMENTATION ANALYSIS IN EUKARYOTIC CELLS

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An accurate description of the cellular metabolism is vital for the rational design of genetic alterations. Metabolic flux analysis, complemented by dynamic ^{13}C labeling experiments, is a comprehensive mathematical tool able to compute the steady-state fluxes in a metabolic network. Mammalian cells exhibit a complex metabolism, which involves compartmentation, simultaneous production and uptake of metabolites, and active alternative pathways. Mitochondrial transporter activity as a part of the metabolic network is scarcely covered by the literature. However, impairment of the function of such transporters is the cause of many known diseases. Carbon labelling experiments require a lot of time and financial resources. Due to the complexity of the mammalian cell metabolism, it is necessary to formulate the appropriate labelling experiment design for accurately computing the metabolic fluxes. For this purpose, CHO K1 cells were cultured in a batch system and metabolic steady state was achieved. Uptake and production rates of extracellular metabolites were computed. The substrates for the labelling experiment were chosen based on their net consumption. A mathematical model that describes the dynamics of ^{13}C label through the metabolic network was systematically built based on data available from databases, literature and further constrained using information from cultivations. The model takes into account the complexity of the central carbon metabolism which is needed to describe the ^{13}C label dynamics. The final combinations of substrate labelling were chosen by simulating the model. The selection criteria was a high confidence for the estimation of the mitochondrial transport fluxes. With the appropriate choice of labelled substrates, subsequent labelling experiments will provide the data for a successful overview of the CHO K1 cells metabolism.

P5.30. A GENOME-SCALE METABOLIC MODEL FOR CHO

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In a joint collaboration with BGI, we have recently completed sequencing and annotating the CHO genome. The availability of this information is now bringing genome-scale science to CHO-based production of biopharmaceuticals. Using our proprietary CHOmics platform and genome-scale model of CHO metabolism, we have successfully developed strategies for media optimization and experimentally shown significant increase in product titers and decrease in byproduct accumulation. This modeling approach has also been successfully used to identify metabolically advantageous selectable markers in CHO cells. Genetically engineered CHO cell lines expressing these novel metabolic markers showed higher integrated viable cell density and lower peak byproduct concentrations compared with the parental cell lines. These results demonstrate that metabolic modeling combined with genome-scale technologies can significantly improve and accelerate research, discovery, and development of therapeutic proteins in mammalian cell lines.

P5.31. ON THE MODEL BASED OPTIMISATION OF SECRETING MAMMALIAN CELL CULTURES VIA MINIMAL GLUCOSE PROVISION

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Animal cell technology produces a wide range of high-value products, including vaccines, recombinant proteins, drugs for cardiovascular, respiratory and immune diseases, and monoclonal antibodies. Commercial synthesis of monoclonal antibodies (MAB) represents one of the most important products in the biopharmaceutical industry because of their diagnostic and clinical applications. However, the production of industrial scale quantities of MAB is an expensive and challenging task. There are a number of complications that make it a difficult process to ensure that the culture is growing under optimal conditions at all times. Optimisation of secreting cultures highly depends on regulation of the delicate balance between prolonged culture viability and increased productivity [1]. Alas, conditions that seem to prolong culture viability usually reduce productivity and vice versa. Optimal feeding profiles based on the provision of glucose and glutamine alone might yield an increase in the final product titre without being the global optimum. Thus far most model based approaches have focused on maximisation of final mAb titre by manipulating the input of Glucose alone or at best Glucose and Glutamine. The omission of other significant nutrients from such model based approaches leads to an overestimation of the required Glucose in an attempt to compensate for the modelling simplification [2]. However, the work of [3] illustrated that excessive feeding of glucose is not the best means towards higher product titres, since it shifts metabolism towards energy inefficient pathways which in turn leads to increased lactate production. Energy metabolism is a significant element of cell culture that has thus far been ignored from a modelling point of view. A truly optimal feeding profile should be derived based on the provision of adequate yet not excessive amounts of energy through controlled quantities of Glucose whilst at the same time ensuring a balanced supply of amino acids. Herein we present a dynamic model that successfully correlates cellular growth and mAb production to the availability of all amino acids in the culture media. Moreover by correlating the availability of energy in the form of ATP to both proliferation and mAb production we are able to derive a feeding profile that successfully maximises final mAb titre whilst restraining the provision of Glucose according to the energetic requirements of the culture. The resulting optimal feeding profile is then applied to fed-batch GS-NS0 cultures in order to prove the validity and applicability of our novel energy - based approach.

REFERENCES

1 Ho Y., Varley J., Mantalaris A. (2006) *Development and analysis of a mathematical model for antibody-producing GS-NS0 cells.* Biot Prog, 22(6), 1560

2 Kiparissides A., Pistikopoulos E.N., Mantalaris A. (2010). *Towards energy-based dynamic optimization …* Comp Aid Chem Eng, 28, 2010, 589-594

3 Xie L., Wang, D.I.C. (1994). *Applications of improved stoichiometric model in medium design and fed-batch cultivation*. Growth Factors, *1*, 17-29

P5.32. GUILT-BY-ASSOCIATION: LARGE SCALE COEXPRESSION ANALYSIS OF THE CHO TRANSCRIPTOME

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To expand our understanding of CHO transcriptomic regulation in the bioreactor an increase in sample numbers and progression beyond differential expression to system level analyses of gene expression is essential. We have applied coexpression network analysis to study gene expression in CHO across a broad range of bioprocess variables.

The dataset set utilised in this study consisted of 295 CHO specific microarrays from 121 individual CHO cultures producing a range of biologics including monoclonal antibodies, fusion proteins and therapeutic factors; non-producing cell lines were also included. Samples were taken from a wide range of process scales and formats that varied in terms of seeding density, temperature, medium, feed medium, culture duration and product type. Cells were sampled for gene expression analysis at various stages of the culture and bioprocess relevant characteristics including cell density, growth, viability, lactate, ammonium, titre and cell specific productivity (Qp) were determined.

Six distinct clusters of co-expressed genes were identified; five of which were found to have associations with bioprocess variables. Two coexpression clusters were found to be associated with culture growth rate, while associations between a further three coexpression modules and Qp were observed. Coexpressed gene clusters were found to be enriched for a number of significant biological processes including cell cycle, protein secretion and vesicle transport in gene set enrichment analysis (GSEA). In addition, trends observed between bioprocess variables have provided important evidence regarding the prioritisation of phenotypes during cell line development to maximise product titre.

P5.33. METABOLIC AND GENE EXPRESSION PROFILING: AN "OMICS" APPROACH TO FED-BATCH STUDIES

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The fed batch process is standard in the industry for the production of biotherapeutic proteins. As it exists today, the process is extremely well controlled, from the engineering of the reactors to the use of completely defined media. However, despite this exquisite control, the behavior of the cell themselves, as factories, is actually very poorly understood. The study presented here was designed to better illuminate the activities of the cells in a production process by analyzing the gene expression and metabolic profiles of CHO cell fed batch culture.
The analytical process for this study began with the generation of samples from a fed-batch process. These samples were then prepared for the two analytical platforms. Both metabolic profiling (by Metabolon, Inc.) and gene expression profiling (using a proprietary CHO microarray that monitors the expression of ~20K transcripts) were conducted to generate information about changes that occur during the cell culture process. Both data sets were used independently for pathway analysis. The results of the pathway analysis were compiled resulting in one final data set reflecting cellular and molecular changes that occur over the course of the fed-batch. We found that, despite what appeared to be a successful process, there were signs of intracellular stress and less than ideal energy metabolism. These are key topics we will need to be aware of and address as we continue to develop our cell culture process.

P5.34. AGE1.HN.AAT - GROWTH OF A NOVEL HUMAN CELL LINE IN GLUCOSE EXCESS AND LIMITATION: COMPARISON OF DIFFERENT METABOLIC STATES VIA STATIONARY METABOLIC FLUX ANALYSIS

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Mammalian cell lines are widely used in pharmaceutical industry for the production of complex drugs. Comparatively low specific growth and production rates, complex fermentation systems and media costs account for high total production costs and enforce constant process optimization.. Comparison of cultivations performed in different process modes clearly show that most established mammalian cell lines have an inefficient metabolism in media containing high glucose concentrations. For example in batch cultivations, the yield coefficient of lactate produced per consumed glucose (Y_{LacGlc}) is rather high with about 1.8 mol lactate/mol glucose [1]. Smaller yield coefficients are found for processes under glucose limitation like fed-batch and continuous cultivation processes.
In our studies we used the novel human designer cell line AGE1.HN.AAT (ProBioGen, Berlin, Germany). It is derived from neuronal tissue, optimized for suspension culture and was further modified to express α1-antitrypsin. Stationary metabolic flux analysis was used to investigate several aspects of metabolism. . The metabolic network is based on a model established for an adherent cell line [2] and comprises 113 reactions [2]. In batch cultivations with this cell line a Y_{LacGlc} of around 1.4 mol lactate/mol glucose was found. In contrast to this value Y_{LacGlc} was much lower in continuous cultivations under glucose limitation. In this case we observed yield coefficients Y_{LacGlc} close to zero.
A similar behavior was found for other metabolites, too. Comparing the minimum requirement of amino acids uptake for the formation of biomass under high and limiting glucose concentrations a positive effect of glucose limitation was detected. I.e., the difference between minimum amino acid uptake according to our model and the measured amino acid uptake was much smaller for cells during steady state in continuous cultivation than for cells during the exponential growth phase of batch cultivations. In addition, differences in intracellular flux distributions were analyzed to identify options for more increasing specific product formation rate.
References:
[1] Mulukutla2010, BC Mulukutla, S Khan, A Lange and WS Hu, *Glucose metabolism in mammalian cell culture: new insights for tweaking vintage pathways,* Trends in Biotechnology 28(2010) 476-484
[2] Wahl2008, SA Wahl, Y Sidorenko, M Dauner, Y Genzel, U Reichl, *Metabolic Flux Model for an Anchorage-Dependent MDCK Cell Line: Characteristic Growth Phases and Minimum Substrate Consumption Flux Distribution,* Biotechnology and Bioengineering 101(2008) 135-152

P5.35. BIOREACTOR CULTIVATION OF CHO DP12 CELLS UNDER SODIUM BUTYRATE TREATMENT – COMPARATIVE TRANSCRIPTOME ANALYSIS WITH CHO CDNA MICROARRAYS

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Sodium butyrate is not only known to inhibit proliferation but also to increase the specific productivity in cultivation of Chinese hamster ovary (CHO) cells – the most commonly used mammalian cell line for pharmaceutical production. So far, little is known about the underlying mechanisms and genes that are affected by butyrate treatment. Besides the proteomic approach to unravel proteins involved in the process, the analysis of transcriptomes presents another promising method. Here we present an application of our CHO cDNA microarray to identify genes associated with high productivity during cultivation of CHO cells under sodium butyrate treatment.
Batch cultivation of CHO DP12 cells was performed in 2 L bioreactor systems under pO_2– and pH–controlled conditions. In the exponential growth phase, 67 hours after inoculation, 2 mM sodium butyrate was added. Samples were taken before as well as repeatedly from 2 h up to 160 h after the addition of butyrate. Cells without sodium butyrate treatment reached a maximum viable cell density of $1 \cdot 10^7$ cells/mL while treated cells reached a plateau at about $6 \cdot 10^6$ cells/mL and kept a viability higher than 90 % for four days longer than untreated cells. Differences between treated and untreated cells could not only be seen in substrate and metabolite concentrations but also in antibody production. Untreated cells showed maximum concentrations of 110 mg/L antibody, whereas cells treated with sodium butyrate reached a maximum of 175 pg/L. Analogously, the specific antibody production was increased by a factor of 3.6 (4.5 pg/c·d compared to 1.2 pg/c·d) in cultivations where butyrate was added. Samples from different points during the cultivation were subsequently analyzed by CHO cDNA microarrays.
(Klausing, S. and Krämer, O. contributed equally)

P5.36. NEXT–GENERATION SEQUENCING OF THE CHINESE HAMSTER OVARY MICRORNA TRANSCRIPTOME: IDENTIFICATION, ANNOTATION AND PROFILING OF MIRNAS

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Chinese hamster ovary (CHO) cells are the predominant cell factory for the production of recombinant therapeutic proteins. Nevertheless, the lack in publicly available sequence information is severely limiting advances in CHO cell biology, including the exploration of microRNAs (miRNA) as tools for CHO cell characterization and engineering.
In an effort to identify and annotate both conserved and novel CHO miRNAs in the absence of a Chinese hamster genome, we deep–sequenced small RNA fractions of 6 biotechnologically relevant cell lines and mapped the resulting reads to an artificial reference sequence consisting of the entire set of currently known miRNA hairpins (miRBase v14). Read alignment patterns and read count ratios of 5' and 3' mature miRNAs were obtained and used for an independent classification into miR/miR* and 5p/3p miRNA pairs and discrimination of miRNAs from other non–coding RNAs. Overall, this strategy resulted in the identification and annotation of 365 conserved and 22 novel mature CHO miRNA sequences originating from 246 miRNA genes, but also 17 hairpin sequences that show clear features of non–coding RNAs. The quantitative content of next–generation sequencing data was analyzed and validated using qPCR, to find that miRNA expression patterns can serve as markers of cell line origin as well as growth conditions: the presence of serum in cultivation media resulted in a distinct transcription signature of 18 miRNAs that is lost upon serum–free adaptation. Likewise, host cells and producer cell lines also exhibit distinct miRNA expression signatures. Finally, using 454 cDNA sequence data of the CHO transcriptome, 26 validated mRNA targets of miR–17–92 were chosen to analyze the conservation of miRNA target sites between human, mouse and Chinese hamster. The results confirm that the majority of miRNA target sites are conserved in CHO, thus, allowing speculations that miRNA functions are conserved as well. This, together with the now publicly available comprehensive miRNA sequence information sets the stage for developing novel RNAi tools for CHO cell engineering.

P5.37. DYNAMIC TRANSCRIPTOME PROFILING OF TWO DIFFERENT CHO-K1 PHENOTYPES: COMPARISON OF GLUTAMINE-DEPENDENT VERSUS GLUTAMINE-FREE ADAPTED CELLS

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Since the establishment of Chinese Hamster Ovary (CHO) cells as the preferred mammalian host cell line for expression of therapeutic proteins, different CHO cell line phenotypes were generated for bioprocess and production optimisation. Due to the inefficient oxidation of glucose in cells cultivated in-vitro and the concomitant lack in energy, glutamine as an alternative energy source is commonly overdosed in feed media to improve cell densities in bioprocesses. However, the resulting by-product ammonia is toxic at high concentrations and has a negative impact on protein glycosylation, a major quality determining parameter of biopharmaceuticals.
To overcome this problem, CHO-K1 suspension cells were previously adapted to growth in glutamine-free media by FACS-assisted evolution. Despite a slightly prolonged lag phase, the newly generated CHO-K1^{Gln-} phenotype showed improved characteristics in viability, an increased life span and an increased integral of viable cell density during batch culture compared to its parental cell line.
As shown in Poster I, the expression of genes undergoes dynamic changes during a batch culture, so that comparison of transcript expression at a single point, for instance in exponential phase, is not sufficient to characterise the differences in growth and metabolism observed. Therefore in the present study we have looked for changes in the pattern of gene expression that characterises this new phenotype, rather than for single point differences. Again several patterns emerge, such as genes whose expression increases in the parent towards the end of the batch, but which are elevated throughout the culture in the Gln- phenotype, or the other way around. The relevant genes will be functionally characterised and discussed in view of the changes in metabolism and the improved viability.

P5.38. PROFILING OF MICRORNA TRANSCRIPTION DURING BATCH CULTIVATION OF CHO-K1 CELLS

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MicroRNA (miRNA) dependent post-transcriptional regulation of gene expression is of utmost importance for orchestrating various cellular processes including cell proliferation, cell death and cell metabolism. It is therefore not surprising that miRNAs have recently been recognized as tools for characterizing and engineering the phenotype of the most prominent bio-industrial workhorse - Chinese hamster ovary (CHO) cells - towards a better performance in modern upstream bioprocesses. For this reason we have studied the transcriptional dynamics of miRNAs over a 12-day batch cultivation of 4 CHO-K1 cell lines that have previously been adapted to grow successfully at different L-glutamine concentrations, ranging from 8 to 0 mM, and identified 118 miRNAs with significant regulation (adj. $p. < 0.05$, fold change > 1.5) in exponential, stationary or decline phase compared to lag phase at day 2. *K*-Means clustering of regulated miRNAs resulted in two predominant transcription profiles which were followed by the majority of miRNAs: profile A representing miRNAs with initial up-regulation during exponential growth followed by down-regulation during stationary and decline phase (65%), and profile B shared by miRNAs with up-regulation upon stationary phase or decline phase (35%). Annotation analysis identified several significantly enriched miRNA families and clusters for profile A such as the miR-17-92 cluster, which is known to possess oncogenic activity by suppressing apoptosis and driving cell proliferation in a complex network with c-myc and E2F. Correlation analysis with mRNA expression data produced from the same samples (see poster I of Bort JA. et al.) was performed to identify miRNA ~ mRNA networks with potential relevance for the regulation and timing of batch growth phases. Indeed, our data show that cooperative down-regulation of miRNAs towards the end of batch-cultivation results in the up-regulation of specific gene-sets, of which a significant fraction is associated with programmed cell death. Finally, by calculating the Euclidean distance of miRNA levels over all timepoints between cell lines adapted to 0 and 8 mM L-glutamine, 24 miRNAs were found to be kinetically differentially expressed (see poster II of Bort JA et al. for according mRNA data).
In conclusion, the presented data gives valuable insights in miRNA function with respect to CHO cell growth and metabolism and identifies several miRNA targets for cellular engineering.

P5.39. PROTEOMIC PROFILING IN USP PLATFORM DEVELOPMENT - METHOD OPTIMIZATION, STATISTICAL EVALUATION & ANALYSIS OF AN ANTIBODY PRODUCING CHO CELL LINE

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The strategic approach of omic tools such as transcriptomics, proteomics and metabolomics can fundamentally improve the understanding of cellular mechanisms within cell culture processes. The knowledge gained may be applied to metabolic engineering as well as to the design and optimization of bioprocesses. In combination with advances in cell line selection and media development it can shorten development time and increase productivity thus raise the efficiency in process development. Within the scope of this study a method for proteomic analysis of a recombinant antibody-producing CHO cell line was established, optimized and statistically evaluated concerning robustness and sensitivity. 2D-DIGE technology was applied to characterize a CHO cell line cultivated in a fed-batch process under different feeding strategies (process A and B). Additionally the proteome database was supplemented through a non gel-based proteome approach.
In designing the experiment, the expected technical and biological variability as well as false positive and false negative errors were thoroughly investigated and allowed the detection of protein expression changes greater than 1.3. For the comparison of different fermentation samples an applicable experimental design was deduced. A 2-way analysis of variance (2-ANOVA) was used to detect significant changes in the expression of proteins. The 2D-DIGE approach revealed that protein expression (i) significantly differed in both processes (78 proteins) due to differences in feed composition and (ii) changed over the fed-batch processes' time course (process A: 608 proteins, process B: 343 proteins). Out of 101 proteins analyzed 43 proteins were clearly identified by MALDI-TOF mass spectrometry. 14 % of the protein spots sequenced were matched to hamster database entries. The majority of the proteins identified were matched to mouse (62 %) and rat sequences (19 %). A substantial fraction of these proteins was categorized to protein biosynthesis/cell proliferation (44), energy metabolism (17) and stress/folding (8).
The results indicate that the process differences showed perturbance on the protein expression profile and demonstrate that protein expression profiling can be an important tool for the analysis and optimization of industrially relevant mammalian cell culture processes.

P5.40. DYNAMIC TRANSCRIPTOME PROFILING OF CHO-K1 SUSPENSION CELLS IN BATCH CULTURES

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Currently nearly 70% of all recombinant protein therapeutics produced worldwide are expressed by Chinese Hamster Ovary (CHO) cells. The ability of CHO cells to adapt rapidly to different culture media, the ease of its genome manipulation and the capacity to produce human-like glycosylated proteins contributed to a large extend to this success. However, the genomic resources available for CHO cells are still quite limited, which currently has led to major efforts to increase knowledge by next generation sequencing technology (see www.CHOgenome.org).
In the last years, microarray analysis was used for the identification of genes that control cellular phenotypes, such as enhanced growth under serum free conditions or high productivity. These genes could later be used for cell engineering and improvement. Most of these analyses were performed with samples taken during exponential growth phase in cell cultures. However, the cellular transcriptome is dynamic, changing in response to external and internal stimuli and thus reflecting the current functional capacity of cells as well as their ability to adapt to a changing environment. Therefore, during a typical production process such as batch or fed-batch culture it can be expected that the transcription pattern of genes will change and that such changes might give indications on the cellular state, may predict a decline in viability and monitor the capacity of the cell to produce a high quality therapeutic. This knowledge can be exploited both for cell line development, by using gene expression patterns to select the right type of production clone, and for process design, by optimising feeding strategies according to the individual needs of the cell line.
In the current study we monitored the change in expression patterns of mRNAs during lag, exponential and late stationary phases in CHO-K1 suspension cell cultures. In total, more than 1400 mRNAs were differentially regulated ($p<0.05$) over the different batch phases (lag, exponential, stationary, decline) relative to the batch starting point. Functional clustering was used to identify groups of genes with similar expression patterns, which were then subjected to functional pathway analysis. Essentially, there are two major patterns of regulated genes: those that are either up- or downregulated in stationary and decline phase, and those that are up- or downregulated during exponential growth relative to phases of low growth such as lag and stationary phase. For validation of microarray analysis data, selected genes from enriched pathways were validated by qRT-PCR using sequenced CHO cDNA for primer design. The significance of enriched pathways on process design and product quality will be discussed.

P5.41. COMBINED "-OMICS" INSIGHTS INTO ANTIBODY-PRODUCING CHO FED-BATCH PROCESSES

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The rapid development of "-omics" technologies in the last decade has led to an increase in the number of "global profiling" studies conducted on recombinant protein-producing mammalian cell cultures. However, the majority of these studies have focused solely on single-level "-omics" analysis, which limits our understanding of cellular mechanisms to that particular "-omics" level. Consequently, recent reports have highlighted the need for an integrative "-omics" approach[1,2], which involves the combined study of multiple "-omics" datasets, in order to better comprehend the complex relationships that exist between different molecular levels intracellularly. The information obtained from such studies can contribute towards the development of strategies for further improving recombinant protein production yield from mammalian cells.

The aim of this study is to utilise a combined "-omics" approach to gain a more comprehensive insight into intracellular behaviour during the fed-batch culture process. A time-course transcriptomics and metabolomics profiling experiment has been conducted on replicate fed-batch bioreactor cultures of monoclonal antibody-producing CHO cells. Data pre-processing is first carried out individually for each "-omics" dataset before both are analysed using a series of common statistical tools. These include the use of hierarchical clustering to identify major trends, as well as Pearson's correlation analysis to shortlist key components with respect to cell growth rates. To date, the analyses have yielded two groups of potentially interesting compounds. These include molecules that are involved in lipid metabolism, as well as those participating in the removal of reactive oxygen species from the cell.

References:

1. Joyce, A.R. and Palsson, B.O. The model organism as a system: integrating 'omics' data sets. (2006) *Nature Rev. Mol. Cell Biol.* **7**:198.
2. Wang, Y-C. and Chen, B-S. Integrated cellular network of transcription regulations and protein-protein interactions. (2010) *BMC Syst Biol.* **4**:20.

P5.42. COMPARATIVE METABOLOMICS ANALYSIS TO UNDERSTAND LACTATE METABOLISM IN CHO FED BATCH CULTURES

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During the development of a chemically defined media, it was observed that the lactate metabolism profile of an individual cell line could be shifted from net lactate production (LP) to net lactate consumption (LC) by simply altering the copper concentration in the basal medium and that different cell lines could demonstrate the LP or the LC profile at the same copper concentration. It was also generally observed that the LC profile correlated with enhanced growth, productivity and process scalability when compared to a batch with the LP profile. Two orthogonal metabolomics studies were conducted using CHO cells in fed-batch cultures to further investigate lactate metabolism shifts with the use of CDM. In the first study, two different cell lines were cultured in the same medium; one lactogenic and the other lactate consuming. In the second study, a single cell line but two different media with different copper concentrations were used to obtain LP (low copper medium) and LC (high copper medium) phenotypes. Time course cell pellet and supernatant from both studies were analyzed for metabolomic profiles.

The primary finding from these two studies was that the cells in LP conditions exhibited a less efficient energy metabolism, with glucose primarily being converted into pyruvate, sorbitol, lactate and others glycolysis intermediates. This decrease in energy efficiency may be due to an inability of pyruvate and acetyl-CoA to progress into the TCA cycle. The lack of progression into the TCA cycle or overflow metabolism in the LP cells, resulted in an inadequate supply of ATP for the cells. As a consequence, glycolysis remained the major contributor for ATP generation, which in turn led to higher lactate production. In addition, free fatty acids accumulation was observed to compensate for the energy requirement of LP cells. These findings provide a strong indication that lactate metabolism is related to the oxidative metabolic capacity of cells. Further, the interaction between lactate metabolism shift and the mitochondrial oxidative capacity will be discussed.

P5.43. CHARACTERIZATION OF THE HUMAN AGE1.HN CELL LINE: A SYSTEMS BIOLOGY APPROACH

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With the emergence of functional genomics approaches in the past decade, powerful tools for the discovery and understanding of cellular mechanisms are accessible. The main purpose of those analyses is to investigate the cell regarding on the level of the transcriptome, proteome and metabolome to retrieve information on possible limitations for growth or productivity. In subsequent steps, these limitations could be addressed by genetic modifications (e.g. overexpression of genes coding for bottle neck enzymes) or supplementation of the media.
In this work, we present a systems biology approach for the analysis of the human protein expression cell line AGE1.HN (ProBioGen AG, Berlin, Germany). To gather insights in the cellular processes, multiple batch cultivations were carried out. The metabolome analysis included both targeted as well as profiling approaches using LC-MS and GC-MS, respectively. The focal point of the analyses was on the central energy metabolism, namely glycolysis, TCA and oxidative phosphorylation. Intracellular metabolite concentrations showed the expected progression during a batch cultivation. Changes within the transcriptome were investigated using a whole genome DNA microarray. Hierarchical Clustering showed a multiplicity of significantly expressed genes, thus indicating a high degree of cellular regulation during the batch experiments. To detect changes within the proteome a 2D-DIGE approach was conducted and >100 differentially expressed protein spots are subsequently analyzed via nanoLC-MS and MALDI-MS.* All polyomic approaches included extensive bioinformatic data processing and analysis. The use of in-house established web-based data processing platforms is shown.
Combining the above mentioned techniques we were able to gather valuable insights in the cellular processes of a human protein production cell line.
* For further information see: “Analysis of the mitochondrial subproteome of the human cell line AGE1.HN – a contribution to a systems biology approach”

P5.44. ANALYSIS OF THE MITOCHONDRIAL SUBPROTEOME OF THE HUMAN CELL LINE AGE1.HN – A CONTRIBUTION TO A SYSTEMS BIOLOGY APPROACH

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Batch cultivations of the human production cell line AGE1.HN (ProBioGen, Berlin, Germany) reveals three distinct growth phases, an initial one with consumption of pyruvate and high glycolytic activity, a second characterized by a highly efficient metabolism with very little energy spilling waste production and a third with glutamine limitation and decreasing viability (Niklas et al.; DOI: 10.1007/s00449-010-0502-y). As many enzymes being involved in central metabolism are located in the mitochondria, we investigated the expression dynamics of mitochondrial proteins during batch cultivations of AGE1.HN cells.
Two standardised batch cultivations in a 20 L stirred tank stainless steel vessel (C-DCU, Sartorius Stedim Biotech, Goettingen, Germany) were carried out with daily sampling for metabolomics, transcriptomics, metabolic flux and proteomics, in order to generate reliable and comparable samples. For subproteomic analysis mitochondria were isolated with sucrose-density-centrifugation. After extracting mitochondrial proteins a 2DE-DIGE-approach was performed with four technical repeats (including dye-swap). 2DE-gel evaluation was performed with Delta2D-software (Decodon, Greifswald, Germany). 696 reliable mitochondrial protein-spots could be detected on a fused 2DE-gel, generated by melting all gel-images from the experiment. Quantitative analysis of this set of spots resulted in 114 proteins that were significantly differently expressed in the first and 206 proteins in the second cultivation during the cultivation time. More than 40 proteins were found to be significantly regulated in both experiments. These proteins are currently identified by LC-MALDI-MS. The resulting data will be integrated with the results of metabolic flux analysis.
Results of metabolomic and transcriptomic analysis of samples from the same cultivations are presented in the abstract of Sebastian Scholz, “Characterization of the human AGE1.HN cell line: a systems biology approach”.
This work is a part of the SysLogics Project: Systems biology of cell culture for biologics, founded by German Ministry for Education and Research (BMBF).

P5.45. ADVANCING BIOPHARMACEUTICAL PROCESS DEVELOPMENT: FROM COMPREHENSIVE BIOPROCESS DATA ANALYSIS TO INTEGRATION OF OMICS DATA

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Development of efficient bioprocesses is essential for cost-effective manufacturing of recombinant therapeutic proteins. In this context, the generation, the analysis, and the interpretation of bioprocess data are key in the pursuit to improve our process understanding and to derive promising strategies for efficient process design in a rational way. Since bioprocess development is increasingly science- and data-driven, both *process* data and *phenotypic cell-level* data analysis is essential to achieve further process improvement.
Here, we present a framework for advanced bioprocess data analysis. This framework consists of the multivariate data analysis (MVDA) methods principal component analysis (PCA), k-means clustering, and partial least-squares (PLS) models to analyze the *macroscopic* bioprocess data (e.g. *extracellular* metabolite concentrations). It also comprises the systems biotechnology methods metabolic flux analysis (MFA) and pathway-oriented, large-scale gene expression profiling which provide *intracellular* information on the characteristics of high-performance cell cultivations as well as metabolomics approaches.
The application of the developed framework for the design of high titer industrial bioprocesses will be discussed on the basis of representative examples from our bioprocess development. It will be shown how the integration of omics technologies enable an improved process understanding, the identification of targets for rational process optimization, and also a reduction of the number of cultivation experiments to be performed due to enhanced process knowledge.
Specifically, we will report on a process development project with an IgG producing Chinese hamster ovary (CHO) cell line in which the maximal mAb product titer could be increased from about 5 g/L to about 8 g/L.

P5.46. A QUANTITATIVE PROTEOMIC ANALYSIS OF THE INSECT *Sf*-9 CELL LINE IDENTIFIES REGULATORY RESPONSES TO GROWTH AND BACULOVIRUS INFECTION

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While several "omic" technologies are now popular in the frame of Systems Biology, their integration in a Systems Biotechnology discipline has yet to fully materialize. Arguably, one of the biggest challenges is the lack of a complete genome annotation for non-standard organisms, which complicates the development of reliable genome-wide technologies. Among these, quantitative mass spectrometry (MS)-based proteomics is perhaps the least exploited, in contrast with DNA microarrays or metabolic flux analysis. *Spodoptera frugiperda* (*Sf*) cells, coupled with baculovirus (BV) infection, are a system of choice to produce a range of recombinant proteins, vaccines and, more recently, gene therapy vectors. In the present work, a first proteomic survey of the *Sf*-9 cell line is presented. After gel-free fractionation of protein extracts, samples were subjected to tandem MS analysis on a LTQ Orbitrap equipment. Protein identifications were maximized by compiling available annotations of related species in a search database. The quantitative proteomic response to BV infection and culture growth was analyzed through stable isotope labelling with amino acids in cell culture (SILAC). A thorough statistical analysis of biological replicates allowed to confidently discriminate differentially expressed proteins due to each effect. Our results provide an overview of the interaction between viral infection and the cell physiological state, suggesting protein targets for the design of improved cell factories. On the whole, this work constitutes a case study of how to take advantage and maximize the information extracted from advanced "omic" technologies for bioprocess optimization.

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P5.47. PROCESS CHARACTERIZATION OF BHK CELL CULTURES USING CHO DNA MICROARRAYS

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Biopharmaceutical processes utilizing mouse or human cell lines are able to reap the greatest benefits from genome-scale technologies as they tap into the vast resource pool of publicly available genomic sequence information. Such resources, however, are lacking for the majority of industrially relevant cell lines including the Baby Hamster Kidney (BHK). Currently, there are fewer than 3,400 nucleotide sequences, including ESTs, deposited in GenBank for Mesocricetus auratus (Syrian hamster, from which BHK cells are derived). In this study, we tested the feasibility of cross-species hybridization of BHK samples on Chinese Hamster Ovary (CHO) Affymetrix microarray. Samples obtained from control and heat-shock treated recombinant protein producing BHK cultures were hybridized onto an Affymetrix oligo-array containing probes for over 22,000 CHO EST sequences. Probe pair level analysis revealed that only a fraction of probes with presumably high degree of orthology between BHK and CHO displayed good quality signal. A comparison with results obtained from similar heat shock treated CHO cells showed that, while same-species hybridization does provide a higher number of statistically significant differentially expressed genes, careful probe level analysis and masking can yield meaningful and reproducible results in cross-species hybridization. Further examples of application of cross-species hybridization in practical bioreactor settings will be presented in this poster.

P5.48. NEXT-GENERATION SEQUENCING OF THE CHO TRANSCRIPTOME

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Since 1957 Chinese hamster ovary (CHO) cells are used for *in vitro* cultivation as they require assimilable low sustenance. Today, CHO cell lines represent the most commonly used mammalian expression system for the production of therapeutic proteins and are considered as the mammalian equivalent of *E. coli* in research and biotechnology. The production of biopharmaceuticals in CHO cells is superior to protein production in bacteria, because mammalian cell lines procure complex folding and post-translational modifications like glycosylation. But contrary to the increasing importance in biotechnology and industry, comprehensive genome and transcriptome information of CHO cell lines are still rare. In this study, we applied 454 sequencing technology to CHO cDNA samples derived from different CHO cell lines and growth conditions, to cover a high variety of transcripts. cDNA sequencing was done with 454 titanium technology (Roche) and generated 1.84 mill. high quality sequencing reads with an average read length of 373 nt summing up to 603 Mb data. Assembly of the sequencing data resulted in 41,039 contigs with 24,149 contigs larger than 500 nt.
With a self-established bioinformatic approach for CHO cell cDNA sequencing and data processing, we assembled more than 36,000 cDNA transcripts from CHO cells and verified their quality exemplarily for the central carbohydrate metabolism. Additionally, we were able to distinguish between different transcript isoforms and assigning them to *Mus musculus,* our model for comparative analysis which provides a high quality genome reliable for annotation. Further, taxonomical and functional analyzes were done.
With the cDNA sequencing data we were able to design a customized CHO microarray. The designed probes are definitely assignable by their contig combination to 31905 splice variants of CHO transcripts. The microarray will be for transcription analysis of CHO cells under process conditions for process and cell line optimization.

P5.50. CHO ENGINEERING – A MODULATOR OF MULTIPLE CELLULAR PATHWAYS SUBSTANTIALLY ENHANCES THE YIELD OF BIOLOGICALS

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An increasing number of biologicals are successfully produced in CHO cells. Over the last two decades transcription levels and viable cell integral were increased to obtain greater yields. More recent cell engineering strategies focus on unfolded protein response or secretion pathways. However, there are many obstacles in recombinant protein expression and cell engineering is limited by the number of transgenes that can be stably expressed in parallel. Moreover, we experienced that often genes promoting protein production have also an inherent pro-apoptotic effect.
We therefore screened for a single upstream modulator target that addresses several potential bottlenecks and identified a candidate that indeed appears to be a key regulator of multiple cellular pathways. An appropriate combination of features was achieved through selection of a specific mutant.
The modulator gene was stably expressed in DG44 producer cell lines expressing either Trastuzumab or a monoclonal antibody drug candidate. We demonstrate that for individual modulator clones specific productivities as well as titers were found to be significantly higher than those obtained for control clones without the modulator. Additionally, CHO host cells have been successfully engineered with the modulator alone or in combination with additional anti-apoptotic genes. Animal origin free platform cell line development was done in parallel for engineered and original host cells to express a biosimilar antibody. Again, specific productivities and titers were substantially enhanced in producer clones derived from engineered host cells. The effect was even more pronounced for the combination with anti-apoptotic genes.

P5.51. CHROMOSOME IDENTIFICATION AND ITS APPLICATION IN CHINESE HAMSTER OVARY CELLS

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Chinese hamster ovary (CHO) cells are the most dependable host cells for the industrial production of therapeutic proteins. At present, CHO cells are the most important “industrial mammalian cell line”, similarly to *Escherichia coli* or *Saccharomyces cerevisiae* cells, among various mammalian cell lines (1). Recently, we constructed CHO genomic bacterial artificial chromosome (BAC) library from a mouse dihydrofolate reductase (DHFR) gene-amplified CHO DR1000L-4N cell line for genome-wide analysis of CHO cell lines (2). The CHO BAC library consisted of 122,281 clones and was expected to cover the entire CHO genome five times.
A CHO chromosomal map was constructed by fluorescence *in situ* hybridization (FISH) imaging using BAC clones as hybridization probes (BAC-FISH). More than 300 BAC clones were mapped on chromosome ideogram. Thirteen BAC-FISH marker clones were necessary to identify all 20 individual chromosomes in a DHFR-deficient CHO DG44 cell line because of the aneuploidy of the cell line. The chromosome homology map showed that a large scale chromosome rearrangement had been occurred in CHO-DG44 cell line. In order to determine the genomic structure of the exogenous *Dhfr* amplicon, a 165-kb DNA region containing exogenous *Dhfr* was cloned from the BAC library using high-density-replica (HDR) filters and Southern blot analysis. The nucleotide sequence analysis revealed a novel genomic structure in which the vector sequence containing *Dhfr* was sandwiched by long inverted sequences of the CHO genome. To investigate the effect of the palindrome structure derived from the BAC clone Cg0031N14 on *Dhfr* amplification in CHO cells, we constructed plasmids that contain part or the whole junction region of the palindrome structure. The transfected CHO DG44 cells containing part or the whole junction region of the palindrome structure could adapt quickly to high methotrexate (MTX) concentrations.

References

- T.Omasa, M.Onitsuka, W.-D. Kim, Curr. Pharm. Biotechnol. 11 (2010) 233.
- T.Omasa *et al.*, Biotechnology and Bioengineering , 104 (2009)986.

P5.52. COMPARATIVE ANALYSIS OF CHO CELL TRANSCRIPTIONAL DYNAMICS UNDER DIFFERENT CELL CULTURE CONDITIONS USING NEXT GENERATION RNA-SEQUENCING TECHNOLOGY

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Next Generation RNA-Sequencing (RNA-Seq) is a methodology for comprehensive measurements of cellular transcription at a scale, accuracy and precision never seen with previous technologies. We conducted a comparative RNA-Seq study of basic cell growth conditions to understand and improve high quality therapeutic protein production in CHO cells. Whole transcriptome analysis is a pre-requisite for full understanding of cellular processes that govern efficient bioproduction. In this study we examined global changes in gene expression in CHO cells under common bioproduction parameters. Eight CHO RNA samples from different cultures were sequenced on two full slides of a SOLiD™ System resulting in approximately 850 million sequence reads. These reads were mapped to multiple references including CHO EST, recently sequenced CHO genome content, as well as known annotated mouse reference transcripts. From this we report estimates of transcript expression levels and use known annotation to infer functional differences that can be associated with changing basic bioproduction growth conditions. These findings may uncover novel genetic mechanisms that could be optimized for improved bioproduction. This analysis of this data set represents the characterization of the CHO transcriptome in an unprecedented way.

Poster Author List

The list was prepared using last names only, therefore each name may contain several individuals.