ESACT

European Society for Animal Cell Technology

23rd ESACT meeting

JUNE 2013
23rd-26th

LILLE
GRAND PALAIS
FRANCE

Better Cells for Better Health

www.esact2013.com

Programme & Book of Abstracts

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Dear Colleagues and Participants of the 23rd ESACT General Meeting,

On behalf of the Organising and Scientific Committees of the 23rd ESACT Conference and Exhibition, as well as on behalf of the Executive Committee of ESACT, I am very pleased to welcome you to Lille, the 4th largest French metropolitan area, an artistic and historic city, but also an important center of economy, higher education and R&D!

This ESACT Meeting will be focused on "Better Cells for Better Health" highlighting both the importance of basic aspects and high-performing bioprocesses, as well as recent advances in stem cells, recombinant proteins and viral vectors. The scientific programme includes keynote and invited lectures, oral communications, posters and workshops. The traditional meeting format, by now a distinguishing feature of ESACT Meetings, is designed to promote scientific and technical interactions among participants, as well as networking: No parallel oral sessions, an unparalleled industrial exhibition featuring the latest technologies, systems and services in animal cell technology, a very rich poster programme and a large variety of social events.

The organization of an ESACT Meeting is an honour, but also a very complex undertaking, which involves a lot of planning and hard work. I would like to thank cordially all those who made possible the organisation of this major event:

- All the members of the Organising Committee who diligently took care of all the practical aspects, scientific and social ones, and I obviously include here the team of Le Public Système (LPS), our PCO, for the excellent, timely and professional support.
- The members of the Scientific Committee who defined the scientific content and selected abstracts and posters, including the team who will be in charge of the poster selection process for the Poster Prize.
- The members of the ESACT Executive Committee for their invaluable support all along the process.

We all cordially look forward to meeting old friends, introducing newcomers to the area of animal cell technology and experiencing lively interactions and discussions on the newest hot topics in the field.

I am sure that your stay in Lille will be an enjoyable and memorable one.

Enjoy the science, the flair and the social program!

Yves-Jacques Schneider
Chairman of the 23rd ESACT Meeting
Full Professor of Biochemistry & Chairman of the Life Science Institute
University of Louvain (UCL), Louvain-la-Neuve Belgium

Organising Committee:

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ISV, University of Louvain, Belgium
Chairman of the Organizing Committee

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KTH-Royal Institute of Technology, Sweden

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SUMMARY

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BD Cell Culture Media

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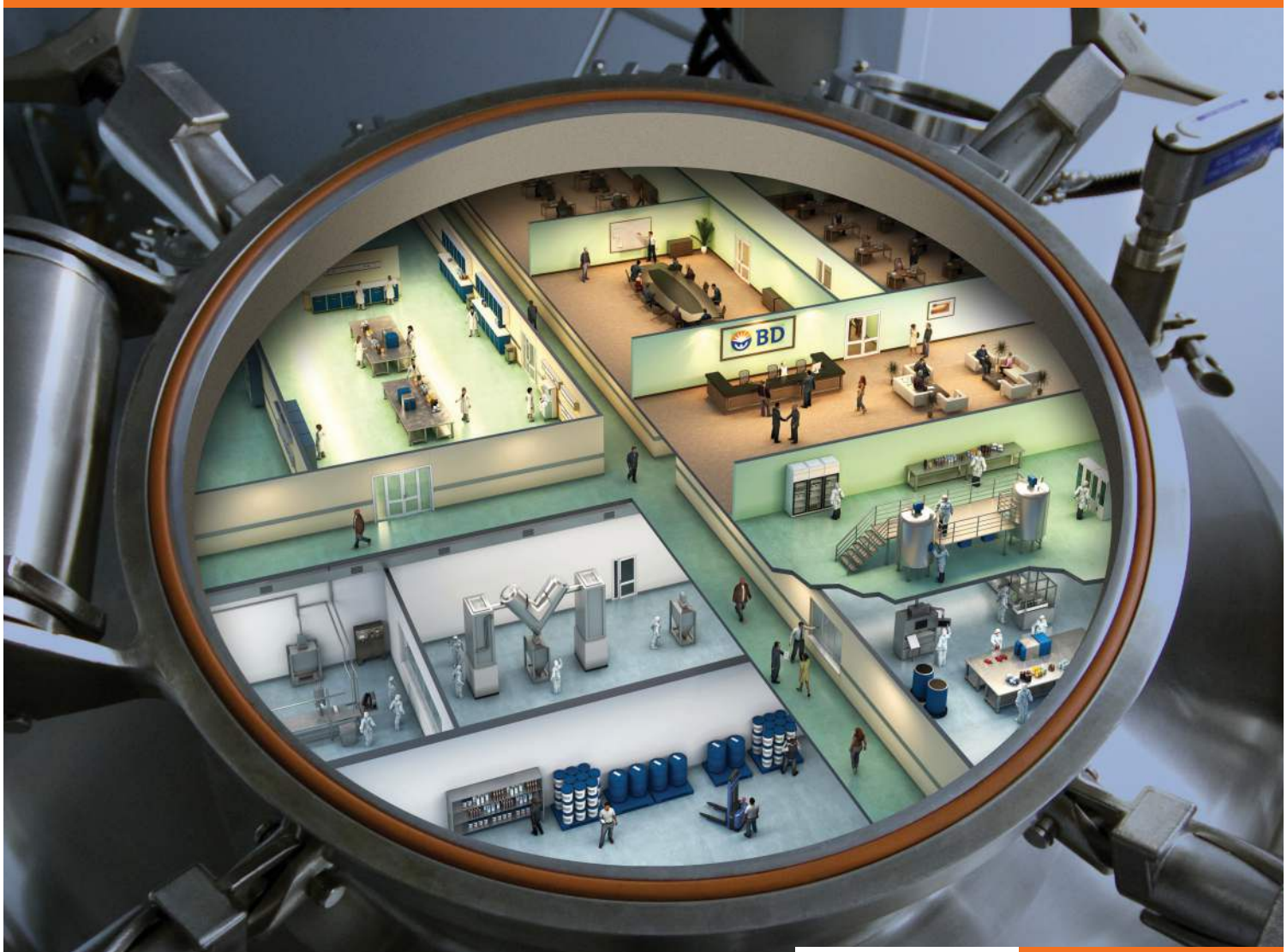

BD

Helping all people live healthy lives

A culture of improving cell culture.

In the constantly evolving business of bioproduction, it is good to know that you have a collaborative, trusted partner that can meet your cell culture media needs.

BD Biosciences – Advanced Bioprocessing leverages an unparalleled depth of experience, advanced technology and proprietary processes to deliver innovative solutions to biopharmaceutical manufacturing, all rooted in our 50 year culture of dependability and consistency.

From catalog and custom media, to complete customized solutions, our portfolio of products and services can help you make advances in cell performance, reduce risk, improve operational efficiency, and increase speed to market. The strength, security and transparency of our supply chain, in addition to the industry's first Animal Free, Antibiotic Free (AF²™) cell culture media and supplement GMP production facility, continue to set new standards for safety and quality.

BD Biosciences – Advanced Bioprocessing is committed to being a dependable partner to support your cell culture needs through the entire biopharmaceutical product lifecycle. Learn more at www.bdbiosciences.com/go/culture

BD Biosciences
Advanced Bioprocessing
7 Loveton Circle
Sparks, MD 21152
bdbiosciences.com

Congress Venue

Lille Grand Palais
1, Boulevard des Cites-Unies
59777 Euralille – France
Metro: Line 2 – Station: LILLE GRAND PALAIS

Opening Hours

	The Welcome Desk Level 1	Exhibition Area Level 3	Hot Line
Saturday 22nd of June	15h00 / 19h00		+33 (0)3 20 14 14 50
Sunday 23rd of June	8h00 / 21h00	14h00 / 21h00	
Monday 24th of June	8h00 / 19h00	8h00 / 19h00	
Tuesday 25th of June	8h00 / 15h30	8h00 / 15h00	
Wednesday 26th of June	8h00 / 19h00	8h00 / 17h00	

Wifi Access

Wifi access: esactmeeting
Password: lille2013

Access to the Congress

The delegate fees give access to the following:

- Access to all the conferences of the ESACT Congress
- ESACT Congress Lunches
- Traders Cocktail on Sunday, June 23rd
- Excursion on Tuesday, June 25th 2013 afternoon (pre-registration compulsory)
- Excursion Dinner on Tuesday, June 25th
- Gala Dinner on Wednesday, June 26th

Accompanying person fees give access to:

- Congress Lunches
- Excursion
- Excursion Dinner
- Gala Dinner

Taxi phone numbers

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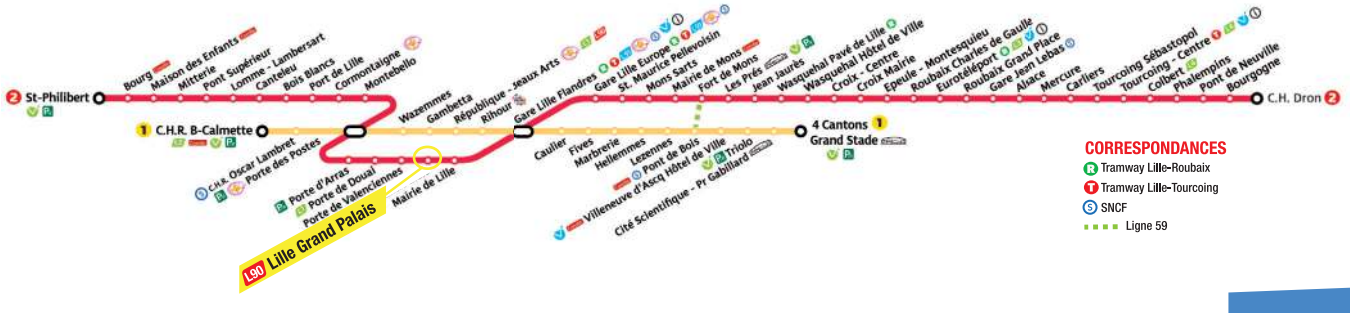
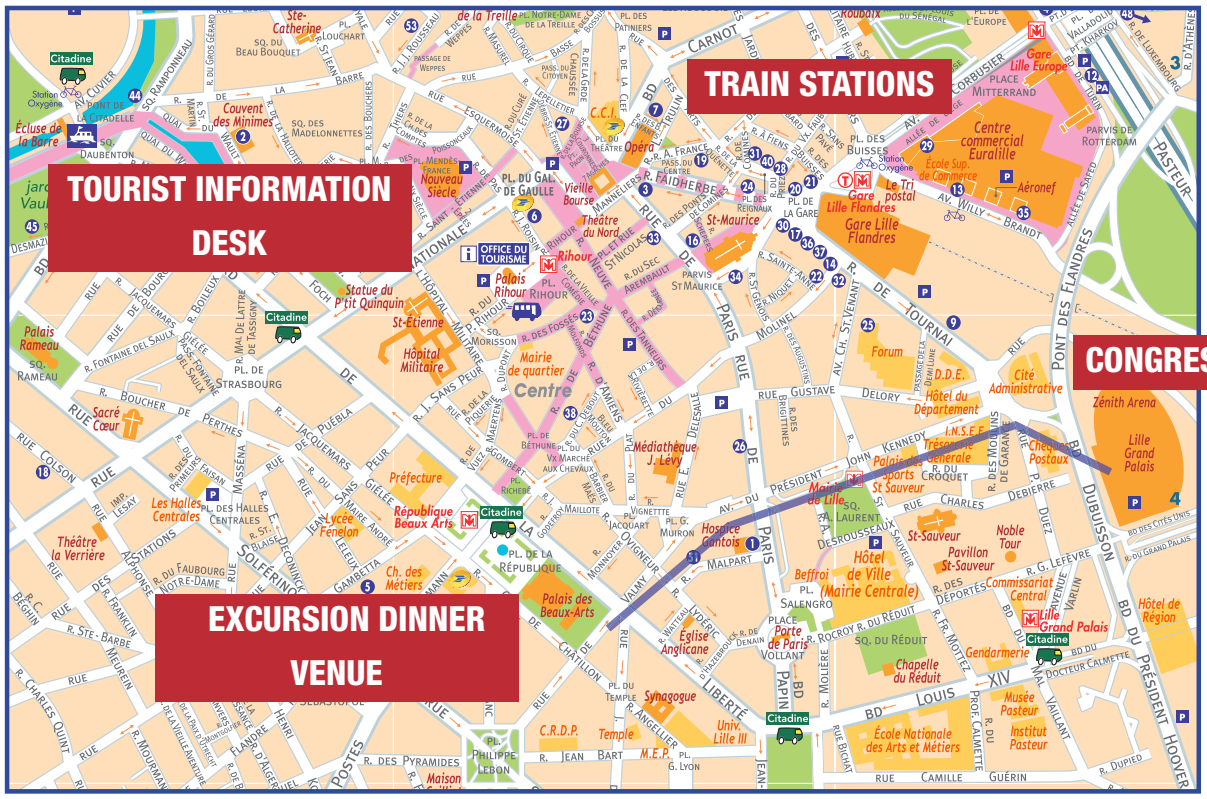
Metro map



Lille Map



Access to Lille Museum of Fine Arts: 15 min walking distance from Lille Grand Palais

Excursions Conditions

One ticket per participant is included in the registration fees. The number of participants is limited for some excursions and strict first-come-first-served order will be observed. No change in excursion choice is possible once registration is made. This excursion is offered by the Organising Committee. There will be no refund if you choose not to attend.

Bus Schedule for Excursions

	Departure from Lille Grand Palais	Return
TOUR 1: LILLE & The Royal district and Charles de Gaulle Birthplace	15h00	The cocktail-dinner will take place at Lille Museum of Fine Arts (see the map above). You can access by walking from Lille Grand Palais. A bus will be at your disposal at 19h00 in front of Lille Grand Palais with a limited number of seats.
TOUR 2: LILLE & The « Dutch gin » trail	14h00	
TOUR 3: LILLE & the LaM	15h00	
TOUR 4: LILLE & The Old Disctrict	15h00	
TOUR 5: BRUGES	14h30	The buses will bring you directly to Lille Museum of Fine Art
TOUR 6: GHENT	14h30	
TOUR 7: ARRAS & The Wellington Quarry	15h00	
TOUR 8: LEWARDE: The mining history	14h30	
TOUR 9: LILLE BY BUS	15h00	

Connecting with patients

UCB has a passionate, long-term commitment to finding more effective treatments for several specific diseases in the central nervous system and immunology disorders. Our challenge is to help patients and families living with the physical and social burden of severe diseases. It holds out the promise of a new generation of therapies that will enable them to enjoy more normal, everyday lives.

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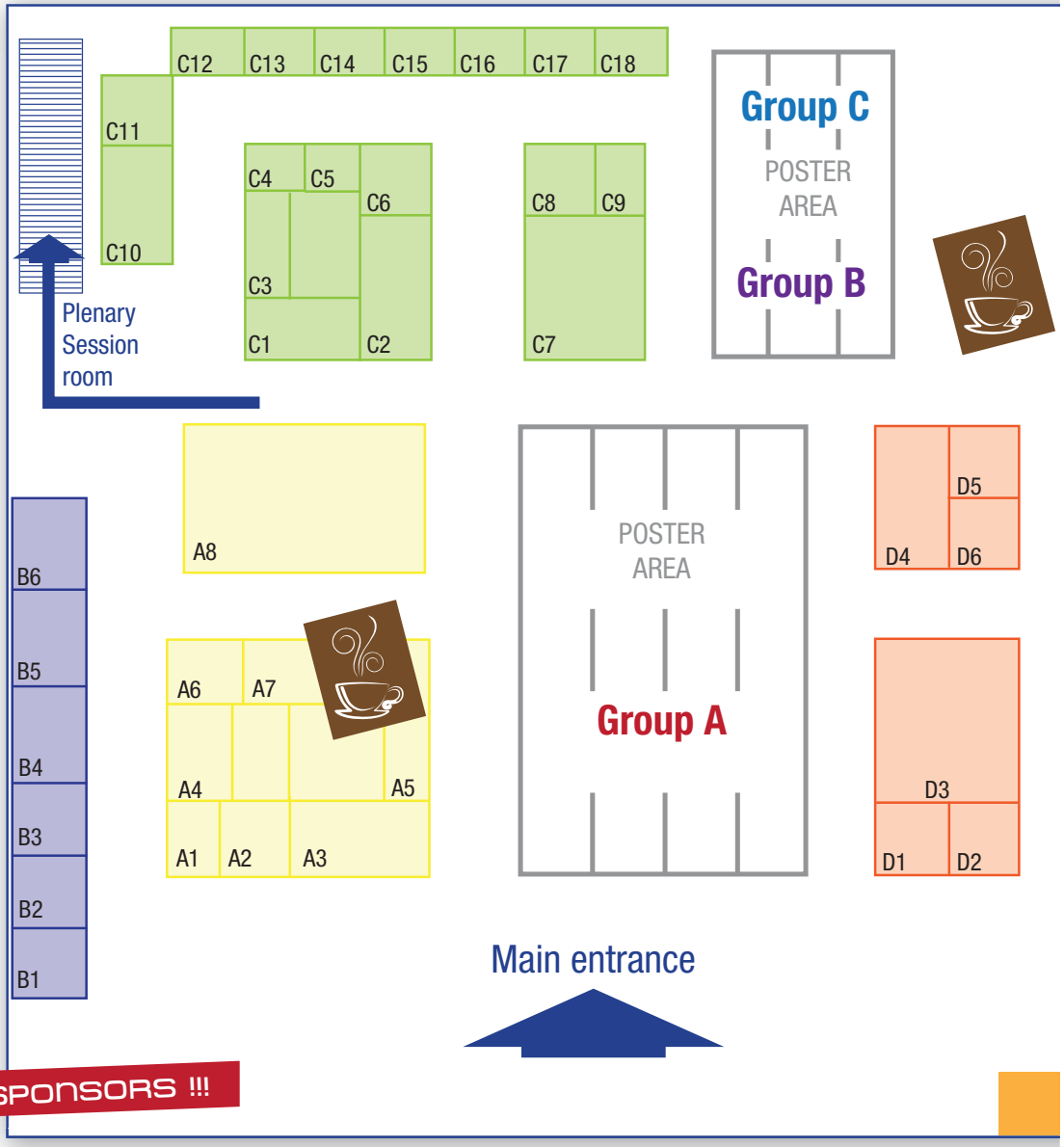
EXHIBITION MAP

VAUBAN AUDITORIUM



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APPLIKON BIOTECHNOLOGY B.V.	C11	CLEAN CELLS	I1
ASI	B3	COBRA BIOLOGICS LTD	H7
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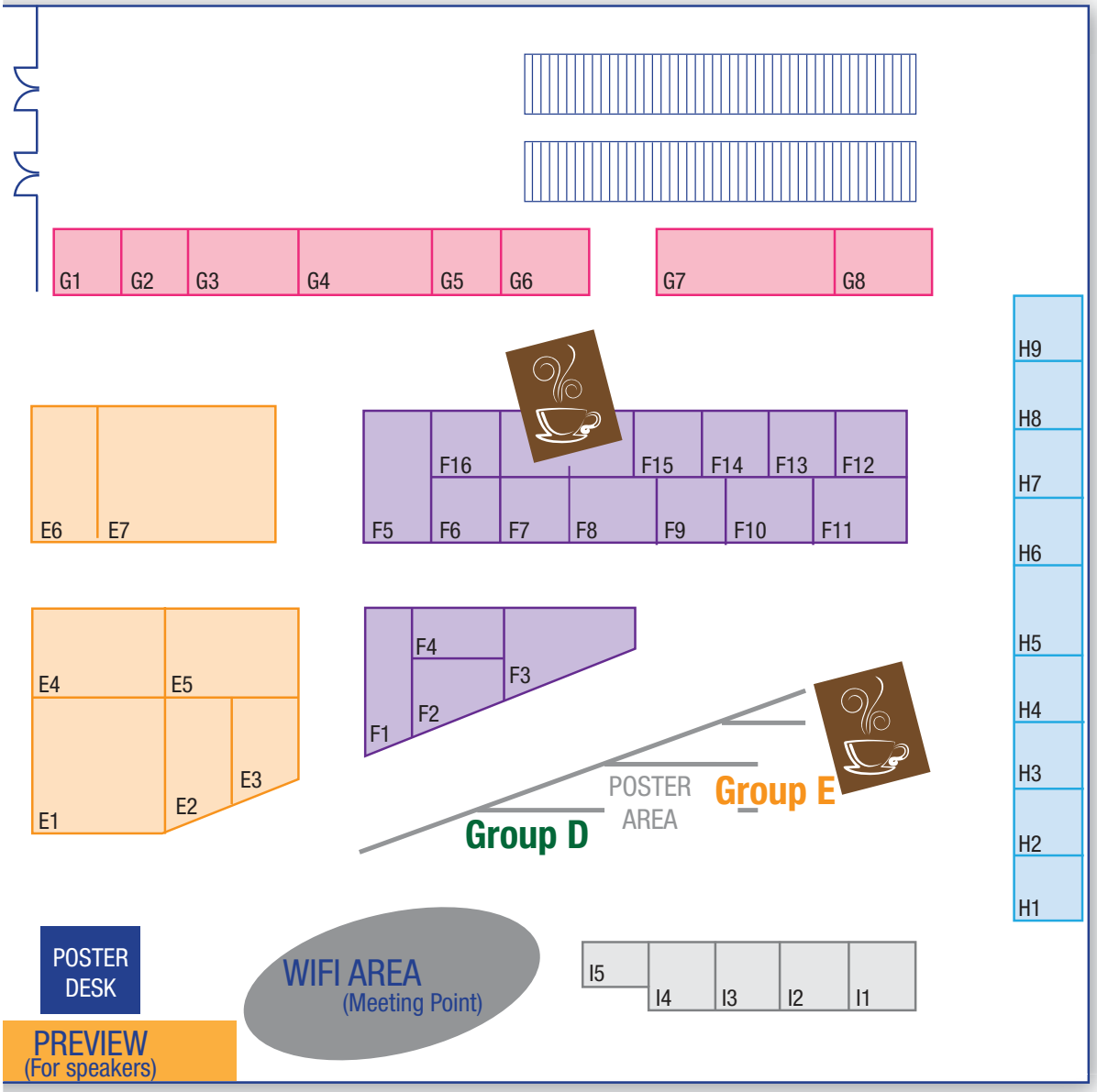
C-CIT AG	F12	GE HEALTHCARE EUROPE GMBH	A8
CELLECTIS BIORESEARCH	I4	GENETIC ENGINEERING & BIOTECHNOLOGY NEWS	A1
CELLON SA	G6		



GYMETRICS SA	C17	OVIZIO IMAGING SYSTEMS	C12
GYROS AB	G1	PALL LIFE SCIENCES	E6
HAMILTON BONADUZ AG	F2	PNEUMATIC SCALES ANGELUS - CARR CENTRITECH	A4
INFORS AG	B6	POLYPLUS TRANSFECTION	H3
INSILICO BIOTECHNOLOGY AG	F14	PRESENS PRECISION SENSING GMBH	F1
IRVINE SCIENTIFIC	G4	PROTAGEN PROTEIN SERVICES GMBH	B2
ISBIO	C4	PROTEIGENE	I2
IUL INSTRUMENTS GMBH	H6	RD-BIOTECH	C9
JM BIOCONNECT	C1	REFINE TECHNOLOGIES	G3
KAISER OPTICAL SYSTEMS	F7	RENTSCHLER BIOTECHNOLOGIE GMBH	H4
KERRY INC.	C2	REPLIGEN	A2
LGC STANDARDS	D6	SAFC AND BIORELIANCE	A3
LONZA	E1	SAINT-GOBAIN PERFORMANCE PLASTICS	B1
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MEDICYTE GMBH	C15	SGS VITROLOGY	G8
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METABOLON INC	E2	SPINNOVATION ANALYTICAL B.V	I5
MILTENYI BIOTEC	A6	TAP BIOSYSTEMS	H2
MOLECULAR DEVICES	F16	THERMO SCIENTIFIC	E7
NOVA BIOMEDICAL	F11	VIRUSURE GMBH	C6
OPTOCELL TECHNOLOGY	H5	WU XI APP TEC	F13

SUNDAY 23RD OF JUNE

	Rooms	EUROTOP Level 5	LIEGE Level 1	VAN GOGH Level 8	MATISSE Level 8	TURIN Level 1	ARTOIS Level 5
	8h00	Doors opening					
INDUSTRIAL	9h00	Thermo SCIENTIFIC	GE Healthcare	BD		ATMI	IrvineScientific
	11h00	Coffee Break in the Hall in front of each Workshop room					
	11h15	Thermo SCIENTIFIC	sartorius stedim	BD	Boehringer Ingelheim	KERRY	SAFC
	13h15	Lunch Boxes offered in the Hall in front of each Workshop room					
TOPICAL	13h45	Therapeutic vaccines	Metabolism as a key for improvement of cell culture processes - status and advances in analytics and data analysis	Is tomorrow's process fed-batch or perfusion?	The www.CHOgenome.org Resource for the International CHO Biootechnology Community		
	15h45	End of Workshop session					

SUNDAY 23RD OF JUNE

Rooms	VAUBAN AUDITORIUM Level 3	EXHIBITION AREA Level 3
16h00	Opening Ceremony	Exhibition & Posters
16h30	Keynote Lecture: A. CAPLAN	
17h15	SESSION I: CELL THERAPY AND VACCINES	
18h35	End of the session	
19h00 to 21h00	TRADERS RECEPTION in the EXHIBITION AREA	

MONDAY 24TH OF JUNE

Rooms	VAUBAN AUDITORIUM Level 3	EXHIBITION AREA Level 3
8h00	Doors opening	
9h00	SESSION II: ADVANCED CELLULAR MODELS	Exhibition & Posters
10h30	Coffee Break	
11h00	SESSION II: ADVANCED CELLULAR MODELS	Exhibition & Posters
12h30	Lunch Break in Jeanne de Flandre room	POSTERS SESSION A
14h30	SESSION III: EPIGENETICS AND SYNTHETIC BIOLOGY	Exhibition & Posters
16h30	Coffee Break	
17h00	SESSION III: EPIGENETICS AND SYNTHETIC BIOLOGY	Exhibition & Posters
18h10	END OF THE DAY	

TUESDAY 25TH OF JUNE

Rooms	VAUBAN AUDITORIUM Level 3	EXHIBITION AREA Level 3
8h00	Doors opening	
9h00	SESSION IV: GENETIC AND PROCESS ENGINEERING	Exhibition & Posters
10h30	Coffee Break	
11h00	SESSION IV: GENETIC AND PROCESS ENGINEERING	Exhibition & Posters
12h30	Lunch Break in Jeanne de Flandre room	POSTERS SESSION B
15h00	END OF THE DAY	
15h00 to 20h00	OUTING & Cocktail-Dinner at the Museum of Fine Arts of Lille	

WEDNESDAY 26TH OF JUNE

Rooms	VAUBAN AUDITORIUM Level 3	EXHIBITION AREA Level 3
8h00	Doors opening	
9h00	SESSION V: NEXT GENERATION MOLECULE FORMATS	Exhibition & Posters
10h30	Coffee Break	
11h00	SESSION VI: CELL INTERACTIONS	Exhibition & Posters
12h30	Lunch Break in Jeanne de Flandre room	POSTERS SESSION C
14h30	Keynote Lecture: C. VERFAILLIE	Exhibition & Posters
15h15	SHORT POSTER PRESENTATIONS & POSTER PRIZE SELECTION	
17h15	Closing Ceremony	
17h30	END OF THE DAY	
19h00	GALA DINNER at Farmhouse of the Templars	

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PROGRAMME OF SUNDAY 23RD OF JUNE
INDUSTRIAL WORKSHOPS

TURIN ROOM LEVEL 1

ATMI

From 9h00 to 11h00

Single-Use Technologies to Fast Track Human Vaccine Production, from Development to Industrial Manufacturing.
Illustrated by a case study related to a new rabies vaccine production, this workshop will address the implementation of single-use technologies to fast track the process. From development to industrial scale, presentations will highlight key decision factors, challenges faced during implementation and current results achieved. Chaired by Jean-Marc Guillaume USP Director, Bioprocess R&D from Sanofi Pasteur, you will discover the perspectives from an industrial stakeholder on the lessons learned from single-use technologies. Conclusions will include insight from the technological partner and the organizational impact from supporting and meeting quality and supply chain requirements of a key partner.

Chairman: *Jean-Marc Guillaume, USP Director, Bioprocess R&D, Sanofi Pasteur*
Speakers: *Jean-Marc Guillaume, USP Director, Bioprocess R&D, Sanofi Pasteur*
Nicolas Seve, Process Scientist, USP, Bioprocess R&D, Sanofi Pasteur
Eric Calvosa, Process Manager, USP, Bioprocess R&D, Sanofi Pasteur
Ahmed Farouk, Single-Use Bioreactor Specialist, Application Lab engineer
Sven Adams, Supply Chain & Purchasing Manager

VAN GOGH ROOM LEVEL 8

BD

From 9h00 to 11h00

Workshop 1:
1. Cell Culture Media and Process Optimization: Diverse Challenges and Optimal Solutions
James W. Brooks, PhD, R&D Manager, BD Biosciences – Advanced Bioprocessing
Diverse cell types utilized in bioprocessing and cell therapy have unique nutritional requirements. To achieve maximal cell performance, the optimal cell culture medium, supplementation, and process are required. We present a multi-faceted approach to media and process optimization, along with case studies demonstrating achieved performance improvement.

2. Rapid Media Design – The Chemically Defined Medium Platform
Karlheinz Landauer, PhD, COO, Celonic
With more than 15 years of experience in process development, Celonic has designed a chemically defined medium platform for the bioprocessing industry. This platform consists of a basal medium composition and a universal feeding solution for virtually all basal media, which is manufactured in BD's state of the art, fully-dedicated animal-free media production facility. This workshop will be presenting case studies in which Celonic tested the platform on several host cell lines and production cell lines in different laboratories around the world.

Workshop 2: From 11h15 to 13h15
The Path to Chemically-Defined Bioprocessing
Elizabeth C. Dodson, PhD, R&D Manager, BD Biosciences – Advanced Bioprocessing
In biopharmaceutical production, optimization of cell culture parameters is a central component of process development. One challenge facing process development scientists is the initial selection of an appropriate cell culture supplement or feed that will give desired titer, growth characteristics, and protein quality. Each biopharmaceutical process requires a unique cell culture environment for optimized performance. The availability of a family of chemically defined (CD) supplements offering a diverse performance profile can significantly improve process development timelines. To facilitate process development, BD will commercialize a diverse set of CD supplements. In this workshop we will share our findings that were made during the discovery process.
- How can you utilize DOEs to facilitate development and optimization of a CD supplement?
- How do you select a base medium for optimal performance with a CD supplement?
- How do you optimize a feeding regimen for top performance with a CD supplement?
- How do you scale up from shake flask to bioreactor to obtain optimal CD supplement performance?
- How do your CD supplements influence protein glycosylation patterns?

MATISSE ROOM LEVEL 8

Boehringer Ingelheim

From 11h15 to 13h15

Producing Value from Discovery to Supply for the Next Generation of Biologics
- Company presentation BI
Stephan Schlenker, Boehringer Ingelheim
- Speed, flexibility in process development
Benedikt Greulich, Boehringer Ingelheim
- Fit for pipeline concepts for antibody discovery and development
Andreas Popp, Morphosys AG
- Developmental challenges with recombinant antibody mixture
Christian Müller, Symphogen A/S
- Getting ready for commercial production
Harald Bradl, Boehringer Ingelheim
- Wrap up
Stephan Schlenker, Boehringer Ingelheim

LIEGE ROOM LEVEL 1

GE Healthcare

From 9h00 to 11h00

Implementation of novel upstream technologies – An integrated/systematic approach
This workshop will offer a unique insight into the impact of novel upstream strategies on bioprocess productivity, economics and reliability provided by globally recognized technologists and visionaries from the biopharmaceutical industry.
End User perspective:
Alain Pralong, GSK Biologics, Belgium, VP New Production Introduction and Industrialisation

- Industry Analyst perspective:
Miriam Monge, Biopharm Services Ltd, Vice President Sales & Marketing
- Contract Manufacturer Organization perspective:
Dethardt Müller, Rentschler Biotechnologie GmbH, Vice President Technology Development
- Technology Provider perspective and moderator:
Gerard Gach, GEHC Life Sciences, Cell Culture Strategy Director – Bioprocess
The Workshop brings together globally recognized technologists and visionaries from the biopharmaceutical industry. Through their in-depth experiences in implementing new processes, they have specific insights on the impact of upstream single use tools and strategies in the bioprocess environment. The audience will benefit from the panels knowledge on implementation and integration of new technologies and will be invited to join in the conversation. They will draw from studies of process modeling, supply chain concerns and CMO use of disposables in order to highlight the flexibility and safety benefits of single use in a full scale production environment. In addition, a preview of a next generation single use seed train bioreactor platform will be presented - the first time this instrument will be shown.

ARTOIS ROOM LEVEL 5

From 9h00 to 11h00

IrvineScientific

Establishing Scale-up Process Using a Platform Chemically Defined Medium in Single-Use Disposable Bioreactor Systems
Tom Fletcher
Case studies will be presented as examples of how innovation has helped overcome particular challenges during cell culture media development.
- Custom Serum-Free Media Development & Optimization for Stem Cells Expansion
Jessie H.-T. Ni, Ph.D. Chief Scientific Officer, Irvine Scientific
- Effects of Cell Culture Media on Therapeutic Protein Quality
Tom Fletcher, Director, Research and Development, Irvine Scientific
- Establishing a Scale-up Process for Single-Use Disposable Bioreactor Systems Using a Platform Chemically Defined Medium
Matt Caple, Scientific Director Cell Culture Development, Gallus BioPharmaceuticals, LLC

TURIN ROOM LEVEL 1

KERRY

From 11h15 to 13h15

Rapid, Cost Effective Supplementation Strategies to Optimize media for the production of rProteins, Biosimilars, and Vaccines while Minimizing Variability
John F. Menton, PhD - Cell Culture R&D Manager
Supplementation of cell culture media is recognized as perhaps the most effective means for delivering enhanced culture/process productivity while reducing development timelines. The task of screening, selecting, and characterizing the vast array of available media supplements can be daunting and costly. In this workshop, we will explain the steps taken by Kerry over the years to minimize variability in our products. We will also demystify and simplify much of the media optimization process, by presenting a technical overview on the most widely used supplements, common practices, recommended approaches to screening and selection, and how to avoid common mistakes with respect to supplementation strategies.

ARTOIS ROOM LEVEL 5

SAFC®

From 11h15 to 13h15

Better Risk Mitigation Strategies: Practical Regulatory and Safety Testing Advice for Biologics Manufacturers
Join us for a practical and interactive workshop discussing the latest in risk mitigation strategies, providing useful regulatory and safety testing advice, and reviewing advances in technologies for risk mitigation.
Presentations will be followed by an «ask the experts» session, so that you can pose your questions directly to our experienced panel, which includes:
- Kevin Kayser, Director, CHOZN® Product Development, SAFC
- Chas Hernandez, Senior. R&D Scientist, Virologist, SAFC
- Martin Wisher, Senior Director, Regulatory Affairs, SAFC

LIEGE ROOM LEVEL 1

sartorius stedim biotech

From 11h15 to 13h15

"Advances in single-use bioreactor technology"
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 1000 L scale. Especially continuous processing has gained tremendous interest as it allows reducing production scale and facility footprint. Besides classical biopharmaceuticals and vaccines, cell therapy products are moving towards industrial relevance. All this increases technical, supply chain and quality requirements on single-use bioreactors significantly.
During the workshop we will discuss the current status of single-use bioreactor solutions covering scale-up, high cell density cultures, qualification and integrity testing.
- Implementation of single-use bioreactors for scale-up and biologics production
Dr. Anne Gilbert, Ph.D, USP Director, Product and Process Development, Novasep Process
- Key considerations for development and qualification of single-use bioreactors
Dr. Gerhard Greller, Director Upstream Technology, Sartorius Stedim Biotech
- Managing challenges of large scale, high cell density cultures; experiences from the XD® process
Dr. Gerben Zijlstra, DSM Biologics
- Converting cell therapy manufacture to pharmaceutical production – quantity Vs biology
Dr. Christian van den Bos, Lonza Collogne
- Integrity testing of single-use bioreactors and bags
Dr. Martin Dahlberg, Sartorius Stedim Biotech

EUROTOP AUDITORIUM LEVEL 5

Thermo SCIENTIFIC

From 9h00 to 13h15

1. Support your validation requirements
David Klinkenberg, BioProduction Scientist and Technical Advisor
The qualification and validation of production, harvest and containment supplies is an integral part of any biopharmaceutical process. Regulatory guidelines around the World recommend that the production, storage and packaging components be assessed for extractables or leachables that may interact with or impact the product being manufactured. Key topics covered:
• Source of extractable and leachable materials from polymeric containers
• How to satisfy requirements for regulatory compliance
• How to obtain the technical data you need from a supplier's documentation
2. 1 Media development and selection strategies that achieve optimal productivity – case studies detailing basal media and feed optimization and application of single-use technologies
Tariq Haq, Senior Product Manager, Media
Production clones have unique bioprocess requirements, making media and feed selection a challenge. As such, it is critical to identify optimal media and feeds, and then combine them with effective cell culture strategies to obtain peak productivity.
A proven method of media and feed optimization that has the ability to thoroughly evaluate nutrient demands of cell culture from high-throughput to production-scale is Metabolic Pathway Design™. Once a medium has been fully optimized through this process, designing the optimal feed strategy to sustain cell growth and generate greater expression in production-scale systems is necessary. Such optimization programs can notably impact productivity, significantly improve process improvement times, and reduce costs. Case studies will discuss successful application of Metabolic Pathway Design for basal media optimization, and feed optimization.
2.2. Development of a novel single-use technology for microcarrier cell culture harvests: Thermo Scientific HyQ Harvestainer
Don Young, Senior Product Manager, BPC
The launch of the Thermo Scientific Single-Use Bioreactor (SUB) in 2006 changed the way biopharmaceutical companies established biological manufacturing processes. Single-use technologies continue to replace stainless steel models – delivering significant advantages including design flexibility and cost-savings. Application-testing and continued integration of leading technologies ensure that single-use systems continue to drive performance in the bioprocessing industry.
A recent innovation, the HyQ Harvestainer, is a fully enclosed single-use solution for processing microcarrier beads in bioreactor cultures. For production processes using anchorage-dependent cells grown on microcarriers, the Harvestainer system provides the customer with a single-use bioprocess container for harvesting and separating the media and expressed biologic or vaccine from the microcarriers.
2.3 Critical process and supply chain services to improve bio-manufacturing performance
Garland Grant, Senior Product Manager, Collaborative Technologies
The bioprocessing industry has undergone rapid growth in the last decade. Technology, cell culture methods, regulatory demands and companies' focus continue to advance – creating new demands on how suppliers and the biologics manufacturers (bio-manufacturer) interact. Media optimization, feed strategy development, on-site process development support, analytical testing, and validation services are just a small handful of the collaborative technologies required to ensure optimal bio-manufacturing performance.
Successful supplier and bio-manufacturer relationships enable internal processes to be streamlined, leveraging the full strength of both organizations to achieve greater efficiency. This presentation will provide an overview of the services critical to achieving process efficiency gains and provide examples of how increased interaction through collaboration efforts enabled suppliers to deliver greater value to the bioprocessing industry.

EUROTOP AUDITORIUM LEVEL 5

THERAPEUTIC VACCINES

Tarit Mukhopadhyay, University College London, UK
Barry Buckland, University College London, UK
Topics to be covered:
Cell delivery of antigens (e.g. Dendritic Cells)
Virus delivery of antigens using a variety of host mammalian cells; for example adenovirus, Herpes, AAV
Development of appropriate safe human cell lines that can be grown in suspension culture
Lessons to be learned from traditional vaccines
Challenge of determining potency of the vaccine
Safety challenges; freedom from unwanted adventitious agents

- Development of a vaccine candidate for Leishmaniasis; a therapeutic vaccine in clinical trials
Paul Kaye, York University, UK
- Regulatory Approval of new cell lines
David Onions, previously CSO, Bioreliance
- Case Study: Retroviral Like particle hepatitis C vaccine
Manuel Carrondo, IBET
- "Plug-and-Play" platform for rapid, scalable, cost-effective manufacture of traditional and novel vaccines.
Peshwa Madhusudan, MAXCYTE, Exec VP Cellular Therapies

LIEGE ROOM LEVEL 1

METABOLISM AS A KEY FOR IMPROVEMENT OF CELL CULTURE PROCESSES - STATUS AND ADVANCES IN ANALYTICS AND DATA ANALYSIS

Yvonne Genzel, Max Planck Institute, Germany
Udo Reichl, Max Planck Institute, Germany
Monitoring of extracellular metabolites such as glucose, glutamine, lactate and ammonium is routinely performed in cell culture since many years. Together with data on cell concentration and viability the development of media, the establishment of basic mathematical models to characterize cell growth and product formation, and the optimization of production processes was supported. With the advent of systems biology, a multitude of new assays to describe basic cell properties have been implemented successfully. These include measurement of intracellular metabolites, nucleotides, proteins, lipids and enzyme activities. On the other hand, basic questions, for instance the impact of compartmentalization, the role of signal transduction processes on cellular metabolism, or the integration of data sets obtained with different tools and from several process stages remain to be addressed properly.

While it is clear that metabolism is a key to achieve robust cell growth and to maximize cell-specific product yields, collecting generic data alone does not enable further development of cell lines or optimization of cultivation processes. Unless the complex information obtained can be integrated into a coherent view on cell-specific properties relevant for production of biologicals, including quantitative models to analyze and to predict at least some aspects of cell behavior, the full potential of new assay technologies cannot be exploited.
Focusing on metabolism-related topics, the workshop intends to present an overview on the analytical portfolio of today and to discuss limitations and new insights from the generated data. The following questions will be addressed:
- What new assays have been developed over the recent years?
- What are the current limits in analyzing cellular metabolism?
- What options are available to design improved media?
- What solutions exist to cope with biological variance and batch-to-batch reproducibility to obtain statistical significant results?
- What is the status of mathematical modeling approaches?
- What would be required for a comprehensive description of relevant aspects of cell growth and product formation in manufacturing of biologicals?

The invited speakers will be
- Tools for metabolic characterization of animal cell processes
Ana Teixeira, IBET, Portugal
- How flexible are animal cells? What can we learn from intracellular metabolite and enzyme activity data?
Yvonne Genzel, Max Planck Institute, Germany
- Cellular compartmentalization: What are the consequences for metabolic studies with CHO cells?
Ralf Takors, University Stuttgart, Germany
- The effect of low nutrient concentrations on product quality during fed-batch cultures
Mike Butler, University Manitoba, Canada
- Options & challenges in modeling metabolism
Udo Reichl, Max Planck Institute, Germany

MATISSE ROOM LEVEL 8

THE WWW.CHOGENOME.ORG RESOURCE FOR THE INTERNATIONAL CHO BIOTECHNOLOGY COMMUNITY

Nicole Borth, Universität für Bodenkultur, Austria
Kelvin H. Lee, University of Delaware, USA
Mike Betenbaugh, Johns Hopkins University, USA

When the first CHO genomic sequence was published in 2011, the immediate need of the scientific community was to obtain easy and fast access to the sequence information. With the release of additional sequencing results also came the realization that additional and new tools are necessary to take full advantage of the available information. This has raised a series of questions: Has the initial goal of easy access been achieved? And should the focus of www.CHOgenome.org change? Should efforts expand to include additional data sets and tools? Should more sequencing data and other ´omics measurements be included?
In this workshop we would like to further the discussion on the next steps for the cell culture community surrounding the CHO Genome effort. Some potential topics include:

- How and for what purposes does the community use genome information?
- Where does the community perceive future requirements and developments?
- What other 'omics data sets and tools should be added?
- What are the future directions of the CHO genome community?

We will start with short presentations on current applications of genome scale information, where it is useful, and how has it helped in different research efforts (contributions are encouraged; please contact nicole.borth@boku.ac.at if you would like to present on two slides maximum) and then proceed to an open forum and discussion addressing questions of interest to the community.

VAN GOGH ROOM LEVEL 8

IS TOMORROW'S PROCESS FED-BATCH OR PERFUSION?

Véronique Chotteau, KTH, Sweden
Tim Charlebois, Pfizer, USA

Fed-batch operation has dominated the biopharmaceutical production horizon over the last several decades. The lower technical risk, high production yields and the importance of therapeutic antibodies and their production platforms have been important drivers of fed-batch supremacy. But is the tide turning in the direction of perfusion-based cell culture and continuous bioprocessing? Several combined factors have been pushing the biopharmaceutical industry to reconsider the long-term future of fed-batch technology: a desire to reduce bioreactor volumes to take advantage of disposable equipment and associated flexibility; the trend toward precision medicine and smaller, more patient-targeted, niche indication products; the availability of more robust perfusion devices; the potential ability of the technology to support consistent, high-quality, high-performance production of a broad range of biopharmaceutical molecules, including but not limited to mAbs; opportunity to leverage downstream processing efficiencies via generation of a more continuous feedstream; potentially favorable plant and process economics.
The workshop will present a review of the historical and factual reasons for perfusion's lower 'popularity', today's views of several industrial actors of fed-batch and perfusion technology and how they see the future of bioprocesses, as well as the comparative economic aspects comparing these modes of operation. This will be followed by a discussion with the workshop participants to explore what the future horizon might look like for the production of recombinant glycoproteins, antibodies, enzymes, viral vectors etc.

- Workshop introduction - Perfusion process perception today
Veronique Chotteau, KTH, Sweden
- Opportunities for driving enhanced performance of CHO-based bioprocesses
Greg Hiller, Pfizer, USA
- Fed-batch vs. perfusion for tomorrows biopharmaceutical
Jakob Rasmussen, NovoNordisk, Denmark
- Fed-batch and Perfusion Processes: Economic and Operational Considerations
Suzanne Farid, University College London, UK
- Fed-batch vs. perfusion for biopharmaceutical production
Bert Frolich, Shire, USA
- When and Why to use perfusion for high quality production of Viral Vectors and Vaccines
Amine Kamen, CNRC, Canada

SPEAKERS & CHAIRPERSONS INDEX

SUNDAY 23RD OF JUNE

VAUBAN AUDITORIUM LEVEL 3

16h00 **OPENING CEREMONY**
Yves-Jacques Schneider, 23rd ESACT Meeting Chair - Martin Fussenegger, ESACT Chairman

16h30 **KEYNOTE LECTURE:**
Adult Human Mesenchymal Stem Cells and their use in pre-clinical animal models of disease
Arnold Caplan, Case Western Reserve University, USA.

17h15 **SESSION I: CELL THERAPIES AND VACCINES**
Chairpersons: Paula Marques Alves & Spiros Agathos

17h15 Human pluripotent stem cells, a versatile tool full of promises for cell therapies
Marc Peschanski, INSERM Évry, France

17h50 Robust cell manufacturing platforms and novel proteomic approaches to streamline the design of cell-based therapies for myocardial infarction
Margarida Serra, IBET, Portugal

18h10 A novel genotype of mva that efficiently replicates in single cell suspensions
Ingo Jordan, ProBiogen, Germany

18h25 Need of rapid and universal quantification methods for influenza vaccine release
Amine Kamen, NRCMC, Canada

18h40 End of the SESSION I

19h00 Traders reception

LILLE GRAND PALAIS

TRADERS COCKTAIL

MONDAY 24TH OF JUNE

VAUBAN AUDITORIUM LEVEL 3

9h00 **SESSION II: ADVANCED CELLULAR MODELS**
Chairpersons: Nicole Borth & Hansjörg Hauser

9h00 Novel hepatocyte cell lines with preserved primary-like phenotype
Christoph Lipps, HZI, Germany

9h20 Aspects of vascularization in multi-organ-chips
Reyk Horland, TU, Germany

9h40 Pyramidal neurons derived from human pluripotent stem cells integrate efficiently into mouse brain circuits in vivo.
Ira Espuny-Camacho, University of Brussels, Belgium

10h00 Human neural in vitro models for preclinical research: 3d culture systems for differentiation and genetic modification of stem cells
Catarina Brito, IBET, Portugal

10h15 Improvement in a human ige-inducing system by in vitro immunization
Hiroharu Kawahara, Kitakyushu National College of Technology, Japan

10h30 Coffee Break in the Exhibition Area

11h00 Special Delivery: Targeted Gene Silencing
Judy Lieberman, Harvard Medical School, USA

11h35 Microrna biogenesis in CHO cells: the impact of dicer mediated Mirna processing on CHO cell phenotpye
Matthias Hackl, BOKU, Austria

11h55 Stable microrna expression improves antibody productivity in Chinese hamster ovary producer cells
Michaela Strotbek, University of Stuttgart, Germany

12h15 Novel strategy for a high-yielding mab-producing CHO strain (overexpression of non-coding rna enhanced proliferation and improved MAB yield)
Hisahiro Tabuchi, Chugai Pharmaceutical, Japan

JEANNE DE FLANDRES LEVEL 11

12h30-14h30 Lunch Break

12h30 Poster Session A in the Exhibition Area (even reference)

VAUBAN AUDITORIUM LEVEL 3

14h30 **SESSION III: EPIGENETICS AND SYNTHETIC BIOLOGY**
Chairpersons: Martin Fussenegger & Terry Papoutsakis

14h30 MicroRNA and omics
Kevin Lee, Delaware Biotechnology Institute, USA

15h05 Engineering of synthetic circuits for biomedical application
Haifeng Ye, ETH, Switzerland

15h25 Crosstalk of synthetic cassettes in defined chromosomal sites
Shawal Spencer, HZI, Germany

15h40 Programmable designer circuits performing biocomputing operations in mammalian cells
Simon Ausländer, ETH, Switzerland

15h50 A red/far-red light-responsive bi-stable toggle switch to control gene expression in mammalian cells
Konrad Müller, University of Freiburg, Germany

16h15 First cpg island microarray for genome-wide analyses of DNA methylation in Chinese hamster ovary cells: new insights into the epigenetic answer to butyrate treatment
Anna Wippermann, Bielefeld University, Germany

16h30 Coffee Break in the Exhibition Area

17h00 Gene regulation in chromatin: Insights from epigenomics and genome editing
Dirk Schübeler, Friedrich Miescher Institute for Biomedical Research, Switzerland

17h35 Targeting FcRn for therapy: from subcellular trafficking analyses to in vivo studies in mice
Sally Ward, UT Southwestern Medical Center, USA

18h40 End of the day

VAUBAN AUDITORIUM LEVEL 3

9h00 **SESSION IV: GENETIC AND PROCESS ENGINEERING**
Chairpersons: Ashraf Amanullah & Stefanos Grammatikos

9h00 Designer nucleases - understanding the basics, improving their application
Toni Cathomen, University Medical Center, Germany

9h35 2D fluorescence spectroscopy for real-time aggregation monitoring in upstream processing
Karen Schwab, Institute of applied Biotechnology, Germany

9h55 Recombination-mediated cassette exchange (RMCE) for monoclonal antibody expression in a chok1-derived host cell line
Lin Zhang, Pfizer, USA

10h15 Implementation of a predictive screening strategy for cell cloning by automation and parallelization
Anke Mayer-Bartschmid, Bayer, Germany

10h30 Rapid construction of transgene-amplified CHO cell lines by cell cycle checkpoint engineering
Kyoungho Lee, University of Osaka, Japan

10h45 Engineering a mammalian cell line toolbox that exhibits multiple productivity and product quality profiles
Chapman Wright, Biogen, USA

11h05 Coffee Break in the Exhibition Area

11h30 Multi-gene engineering of mammalian cell metabolism: walking the steps towards hyper-productivity
Ana Filipa Rodrigues, IBET, Portugal

11h50 Monitoring intracellular redox changes in biotechnologically relevant mammalian cell lines with genetically encoded fluorescent biosensors
Karen Perelmuter, Institut Pasteur, Uruguay

12h10 A comprehensive view on an old metabolite: lactate
Martin Jordan, Merck Serono, Switzerland

12h25 Comprehensive understanding of heparan sulfate proteoglycan biosynthesis in CHO and hek293 cells
Sojeong Lee, Kaist, Korea

JEANNE DE FLANDRES LEVEL 11

12h30-14h30 Lunch Break

12h40 Poster Session B in the Exhibition Area (odd references)

TURIN ROOM LEVEL 1

12h40 - 14h30 **ESACT GENERAL ASSEMBLY**

14h30 End of lunch break

14h00 Outing - departure according to your TOUR (see page 6)

+ Dinner at the Museum of Fine Arts of Lille

19h30 – 21h00: Free exhibition visit of the Museum with conference guides.

21h00 – 23h00: Cocktail-Dinner

VAUBAN AUDITORIUM LEVEL 3

9h00 **SESSION V: NEXT GENERATION MOLECULE FORMATS**
Chairpersons: Francesc Godia & Hitto Kaufmann

9h00 Gene therapy: progress and challenges
Thierry Vanden Driessche, Free University of Brussels, Belgium

9h35 Targeting of siRNA to inflammatory diseases
Jorgen Kjems, Interdisciplinary Nanoscience Center iNANO, Denmark

10h10 Beat™ the bispecific challenge: a novel and efficient platform for the expression of bispecific IgGs
Pierre Moretti, Glenmark, Switzerland

10h25 Next generation bispecific antibody design and the influence thereof on product yield and stability
Karin Taylor, University of Queensland, Australia

10h45 Investigating the determinants of novel-format antibody expression in CHO cells
Claire Gaffney, University of Manchester, UK

11h00 A quantitative and mechanistic model for monoclonal antibody glycosylation as a function of nutrient availability during cell culture
Ioscani Jimenez Del Val, Imperial College, UK

11h15 Coffee Break in the Exhibition Area

11h30 **SESSION VI: CELL INTERACTIONS**
Chairpersons: Alan Dickson & Otto-Wilhelm Merten

11h30 From matrix mechanics to the nucleus
Dennis Discher, University of Pennsylvania, USA

12h05 Electrically modulated attachment and detachment of animal cells cultured on an ITO electrode.
Sumihiro Koyama, Jamstek, Japan

12h20 Announcements (ESACT courses, JAACT, ECI CCE etc…)

JEANNE DE FLANDRES LEVEL 11

12h30-14h30 Lunch Break

12h30 Poster Session C in the Exhibition Area

VAUBAN AUDITORIUM LEVEL 3

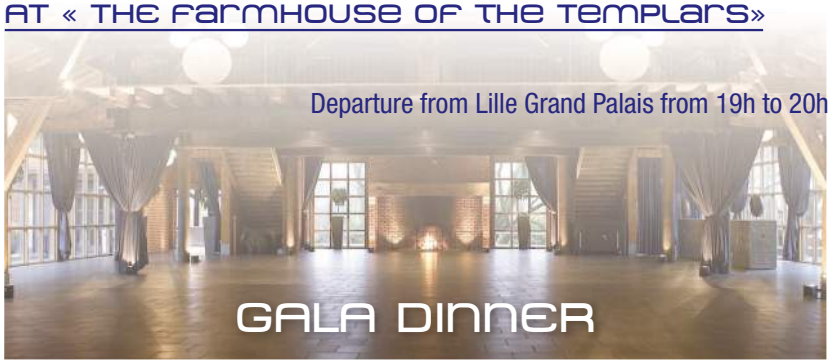
14h30 **KEYNOTE LECTURE:**
Engineering liver tissue from stem cells
Catherine Verfaillie, KU Leuven, Belgium

15h15 MERCK MILLIPORE POSTER PRIZE SESSION

17h15 CLOSING CEREMONY
Yves-Jacques Schneider - Francesc Godia - Hansjörg Hauser

17h30 END OF THE CONGRESS

AT « THE FARMHOUSE OF THE TEMPLARS»



ABSTRACTS SUMMARY

Sunday 23rd of June- 17h50
001

ROBUST CELL MANUFACTURING PLATFORMS AND NOVEL PROTEOMIC APPROACHES TO STREAMLINE THE DESIGN OF CELL-BASED THERAPIES FOR MYOCARDIAL INFARCTION

Margarida SERRA [1,2], Cláudia CORREIA [1,2], Patrícia GOMES-ALVES [1,2], Ana S. ALMEIDA [1,2], Marcos F.Q. SOUSA [1,2], Catarina BRITO [1,2], Karsten BURKERT [3], Tomo SARIC [3], Jurgen HESCHELER [3], Luis RODRIGUEZ [4], Maria T. LAIN [4], Jose A. LÓPEZ [5], Antonio BERNARD [5], Manuel J.T. CARRONDO [2,6], Paula M. ALVES [1,2]

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KEY WORDS:
INDUCED PLURIPOTENT STEM CELLS / HUMAN CARDIAC STEM CELLS / ACUTE MYOCARDIAL INFARCTION / BIOREACTORS / HIGH-THROUGHPUT PROTEOMIC TOOLS

BACKGROUND AND NOVELTY:
Stem cell (SC) transplantation has emerged as an exciting treatment for patients with acute myocardial infarction (AMI). The major challenges in this field are lack of expertise in product characterization and specialized cell manufacturing which are imperative to bring SC-based products to clinic [1]. Within this context, our work has been focused on production and characterization of two challenging SC-based products: i) cardiomyocytes (CM) derived from induced pluripotent SCs (iPSC), which are capable to regenerate myocardium [2], and ii) adult cardiac SCs (hCSC), which trigger paracrine mechanisms that activate endogenous SCs to promote regeneration[3].

EXPERIMENTAL APPROACH:
Our strategy for CM production consisted in integrating cardiac differentiation and cell lineage purification steps in environmentally controlled stirred tank bioreactors. iPSC were cultivated as aggregates and the impact of different parameters on bioprocess yields was evaluated. Regarding hCSC expansion, different microcarriers were screened for their ability to support hCSC growth. Cell characterization was performed along culture time using flow cytometry, qRT-PCR and microscopy analysis. High-throughput proteomic tools have also been applied to uncover novel molecules of hCSC Receptome.

RESULTS AND DISCUSSION:
Our results showed that hypoxia conditions and an intermittent stirring profile are key parameters in iPSC differentiation towards functional CM. Using these conditions, we were able to improve by 1000-fold the final yields of CM (purity>98%).
An efficient protocol for hCSC cultivation using microcarrier technology was successfully implemented and hCSC retained their phenotype, multipotency and ability to secrete key growth factors after expansion in bioreactors. From hCSC Receptome analysis, more than 2000 proteins were identified, including proteins involved in the cardiac function.
The knowledge generated from our study will establish a new way to streamline the design and manufacturing of novel cell-based therapies for AMI.

Sunday 23rd of June- 18h10
002

A NOVEL GENOTYPE OF MVA THAT EFFICIENTLY REPLICATES IN SINGLE CELL SUSPENSIONS

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KEY WORDS:
VACCINES / MODIFIED VACCINIA ANKARA / AVIAN CELL LINE / AGE1. CR.PIX

BACKGROUND AND NOVELTY:
Vectored vaccines based on modified vaccinia Ankara (MVA, a hyperattenuated poxvirus) may lead to new treatment options against infectious diseases and certain cancers. We established avian suspension cell lines (CR and CR.pIX) and developed chemically defined media for production of MVA. Because cell-to-cell spread is an important mechanism for vaccinia virus replication one common hurdle in suspension processes appears to be that induction of cell aggregates is required to obtain high yields. We now describe a novel genotype of MVA that replicates to high titers in the CR single cell suspensions without aggregate induction.

EXPERIMENTAL APPROACH:
After passaging MVA in CR suspension cultures in chemically defined media we observed that infectious titers increased. 135 kb of genomic DNA sequence recovered from a passage eleven population revealed accumulation of a novel genotype (MVA-CR) with point mutations in three genes. Remaining traces of wildtype virus were removed by plaque purification and the pure isolate (MVA-CR19) was further characterized.

RESULTS AND DISCUSSION:
Compared to wildtype MVA, plaques formed by MVA-CR19 on adherent CR cells appear to be larger and to develop earlier. Titers are slightly higher in complete lysates and significantly elevated in cell-free supernatants. Most surprisingly, MVA-CR19 replicates efficiently without aggregate induction also in single cell suspension cultures. We hypothesize that a greater fraction of MVA-CR19 escapes the hosts to also infect distant targets. In such a model the new genotype should not confer a significant advantage to viruses spreading in cell monolayers, and indeed we could not generate the MVA-CR genotype by passaging in adherent cultures. Production and purification of MVA-based vaccines may be simplified with this strain as processes based on single cell suspensions are less complex compared to the current protocols and because infectious units accumulate in the cell-free volume where burden with host-cell derived contaminants is lower.

NEED OF RAPID AND UNIVERSAL QUANTIFICATION METHODS FOR INFLUENZA VACCINE RELEASE

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KEY WORDS:
INFLUENZA VACCINE / QUANTITATION / LOT RELEASE / PROCESS ANALYTICAL TECHNOLOGIES (PAT) / CELL CULTURE

BACKGROUND AND NOVELTY:
Hemagglutinin (HA), the major surface protein of the influenza virus, is used as the influenza vaccine potency marker and measured by Single Radial Immunodiffusion (SRID) assay. To date, SRID remains the only method approved by the regulatory agencies to release influenza vaccines. The method relies on the availability of reference reagents which need 2-3 months to be updated and produced. Clearly, novel rapid, robust and reliable in-process quantification methods to monitor viral particles and viral antigen content are highly needed to accelerate development and approval of candidate influenza vaccines within the timelines required by urgent interventions in case of pandemics.

EXPERIMENTAL APPROACH:
To respond to these urgent needs, many technologies are under development, but among different methods evaluated, a universal SRID assay using antibodies recognizing conserved HA regions and RP-HPLC method to quantify total HA content in vaccine doses were the most promissing. Whereas, anion-exchange HPLC to in-process monitor the total viral particles stands out as a valuable method to accelerate process development for influenza vaccine candidates.

RESULTS AND DISCUSSION:
The three methods have been successfully evaluated and compared to specific strain-SRID assay to quantify different HA strain subtypes in vaccine preparations. Furthermore, RP-HPLC and anion-exchange HPLC methods were successfully used to monitor HA antigen and total viral particles in samples at all stages of the manufacturing process. This set of universal, rapid and reliable methods is currently validated and is used to support an accelerated development of a cell culture process for influenza vaccine manufacturing. Also it is used to better define the quality attributes of the bulk product and accelerate the release of vaccine lots.

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Petiot E, Jacob D, Lanthier S, Lohr V, Ansorge S, Kamen AA: Metabolic and kinetic analyses of influenza production in perfusion HEK293 cell culture. BMC Biotechnol 2011, 11:84.

NOVEL HEPATOCYTE CELL LINES WITH PRESERVED PRIMARY-LIKE PHENOTYPE

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KEY WORDS:
HEPATOCYTES / IMMORTALIZATION / HEPATOCYTE LONG TERM CULTIVATION / ENGRAFTMENT / MAINTENANCE OF FUNCTIONALITY

BACKGROUND AND NOVELTY:
Hepatocytes (HCs) are highly specialized cells that display a number of specific/unique functions such as protein synthesis, carbohydrate metabolism and detoxification. These properties render hepatocyte cultures an attractive option for animal toxicology testing, screening for new drugs as well as regenerative approaches. However, such approaches are limited since primary HCs lose their specific features within few days of in vitro cultivation. Thus, they cannot be expanded to sufficient numbers. Since currently available cell lines are insufficiently expressing specific hepatic factors, we explored novel strategies for expansion of HCs by controlled immortalization. We show the generation of expandable HC cell lines which maintain many properties of freshly isolated hepatocytes and can even rescue animals that display compromised liver functions.

EXPERIMENTAL APPROACH:
A lentiviral screening library comprising more than 30 immortalizing genes was used to randomly infect primary mouse HCs of different genetic background. Cell cultures were screened for proliferation and characterized for the hepatic properties in 2D and 3D culture conditions.

RESULTS AND DISCUSSION:
Immortalized cell lines were established that showed robust proliferation and underwent more than 70 cumulative population doublings within the first 170 days. Combinations of genes were identified that reproducibly support expansion and high levels of hepatic markers including albumin, HNF4 , Foxa2 and C/EBP . Subjecting the cell lines to three-dimensional cultivation conditions, hepatic marker expression levels were found to increased and even closer to primary HCs. To evaluate if these cells can engraft and overtake functionality in vivo, they were transplanted into FAH-/- mice. While non-transplanted control animals died from liver failure, transplanted animals were rescued. Immunohistochemistry confirmed successful engraftment. Together, the results show that this strategy supports the expansion of HCs while preserving their specific functions.

Monday 24th of June- 9h20
005

ASPECTS OF VASCULARIZATION IN MULTI-ORGAN-CHIPS

Reyk HORLAND [1], Reyk HORLAND [1], Sven BRINCKER [1], Benjamin GROTH [1], Ulrike MENZEL [1], Ilka WAGNER [1], Eva-Maria MATERNE [1], Gerd LINDNER [1], Alexandra LORENZ [1], Silke HOFFMANN [1], Mathias BUSEK [1], Frank SONNTAG [3], Udo KLOTZBACH [3], Roland LAUSTER [1], Uwe MARX [1,2]

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KEY WORDS:
MULTI-ORGAN-CHIP / MICRO-BIOREACTOR / ENDOTHELIAL CELLS / ARTIFICIAL VASCULATURE / CIRCULATION SYSTEM

BACKGROUND AND NOVELTY:
Enormous efforts have been made to develop circulation systems for physiological nutrient supply and waste removal of in vitro cultured tissues. However, none of the currently available systems ensures long-term homeostasis of the respective tissues over months. This is caused by a lack of in vivo-like vasculature, which leads to continuous accumulation of protein sediments and cell debris in the systems. Here, we demonstrate a closed and self-contained circulation system emulating the natural blood perfusion environment of vertebrates at tissue level.

EXPERIMENTAL APPROACH:
The system uses a miniaturized physiological blood-like circulatory network with an integrated micro-pump to provide circulation of microliter-volume to support milligrams of tissue. This mimics the physiological ratio of humans, where liters of blood-volume support kilograms of tissue, at a chip-compatible micro-scale. The self-contained circulation systems were formed in PDMS by replica molding from master molds and were afterwards bonded to a cover-slip by oxygen plasma treatment. Human microvascular endothelial cells (HMVEC) were seeded into the channels, attached to all channel surfaces, and afterwards cultured up to 14 days under pulsatile flow conditions.

RESULTS AND DISCUSSION:
We evaluate the impact of artificial vessels in an approach for systemic substance testing in multi-organ-chips. Creating the conditions for the circulation of nutrients through the tissue-chamber, will allow for in vivo-like crosstalk between endothelial cells and tissues and prevent clumping inside the channels. Data on the colonization of the microfluidic 3D channels and long-term viability of the endothelial cell layers will be presented. In addition, challenges and opportunities of this platform technology in comparison to the existing dynamic bioreactor systems will be addressed.

Monday 24th of June- 9h40
006

PYRAMIDAL NEURONS DERIVED FROM HUMAN PLURIPOTENT STEM CELLS INTEGRATE EFFICIENTLY INTO MOUSE BRAIN CIRCUITS IN VIVO

Ira ESPUNY-CAMACHO [1], Kimmo MICHELSEN [1], David GALL [2], Daniele LINARO [3], Anja HASCHE [1], Jerome BONNEFONT [1], Camilia BALI [1], David ORDUZ [2], Nelle LAMBERT [1], Nicolas GASPARD [1], Sophie PERON [4], Serge SCHIFFMANN [2], Michele GIUGLIANO [3], Afsaneh GAILLARD [4], Pierre VANDERHAEGHEN [1]

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KEY WORDS:
HUMAN PLURIPOTENT STEM CELLS / CORTICAL DIFFERENTIATION / TEMPORAL PATTERNING / IN VIVO TRANSPLANTATION AND INTEGRATION INTO THE HOST / AXOGENESIS AND NEURONAL MATURATION

BACKGROUND AND NOVELTY:
The cerebral cortex is the most complex structure of our brain. During evolution, the relative size of the cortex has increased considerably among higher mammals and new cortical areas involved in higher evolved functions have emerged. The study of human cortical development has major implications for brain evolution and cortical related diseases, but has remained elusive due to paucity of experimental models.
Here, we describe an intrinsic pathway of corticogenesis from human embryonic (ESC) and induced pluripotent (iPSC) stem cells leading to the sequential generation of first forebrain progenitors and later pyramidal neurons of all six layers identities in a time-dependent fashion, highly reminiscent of the in vivo situation.

EXPERIMENTAL APPROACH:
We describe an in vitro model for the directed differentiation of human pluripotent stem cells in a monolayer fashion and devoided of morphogens, but supplemented with noggin, and inhibitor of the BMP pathway, that has been shown to be required for neuroectoderm specification. Specified progenitors and neurons are later transplanted into mouse newborn brain and analysed after several months in vivo by immunofluorescence analysis and by patch-clamp recordings.

RESULTS AND DISCUSSION:
Following this in vitro differentiation human pluripotent stem cells efficiently differentiated into forebrain and telencephalic progenitors based on the expression of various genes tested by immunofluorescence, quantitative PCR and microarray analysis. At later stages, these cells exited cell cycle and became cortical pyramidal neurons, as attested by their pyramidal morphology, but also by the expression of various markers of cortical neurons and cortical layer specific genes. The cortical neurons present markers of connectivity and a mature electrophisiological profile at later stages. Moreover, following grafting into the mouse newborn cortex, the human ESC-derived neurons extend axons to endogenous cortical targets, present numerous synapses and functionally integrated into the host.

HUMAN NEURAL IN VITRO MODELS FOR PRECLINICAL RESEARCH: 3D CULTURE SYSTEMS FOR DIFFERENTIATION AND GENETIC MODIFICATION OF STEM CELLS

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KEY WORDS:
I3D CELL MODELS / NEURAL DIFFERENTIATION / DYNAMIC CULTURE SYSTEMS / HUMAN STEM CELLS / GENE DELIVERY

BACKGROUND AND NOVELTY:
There is an increasing need for more relevant human cell models for the early stages of drug development. These models should closely recapitulate the in vivo cell-cell interactions and present higher physiological relevance. Here we describe the development of human 3D neural in vitro models for target validation and toxicological assessment, by combining human stem cells (SCs) as scalable supply of neural-subtype cells and dynamic culture systems.

EXPERIMENTAL APPROACH:
Dynamic 3D culture system-based strategies were adopted for expansion and differentiation of human SCs, namely NT2 embryocarcinoma SC line and midbrain-derived neural SCs (hNSC). Process parameter optimization included stirring rate, process duration, media composition and oxygen levels. Furthermore, in order to increase the versatility of the 3D models, we assessed the possibility of genetic manipulation strategies via helper-dependent canine adenovirus type 2 (hd-CAV2) vectors. Differentiation and transduction efficiencies were analyzed by phenotypic and functional characterization using confocal microscopy, electron microscopy, qRT-PCR, Western Blot and metabolic profiling.

RESULTS AND DISCUSSION:
NT2 differentiation in stirred tank vessels resulted in 3D co-cultures of neurons capable of synaptic activity and mature astrocytes, with 10 and 2.5 fold higher cell yields, respectively, in comparison with 2D cultures. Moreover, this process was reproducible and robust enabling an efficient cell source to feed high throughput toxicological assays. Concerning hNSC, differentiated neurospheres were enriched in neurons which expressed the dopaminergic markers tyrosine hydroxylase (TH) and Nurr1. Furthermore, hd-CAV2-mediated gene delivery strategies allowed an efficient gene transfer into differentiated neurospheres, with transgene expression detectable also in the inner layers.
The 3D models developed herein contribute to increase the collection of tools available for more accurate pre-clinical evaluation, accelerating the drug development pipeline.

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IMPROVEMENT IN A HUMAN IGE-INDUCING SYSTEM BY IN VITRO IMMUNIZATION

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KEY WORDS:
IGE INDUCTION / ALLERGY / IN VITRO IMMUNIZATION / HUMAN LYMPHOCYTES

BACKGROUND AND NOVELTY:
The immune system, which is the self-defense system of the body, occasionally responds in a manner that is harmful to the body. The incidence and severity of allergies caused by the environment are increasing and have recently become a serious social problem. We have previously developed an in vitro system for inducing human IgE antibody specific to a designated antigen that can be used to study various allergic reactions.

In this study, we attempted to improve this system to stimulate IgE levels in its medium for a highly sensitive screening.

EXPERIMENTAL APPROACH:
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 previously. Human lymphocytes were cultured in 96- or 24-well plates at a final density of one million cells/ml in the medium and incubated in a CO2 incubator at 37°C for 10 days. After 10 days, approximately 700 ng/ml of IgE antibody was secreted into the medium.

RESULTS AND DISCUSSION:
The IgE-inducing system was used to investigate various factors stimulating IgE production. When human lymphocytes obtained from naturally immunized donors with allergens were used, elimination of IL-2 from the medium gave higher IgE production. These results indicate that IL-2 may be required to initially immunize humans with allergens.
The level of secreted IgE reported in this study may be the highest compared to those reported elsewhere. This improved system for human IgE production in a medium without IL-2 is considered to be of profound use for studying allergy mechanisms and investigating allergy-alleviating materials.

Monday 24th of June- 11h35
009

MICRORNA BIOGENESIS IN CHO CELLS: THE IMPACT OF DICER MEDIATED MIRNA PROCESSING ON CHO CELL PHENOTPYE

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KEY WORDS:
CHO / MICRORNA / CELL ENGINEERING / GROWTH

BACKGROUND AND NOVELTY:
MicroRNAs are 19-24 nt long RNAs that control gene expression by translational repression or mRNA degradation. Applications of miRNAs in cell culture technology are the use as biomarkers during cell line development as well as gene engineering targets to enhance space-time yields of bioprocesses. The analysis of miRNA expression in CHO cells in response to serum supplementation showed a predominant up-regulation, which was reflected in higher expression of Dicer, the key enzyme in the production of mature miRNA duplexes. Since serum significantly enhances growth of CHO cells, we set out to study the relevance of Dicer for CHO cell growth.

EXPERIMENTAL APPROACH:
High-throughput analysis of miRNA transcription was done using Illumina and microarrays. MiRNA content was measured by chip-based gel electrophoresis. Dicer expression was analyzed by real-time PCR and Western Blot, and expression was manipulated using specific shRNA constructs and human Dicer cDNA expression constructs.

RESULTS AND DISCUSSION:
Based on the effect of serum supplementation on miRNA levels, Dicer expression was analyzed in response to growth arrest by serum-removal or nutrient depletion, which showed a strong down-regulation under these conditions. To further assess the link between CHO cell growth and Dicer expression, mRNA and protein levels were determined in five serum-free adapted CHO cell lines with specific growth rates ranging between 0.45 and 1.00 d-1. This data showed a strong correlation between Dicer expression and specific growth rate. A CHO DUKX-B11 host cell line was transfected with a human Dicer1 cDNA construct to generate two stable Dicer overexpressing host cell lines exhibiting a 1.5 – 2.5 fold overexpression. Compared to the untransfected host, a ~20-30% increase in growth rate and cumulative viable cell days was observed.
These data suggest that Dicer expression and hence the production of mature miRNAs is strongly linked to cell specific growth rate and stress response to nutrient limitation in CHO cells.

Monday 24th of June- 11h55
010

STABLE MICRORNA EXPRESSION IMPROVES ANTIBODY PRODUCTIVITY IN CHINESE HAMSTER OVARY PRODUCER CELLS

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KEY WORDS:
MICRORNA SCREEN / THERAPEUTIC PROTEINS / CELL LINE ENGINEERING / FED-BATCH

BACKGROUND AND NOVELTY:
MicroRNAs (miRNAs) are short non-coding RNAs that post-transcriptionally regulate the expression of different target genes and, thus, potentially offer the opportunity to engineer networks of genes in order to achieve complex phenotypic changes in mammalian cells. We hypothesized that this feature of miRNAs could be exploited as a strategy to improve therapeutic protein production processes by increasing viable cell densities and/or productivity of mammalian host cells. This first functional miRNA screen in Chinese hamster ovary (CHO) producer cells led to the identification of miRNAs that enhance IgG productivities not only transiently but also in fed-batch cultures using stable cell lines.

EXPERIMENTAL APPROACH:
To identify miRNAs that increase the productivity of producer cells, a global miRNA screen was performed in CHO cells stably expressing an IgG1. In the primary screen, antibody titers in cell culture supernatants were determined upon transient transfection of a human miRNA library. Candidate miRNAs were validated in a secondary screen in terms of specific productivity in three different CHO producer cell lines. Finally, stable miRNA-expressing CHO producer cells were generated and their performance was analyzed in fed-batch cultures.

RESULTS AND DISCUSSION:
Our global screen identified 16 human miRNAs that reproducibly improved IgG titers when transiently introduced into CHO producer cell lines. Two miRNAs positively impacting the viable cell density and specific productivity, respectively, were selected and stably co-expressed in CHO producer cells. Preliminary results with these cells revealed higher IgG titers in fed-batch cultures while conserving product quality. MiRNA-based cell line engineering is thus an attractive approach toward the genetic optimization of CHO host cells for industrial applications.

NOVEL STRATEGY FOR A HIGH-YIELDING MAB-PRODUCING CHO STRAIN (OVEREXPRESSION OF NON-CODING RNA ENHANCED PROLIFERATION AND IMPROVED MAB YIELD)

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KEY WORDS:
LONG NON-CODING RNA / OVEREXPRESSION / HIGH-TITER / NFKBIA / COMPLEMENTARY SEQUENCE

BACKGROUND AND NOVELTY:
Innovation in mAb production is driven by strategies to increase yield. A host cell line constructed to overexpress TAUT (taurine transporter) produced a higher proportion of high-mAb-titer strains. From these we selected a single TAUT/mAb strain that remained viable for as long as 1 month. Its improved viability is attributed to improved metabolic properties. It was also more productive (>100 pg/cell/day) and yielded more mAb (up to 8.1 g/L/31 days) than the parent cell line. These results suggested that this host cell engineering strategy has great potential for the improvement of mAb-producing CHO cells.

EXPERIMENTAL APPROACH:
Our present challenge was to achieve a high yield in a shorter culture period by modulating events in the nucleus by using non-coding RNA (ncRNA). We looked for long ncRNA (lncRNA) that was abnormally expressed in high-titer cells. A Mouse Genome 430 2.0 array (Affymetrix) identified the lncRNA as a complementary sequence of the 3' non-coding region of mouse NFKBIA (NF-kappa-B inhibitor alpha) mRNA. NFKBIA is an important regulator of the transcription factor NFKB, a positive regulator of cell growth. Since NFKBIA suppresses NFKB function, inhibition of NFKBIA by overexpression of the lncRNA might further enhance cell proliferation. We genetically modified the TAUT/mAb strain to overexpress part of the lncRNA.

RESULTS AND DISCUSSION:
The resulting co-overexpression strains gave increased yield, and one strain increased yield in a shorter culture period (up to 6.0 g/L/14 days from 3.9 g/L/14 days). Interestingly, however, this effect might not be due to enhancement of the NFKB-dependent promoter activity of the mAb expression plasmid because mAb production under EF-1 promoter without an NFKB binding site was also enhanced by overexpression of part of the lncRNA. Since overexpression of the partial sequence still functions as an antibody production enhancing sequence in mAb-producing cell lines, many unexpected functions from ncRNA-containing microRNA might exist.

ENGINEERING OF SYNTHETIC CIRCUITS FOR BIOMEDICAL APPLICATIONS

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KEY WORDS:
SYNTHETIC BIOLOGY / SYNTHETIC GENE CIRCUITS / PROSTHETIC NETWORKS / GENE REGULATION / CELL THERAPY

BACKGROUND AND NOVELTY:
Synthetic biology has significantly advanced the design of genetic devices that can reprogram metabolic activities in mammalian cells and provide novel therapeutic strategies for gene- and cell-based therapies. In this study we designed a novel synthetic optogenetic transcription device used for the treatment of diabetes. Further more, we have assembled a designer circuit which allowed a three-in-one treatment strategy to simultaneously address metabolic syndrome: hypertension, hyperglycemia, and obesity.

EXPERIMENTAL APPROACH:
A synthetic signaling network enabling light-inducible transgene expression in mammalian cells was designed by linking the signal transduction of melanopsin to the control circuit of the nuclear factor of activated T cells. The engineered cells containing the designed circuit were subcutaneously or transdermally implanted into mice to remote control glucacon-like peptide 1 expression through illumination. Another assembled synthetic circuit was also designed which allowed an antihypertensive drug to dose-dependently regulate a chimeric trace amine-associated receptor whose singling was rewired to expression of the bifunctional peptide GLP-1-Leptin. This synthetic circuit was engineered into mammalian cells and further implanted into mouse models for disease therapy.

RESULTS AND DISCUSSION:
The optogenetic transcription device could be used to precisely program gene expression in mammalian cells and mice when illuminated by light. In type 2 diabetic mice subcutaneously implanted with the engineered light-responsive cells, the light-triggered expression of GLP-1 could successfully attenuate glycaemic excursions in db/db mice. The designer circuit was successfully tested in a mouse model of the metabolic syndrome. Mice containing engineered cell implants treated with guanabenz showed decreased blood pressure and significantly increased GLP-1 and Leptin levels which in turn attenuated glycemic excursions, decreased food intake, body weight, plasma cholesterol and free fatty acid levels.

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Monday 24th of June- 15h25
O13

CROSSTALK OF SYNTHETIC CASSETTES IN DEFINED CHROMOSOMAL SITES

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KEY WORDS:
PROMOTER CROSSTALK / RMCE / ROSA26/TET-PROMOTER INTERACTION / EPIGENETIC SILENCING / DNA METHYLATION

BACKGROUND AND NOVELTY:
Development of recombinant cell lines relies mostly on illegitimate recombination followed by extensive screening to select the best clones. This is required since chromosomal elements next to the transgene integration sites affect its expression ("position effect"). To investigate the expression of the Dox-inducible Tet-promoter within the well-known Rosa26 locus, we employed recombinase mediated cassette exchange. Unexpectedly, we saw stochastic transgene expression. We show that the Tet-promoter is prone to DNA methylation in this integration site suggesting a crosstalk between the incoming synthetic promoter and the endogenous Rosa26 locus.

EXPERIMENTAL APPROACH:
Site specific transgene integration in ES cells and NIH3T3 cells was pursued by Flp mediated RMCE. Transgene expression was quantified using FACs analysis, Luciferase assay as well as RT PCR. To investigate the epigenetic crosstalk DNA methylation was studied using bisulfite sequencing.

RESULTS AND DISCUSSION:
Various Tet-cassettes were targeted into the Rosa 26 promoter by employing Flp mediated Recombinase mediated cassette exchange (RMCE) technology. Depending on the design, the Tet-cassettes were found to be insufficiently expressed in various cell lines but also in transgenic mice. Unexpectedly, we observed only stochastic transgene activation within individual cells of the genetically identical clones. Epigenetic characterization revealed that the Tet promoter is highly affected by DNA methylation in the Rosa 26 locus in spite of the Rosa locus staying largely unmethylated itself. This work increases our understanding of the transgene behaviour upon targeting in various different loci and thus holds significant importance in establishment of transgenic cell lines and animal models.

Monday 24th of June- 15h37
O14

PROGRAMMABLE DESIGNER CIRCUITS PERFORMING BIOCOMPUTING OPERATIONS IN MAMMALIAN CELLS

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KEY WORDS:
SYNTHETIC BIOLOGY / BIOCOMPUTING / BIOENGINEERING / GENE NETWORKS

BACKGROUND AND NOVELTY:
Cells operate as information-processing systems that dynamically integrate and respond to environmental input signals. In Synthetic Biology, bioengineers hijack existing or create novel gene circuits to perform tailored functions in living cells. In this study, existing biological parts are rewired to multi-component designer circuits operating in single mammalian cells. Programmed by external input signals, circuit-transgenic cells are capable of executing a set of basic Boolean logic gates as well as basic arithmetic operations that are reminiscent to digital circuits in electronics.

EXPERIMENTAL APPROACH:
The design strategy includes small molecule-dependent transcriptional regulators that integrate input signals and drive the transcription of downstream translational controllers or reporter genes. Functional interconnection of orthogonal gene controllers enables precise regulation of reporter protein production based on combinatorial control of transcriptional and translational gene switches. Fluorescent reporter proteins served as output signals and enabled single-cell analysis using fluorescence microscopy and flow cytometry.

RESULTS AND DISCUSSION:
Inspired by digital electronics, we first developed a set of cellular Boolean logic gates that are programmed by external inputs. Further connection of basic logic gates remarkably increased the computational capacity in single cells, which is exemplified by the design of the XOR gate. Additionally to logic gates, we developed two arithmetic circuits, the half-adder and half-subtractor, which both performed binary calculations in living cells. Biocomputing cells that are capable of executing basic arithmetic operations represent fundamental building blocks for future (multi-)cellular systems executing functions with increasing complexity. Linking biocomputing circuits to the detection of disease-related biomarkers could increase the precision of prosthetic gene networks and may pave the way for a new generation of cell-based treatment strategies.

A RED/FAR-RED LIGHT-RESPONSIVE BI-STABLE TOGGLE SWITCH TO CONTROL GENE EXPRESSION IN MAMMALIAN CELLS

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KEY WORDS:
OPTOGENETICS / GENE EXPRESSION / PHYTOCHROME / SYNTHETIC BIOLOGY / LIGHT-INDUCED

BACKGROUND AND NOVELTY:
Processes in multicellular systems are orchestrated by gene expression programs that are tightly regulated in time and space. The targeted manipulation of such processes by synthetic tools with high spatiotemporal resolution could, therefore, open new opportunities in tissue engineering and enable a deepened understanding of developmental processes. Here, we describe the first red/far-red light-triggered gene switch for mammalian cells for achieving gene expression control in time and space.

EXPERIMENTAL APPROACH:
We constructed the red light-switchable gene expression system based on the concept of a split transcription factor. In doing so, we capitalized on the red light-dependent interaction of the A. thaliana proteins phytochrome B (PhyB) and the phytochrome interacting factor 6 (PIF6). We optimized and characterized the system using secreted alkaline phosphatase (SEAP) as reporter and quantitatively analyzed the light-induced expression kinetics by a mathematical model. Finally, we used an in-vivo angiogenesis assay as a proof-of-concept for the system's suitability for applications in tissue engineering.

RESULTS AND DISCUSSION:
We show that the system can be toggled between stable on- and off-states using short light pulses at 660 or 740 nm. Gene expression correlated with the applied photon number and was compatible with different cell lines, including human primary cells. Experimental data and modeling results confirmed that expression shut-off occurs immediately upon illumination with far-red light. We demonstrate the system's potential in tissue engineering by applying it to induce angiogenesis in chicken embryos. The system's performance combined with tissue-compatible regulating red light will enable unprecedented spatiotemporally controlled molecular interventions in cells, tissues and organisms. Moreover, non-invasive induction by light represents an excellent alternative to existing processes for the production of biopharmaceuticals that are unstable or have cytotoxic or cytostatic properties.

FIRST CPG ISLAND MICROARRAY FOR GENOME-WIDE ANALYSES OF DNA METHYLATION IN CHINESE HAMSTER OVARY CELLS: NEW INSIGHTS INTO THE EPIGENETIC ANSWER TO BUTYRATE TREATMENT

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KEY WORDS:
CHINESE HAMSTER OVARY (CHO) CELLS / EPIGENETICS / DNA METHYLATION / CPG ISLAND MICROARRAY / BUTYRATE EFFECT

BACKGROUND AND NOVELTY:
Today a majority of biopharmaceuticals is produced in CHO cells. Optimisation of productivity and growth requires insight into regulatory processes which is to some extent accomplished by 'omics' approaches. A promising aspect in this context is the epigenetic process of DNA methylation. Supplementation of butyrate provides an opportunity to enhance cell specific productivities in CHO cells and leads to alterations of epigenetic silencing events. Genome-wide studies of changes in DNA methylation following butyrate treatment promise valuable information regarding the optimisation of cultivation processes. However, DNA methylation has not been investigated on a genomic scale in CHO cells so far, and suitable tools did not exist until recently. Here, we present a customised microarray allowing us to conduct genome-wide analyses of DNA methylation in CHO cells.

EXPERIMENTAL APPROACH:
In order to screen for differential methylation of genomic regions prone to encounter epigenetic modifications, so-called CpG islands (CGIs), we developed a 60 K microarray covering 19,598 promoter-associated and intragenic CGIs. The design was based on the genomic and transcriptomic information currently available for CHO cells. We analysed four replicate CHO DP-12 cultures prior to treatment with butyrate as well as 24 h and 48 h after butyrate addition.

RESULTS AND DISCUSSION:
We found 1,340 genes to be differentially methylated 24 hours after butyrate addition. GO terms regarding apoptosis, chromatin modification and DNA repair were significantly enriched. Functional classifications indicated involvement of several major signalling systems such as the Wnt and MAPK pathways. Moreover, the results hint towards a role of the miRNA system in the epigenetic answer to butyrate treatment. Genes of the major mediators of maintenance and de novo methylation, DNMT3A and UHRF1, showed differential methylation. Surprisingly, the observed regulations proved to be temporary, as 91 % of them were not detectable anymore another 24 hours later.

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Tuesday 25th of June- 9h35
O17

2D FLUORESCENCE SPECTROSCOPY FOR REAL-TIME AGGREGATION MONITORING IN UPSTREAM PROCESSING

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KEY WORDS:
SYNCHRONOUS FLUORESCENCE SPECTROSCOPY / EXTRINSIC FLUORESCENCE DYES / PROTEIN AGGREGATION / PROCESS CONTROL

BACKGROUND AND NOVELTY:
Product aggregation is one side effect of rising yields due to process improvement and therefore accompanied with massive product loss during downstream processing. Hence, real time bioprocess monitoring and on-line product quality control during upstream processing (USP) comes more into focus, addressing this issue. For bioprocess control, synchronous fluorescence spectroscopy (SFS) in combination with multivariate data analysis (MVA) based on intrinsic cell culture fluorescence is a promising tool. Furthermore extrinsic fluorescence dyes are widely used to detect and quantify aggregated protein. In this study, SFS in combination with extrinsic fluorescence dyes for further process optimization was investigated, in order to establish real-time aggregation monitoring in USP.

EXPERIMENTAL APPROACH:
The sensitivity of the extrinsic dyes and their detection limit regarding aggregated protein was estimated based on SFS and MVA for cell free model systems and in cell culture experiments. SEC-MALS and Protein A-HPLC were used as reference methods for determination of protein concentration, aggregation levels and molecular weight estimation. Furthermore, a CHO cell line producing a monoclonal antibody was used for cell culture experiments. The toxicity and biocompatibility of different fluorescence dyes were compared based on LC50 via WST1 assays and flow cytometry.

RESULTS AND DISCUSSION:
The detection limit within cell free model systems for protein aggregation monitored via extrinsic fluorescence dyes and SFS was strongly depending on the dye. As expected, fluorescence signal intensities and dye concentrations were correlated and the biocompatibility of extrinsic dyes varied. Suitable candidates were selected based on their sensitivity and toxicity. Additionally, dye accumulations within cells or onto membranes were identified and possible side effects towards aggregation real-time monitoring were analyzed. Cell culture experiments with extrinsic dyes gave additionally promising results towards detection limits.

Tuesday 25th of June- 9h55
O18

RECOMBINATION-MEDIATED CASSETTE EXCHANGE (RMCE) FOR MONOCLONAL ANTIBODY EXPRESSION IN A CHOK1-DERIVED HOST CELL LINE

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KEY WORDS:
MAB / RECOMBINATION-MEDIATED CASSETTE EXCHANGE / SITE-SPECIFIC INTEGRATION / FRT FLP / CELL LINE DEVELOPMENT

BACKGROUND AND NOVELTY:
Cell lines suitable for therapeutic monoclonal antibody (mAb) production require excellent growth, stability and productivity characteristics. The development of such cell lines has classically been a time-consuming and resource-intensive process. The objective of current study is to develop a FRT-/FLP based site-specific integration (SSI) to efficiently target gene of interest to a specific locus in CHOK1 genome and rapidly generate stable cell lines with desired performance characteristics. The presentation will demonstrate with examples how SSI has been successfully applied to the generation of high-performing mAb expressing cell lines. To our knowledge this is the first successful demonstration of FLP-based site-specific targeted mAb production in a widely used commercially relevant cell line.

EXPERIMENTAL APPROACH:
We engineered FRT sequences into a mAb expression vector and then performed a standard cell line construction process in a CHOK1-derived host cell line. High performance recombinant cell lines, harboring the modified mAb expression vector containing FRT sequences were isolated. One particular clone with the best combination of growth, productivity and stability characteristics combined with a low copy number and single integration site was used as a progenitor for the creation of the SSI host cell line by RMCE with a null vector. RMCE reaction replaces the original mAb transcription units in the progenitor cell line with marker genes to generate the new host cell line. This allows a more regulatory favorable RMCE, as the SSI host cell line is free of pre-existing mAb gene.

RESULTS AND DISCUSSION:
The resulting SSI recombinant cell lines not only exhibited excellent and consistent growth/productivity profiles, but importantly inherited the production stability trait observed in the progenitor. We have subsequently determined the genetic context of FRT-tagged loci in the SSI host by using Illumina HiSeq sequencing technology.The locus has been mapped to the reference CHOK1 genome published by BGI.

IMPLEMENTATION OF A PREDICTIVE SCREENING STRATEGY FOR CELL CLONING BY AUTOMATION AND PARALLELIZATION

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KEY WORDS:
CELL LINE DEVELOPMENT / AUTOMATION / PREDICTIVE CLONE SELECTION / SHORTEN DEVELOPMENT TIMELINES

BACKGROUND AND NOVELTY:
Despite many advances and novel techniques, cell line development is often rate-limiting in the overall progress of a specific project. To save on overall project timelines, it is Bayer's approach to push up cell line development into late research phase to have a high performing cell line ready before entering into clinical development. This comes at the expense of having to perform cell line development for multiple clinical candidates for a given project at the same time, with project load ever increasing as often observed in industry. To overcome these limitations the workflow has been optimized including a compact flexible automation platform as key component with integrated data management connected to our Biologics data platform.

EXPERIMENTAL APPROACH:
A new automation friendly cell line development workflow was created aiming at enhanced predictivity for scale-up while enabling automate screening of high numbers of different clones. Key steps were tested manually. The newly designed workflow is being transferred onto a specifically designed automation platform.

RESULTS AND DISCUSSION:
The newly designed workflow was manually tested and yielded clones of good productivity. An automation platform consisting of different liquid handlers, a cell imaging system, an incubator, a cryo vial decapper and a robo arm was established. First test runs were successfully started. The automation enables an efficient high throughput cell line development process. Appropriate data management is employed to cope with the associated increase in complexity of several candidates/projectes in parallel screening programs. A stringent workflow has been devised which is amenable to automation and still compatible with predictive clone selection and early assessment of product quality attributes.

RAPID CONSTRUCTION OF TRANSGENE-AMPLIFIED CHO CELL LINES BY CELL CYCLE CHECKPOINT ENGINEERING

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KEY WORDS:
CHINESE HAMSTER OVARY CELLS / GENE AMPLIFICATION / MONOCLONAL ANTIBODY / ATR / CELL CYCLE CHECKPOINT

BACKGROUND AND NOVELTY:
The process of establishing high-producing cell lines for the manufacture of therapeutic proteins is both time-consuming and laborious due to low probability of obtaining high-producing clones from a pool of transfected cells. Usually, many rounds of MTX selection to amplify the transgene and screening of over several hundred individual clones are required to obtain high-producing cells. Here, we present a novel concept to accelerate gene amplification through cell-cycle checkpoint engineering. In our knowledge, there is no previous report which focused on controlling cell cycle checkpoint to enhance the efficiency of DHFR gene amplification system.

EXPERIMENTAL APPROACH:
A small interfering RNA (siRNA) expression vector against Ataxia-Telangiectasia and Rad3-Related (ATR), a cell cycle checkpoint kinase, was transfected into Chinese hamster ovary (CHO) cells. The effects of ATR down-regulation on gene amplification and productivity in CHO cells producing green fluorescent protein (GFP) and monoclonal antibody (mAb) were investigated.

RESULTS AND DISCUSSION:
The ATR down-regulated cells showed up to 6- fold higher ratio of GFP-positive cells than that of the control cell pool, and had about 4- fold higher specific productivity and 3- fold higher volumetric productivity as compared to the control cell pool during the construction of mAb-producing cells. ATR down-regulated cells showed much faster increase of transgene copy number during gene amplification process via methotrexate (MTX) treatment in both GFP- and mAb- producing cells. Our results suggest that a pool of high producing cells can be more rapidly generated by ATR down-regulation as compared to the conventional gene amplification method by MTX treatment. This novel method is a promising approach to reduce the duration and labor in the process of cell line construction.

Tuesday 25th of June- 10h39
021

ENGINEERING A MAMMALIAN CELL LINE TOOLBOX THAT EXHIBITS MULTIPLE PRODUCTIVITY AND PRODUCT QUALITY PROFILES

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KEY WORDS:
HOST CELL ENGINEERING / PRODUCT QUALITY / PRODUCTIVITY

BACKGROUND AND NOVELTY:
Although the simplicity of having a single, well-characterized host upon which to initiate cell line development has many advantages, a one size fits all approach does have drawbacks. The range of product quality attributes achievable by a particular host will be limited by its intrinsic genotype and phenotype, which may not overlap with the optimum profile for a given therapeutic. The host proteome, particularly as it relates to folding and trafficking pathways, may differ in a manner that could influence the ability to effectively express some therapeutics. With this in mind, we have begun engineering a "toolbox" of different CHO and HEK293 cell lines to make them suitable hosts for the manufacturing of biologics.

EXPERIMENTAL APPROACH:
With the increased sophistication of engineering tools enabling precise genome editing now at our disposal, we endeavored to create and characterize modified mammalian hosts with tailored made phenotypes. This work describes the initial three step process in which new candidate hosts were i) adapted and evolved for enhanced performance in the platform process, ii) engineered for effective selection and amplification phases, and iii) subsequently auditioned with model proteins to assess the resulting diversity of the hosts. Particular interest was paid to individual engineered host growth rate, productivity and product quality attributes.

RESULTS AND DISCUSSION:
Across the different host cell lines generated and characterized, we saw variations in the ability to express the model proteins, frequency of potentially immunogenic glycans such as alpha-galactose and hydroxylated sialic acid, and different propensities for aggregation. This supports our original premise that each engineered host could contribute unique growth, productivity and product quality attributes resulting in a "cell line toolbox". We believe that moving forward this host cell toolbox affords us far greater flexibility and capabilities than a single host.

Tuesday 25th of June- 11h30
022

MULTI-GENE ENGINEERING OF MAMMALIAN CELL METABOLISM: WALKING THE STEPS TOWARDS HYPERPRODUCTIVITY

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KEY WORDS:
METABOLIC ENGINEERING / MULTI-GENE MANIPULATION / MAMMALIAN CELLS

BACKGROUND AND NOVELTY:
Mammalian cell metabolic engineering holds the potential to develop high-producing hosts for the manufacture of complex biopharmaceuticals. In 2007, G. Seth and colleagues conceptualized hyperproductivity as the orchestrated combination of superior attributes from different biochemical pathways [1]. But metabolic manipulation involves labor-intensive steps, from the introduction of the target gene to the isolation and characterization of the candidate clones, turning multiple manipulations extremely difficult. Multi-gene engineering has typically been restricted to 2-3 genes, with the most outstanding achievement reporting up to 3 siRNAs and 3 transgenes off a single genetic platform [2]. In this work, we experimentally challenge the theoretical concept of hyperproductivity to improve a mammalian cell host producing a recombinant enveloped virus, by the manipulation of more than 30 genes.

EXPERIMENTAL APPROACH:
A novel method was implemented for high-throughput screening of hundreds of clones in the early stages of cloning – single step cloning-titration method – allowing for fast isolation of the high-producing phenotypes in a metabolically engineered population. Assisted by a previous functional genomics study on the networks recruited when establishing a producer cell line, more than 30 candidates, including metabolic and regulatory genes, were chosen for manipulation. Targeted pathways included energy generation, oxidative stress, apoptosis, protein processing, lipid biosynthesis and nucleotide metabolism. Manipulated genes span across cytosol, mitochondria and endoplasmic reticulum.

RESULTS AND DISCUSSION:
When single genes were used, specific productivity raised up to 20-fold increase. Genes found to yield high-producing phenotypes are now being combined and the resulting clones characterized. The diversity of metabolic pathways targeted in this study is likely to be of relevance to several biopharmaceuticals produced in mammalian hosts, including complex proteins and other types of recombinant viruses.

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MONITORING INTRACELLULAR REDOX CHANGES IN BIOTECHNOLOGICALLY RELEVANT MAMMALIAN CELL LINES WITH GENETICALLY ENCODED FLUORESCENT BIOSENSORS

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KEY WORDS:
BIOSENSOR / REDOX / CHO-K1 / HEK-293 / HT-29

BACKGROUND AND NOVELTY:
Cellular functions such as DNA replication and protein translation are influenced by changes in intracellular redox homeostasis. Recently, redox-sensitive variants of the green fluorescent protein (roGFP2 and rxYFP) have been developed [1-3]. The biosensors were engineered to equilibrate with the intracellular pool of oxidized and reduced glutathione, the major redox buffer of most eukaryote, allowing the in situ and time-resolved analysis of the cellular redox state. The monitoring of intracellular redox changes can be useful to guide intervention strategies aimed at optimizing cell production processes. The goal of this work was to generate and characterize stable cell lines expressing a redox biosensor.

EXPERIMENTAL APPROACH:
CHO-K1-hGM-CSF (CHO-k1 expressing human granulocyte macrophage colony-stimulating factor), HEK293 and HT-29 cells were lipotransfected with pcDNA3.1-rxYFP plasmid. Cells were selected with zeocin or geneticin and cloned using a MoFlo cell sorter. The functional analysis of the biosensor was performed by flow cytometry. The growth, metabolism and productivity of the reporter CHO-K1-hGM-CSF-YFP and the parental cell line were compared in batch cultures.

RESULTS AND DISCUSSION:
All reporter cell lines displayed a sensitive and reversible response to different redox stimuli (H2O2, pro-oxidant and reducing agent). Cell density, glucose consumption, lactate and rhGM-CSF production were not affected by the introduction of the sensor in CHO-K1-hGM-CSF cells. Prior to entrance to the late log-phase (i.e. growth retardation) the biosensor revealed a significant and sustained shift to a more oxidative intracellular milieu. This suggests that the biosensor is capable to detect early metabolic deficiencies that alter the cellular redox balance in batch culture systems.
Ongoing work aims to test different additives and replenishment strategies to restore the cellular redox homeostasis to increase cell mass and productivity.

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The rxYFP-gene was a kind gift from Dr. G. Pani (Istituto di Fisica, Universitá Cattolica S. Cuore, Italy).

OTHER INFORMATION
K. Perelmuter and I. Tiscornia contributed equally to this work. This work was supported by Agencia Nacional de Investigación e Innovación, Uruguay (project FMV_2009_2617).

A COMPREHENSIVE VIEW ON AN OLD METABOLITE: LACTATE

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KEY WORDS:
LACTATE METABOLISM / MITOCHONDRIAL ACTIVITY / CELL CULTURE MEDIUM

BACKGROUND AND NOVELTY:
In animal cell culture processes, lactate belongs since years to the most intensively monitored metabolites. Lactate can strongly impact the productivity, mainly by influencing the culture pH. In bioreactors, the pH drop induced by excessive lactate production can be neutralized by base addition. However, in diverse non-regulated culture systems, the lactate accumulation can be fatal for the culture performances as soon as it causes the pH to drop below critical physiological values. Conversely, a moderate lactate accumulation at the beginning of the batch culture might be desirable, since rapid cell growth requires a highly active energy metabolism, which is characterized by aerobic glycolysis or "Warburg effect", first described as a criterion to distinguish fast growing cancer cells from normal tissue.

EXPERIMENTAL APPROACH:
In a comparative study involving several CHO clonal cell lines grown under various conditions we noticed different lactate profiles. In further tests under specific culture conditions, each clone generated its distinctive lactate profile. Additional data on a few model clones correlated the lactate profiles to the mitochondrial activity and the expression levels of certain genes such as aralar1 and timm8a. The lactate profiles of clones could be easily altered by feeding certain media components. Moreover we demonstrated that the culture medium can strongly affect the lactate profile as well as the expression of aralar1, which is known for its role in energy metabolism.

RESULTS AND DISCUSSION:
Our data clearly confirm that multiple strategies, including clone screening, medium composition change and cell line genetic engineering, can be employed to obtain a process in which cells switch their metabolism after a few days and start to consume lactate.

BIBLIOGRAPHY, ACKNOWLEDGEMENTS:
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Tuesday 25th of June- 12h22
025

COMPREHENSIVE UNDERSTANDING OF HEPARAN SULFATE PROTEOGLYCAN BIOSYNTHESIS IN CHO AND HEK293 CELLS

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KEY WORDS:
TRANSIENT GENE EXPRESSION / HEPARAN SULFATE PROTEOGLYCAN / ENDOCYTOSIS / CHO / HEK293

BACKGROUND AND NOVELTY:
Chinese hamster ovary (CHO) and human embryonic kidney 293 (HEK293) cells are the most popular host cells for transient production of therapeutic proteins. These host cells require high transfection efficiency in order to enhance productivity. While most mammalian cells express heparan sulfate proteoglycans (HSPGs) on the cell surface, recent studies have shown the close relationship between HSPGs and gene delivery via endocytosis. Albeit many researchers have revealed the direct involvement of HSPGs in transfection, metabolic process of HSPG biosynthesis in CHO and HEK293 cells is yet unknown.

EXPERIMENTAL APPROACH:
In this study, we performed immunofluorescence staining and detection of mRNA to demonstrate the biosynthesis, secretion, and degradation of HSPG in CHO DG44 and HEK293 EBNA cells.

RESULTS AND DISCUSSION:
The localization pattern of HSPG in both cells changed simultaneously with cell growth. The time course study and immunofluorescence showed that the synthesis of HSPG increased on day 2 after plating and the secretion and/or fading of HSPG became significant on day 4 after plating. Flow cytometry analyses showed fluctuated patterns possibly due to continuous biosynthesis-secretion-degradation circulation. The relative mRNA amount of 15 genes related with each step of HSPG biosynthesis showed parallel mode with immunofluorescence results. The expression level of enzymes related with glycosaminoglycan chain modification was especially high in both cells. In addition, overall mRNA expression level in HEK293E cells was higher than CHO DG44 cells. Furthermore, heparanase assay suggested that the gradual degradation of HSPG is related with the increased heparanase activity in the cells and media.
Taken together, our data show the relationship between morphological changes of HSPGs and mRNA expression level during the culture in CHO DG44 and HEK293E cells. This study provides clues to enhance the transfection efficiency in transient gene expression system by engineering cell surface HSPGs.

Wednesday 26th of June- 10h10
026

BEAT™ THE BISPECIFIC CHALLENGE: A NOVEL AND EFFICIENT PLATFORM FOR THE EXPRESSION OF BISPECIFIC IGGS

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KEY WORDS:
BISPECIFIC ANTIBODY / HETERODIMERIC IGG / BEAT / T CELL RECEPTOR / CHO

BACKGROUND AND NOVELTY:
The binding of two biological targets with a single IgG-based molecule is thought to be beneficial for clinical efficacy. However the technological challenges for the development of a bispecific platform are numerous. While correct pairing of heterologous heavy and light chains (HC and LC) can be achieved by engineering native IgG scaffolds, crucial properties such as thermostability, effector function or low immunogenicity should be maintained. The molecule has to be expressed at industrially relevant levels with a minimum fraction of contaminants and a scalable purification approach is needed to isolate the product from potentially complex mixtures. This presentation will introduce a novel bispecific platform based on the proprietary BEAT technology (Bispecific Engagement by Antibodies based on the T cell receptor) developed by Glenmark and will present the CLD strategy used for stable clone generation.

EXPERIMENTAL APPROACH:
The challenge of HC heterodimerization is solved by mimicking the natural association of the heterodimeric T-cell surface receptors and between two CH3 domains of IgG. LC mispairing is avoided by replacing one Fab arm of the bispecific IgG by a scFv. The protein A binding site of the HC is abrogated to facilitate the isolation of the BEAT-antibody by affinity chromatography.

RESULTS AND DISCUSSION:
The molecule shows good thermostability and low likelihood of immunogenicity. Stable cell lines are generated by co-transfection of proprietary expression vectors in CHO-S cells. The asymmetry of our bispecific format allows the characterization of the secretion profiles of generated clones using high throughput analytics based on the molecular weight. Using this approach, clones with volumetric productivity of several g/L and a high heterodimerization level (>90%) could be generated within 5 months. In summary, our platform combining the BEAT technology for heterodimerization and an efficient cell line selection strategy allows production of pure bispecific antibody at several g/L.

NEXT GENERATION BISPECIFIC ANTIBODY DESIGN AND THE INFLUENCE THEREOF ON PRODUCT YIELD AND STABILITY

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KEY WORDS:
BISPECIFIC ANTIBODY / MAMMALIAN EXPRESSION / CHO-S / STABILITY

BACKGROUND AND NOVELTY:
First-line cancer treatments such as surgical removal of tumours is highly invasive and if unbeknownst to patient or physician the cancer has spread to other organs, not an effective means of providing a therapeutic benefit; strengthening the need to develop targeted therapies capable of overcoming drug resistance and limiting the immunogenic effects associated with chemotherapeutics. One novel approach to circumvent this phenomenon includes the engineering of new antibodies capable of targeting cell surface antigens for which natural antibodies don't exist. Monoclonal antibodies (mAbs) provide a means to circumvent systemic drug administration when conjugated to drugs or radio-labels. Bispecific antibodies (BsAbs) can be engineered to target multiple antigens; whether these are cancer cell surface antigens, specific drugs or targets on a drug delivery nanoparticle; explaining why these next generation biomolecules have gained popularity in recent years.

EXPERIMENTAL APPROACH:
Development of processes to produce high levels of BsAbs has proved to be more complicated than standard production techniques required for mAb development owing to the lower expression levels of BsAbs and their inherent downstream instability. We have engineered a number of standard BsAb formats for production in a CHO-S expression system and will relate changes in BsAb structure to the total yield following chromatographic purification.

RESULTS AND DISCUSSION:
BsAbs are expressed at a significantly lower level than standard mAbs. Slight modifications at the BsAbs DNA level can results in two similar constructs having 6-fold differences in product yield. However, the same changes in the BsAb DNA sequence results in improved downstream and long-term stability of the product. Developing the mammalian expression and chromatographic techniques to enhance BsAb production is therefore a pivotal component in improving product yield and stability.

INVESTIGATING THE DETERMINANTS OF NOVEL-FORMAT ANTIBODY EXPRESSION IN CHO CELLS

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KEY WORDS:
EXPRESSION / AMINO ACID SEQUENCE / PHAGE DISPLAY / TRANSIENT HEK / WESTERN BLOTTING

BACKGROUND AND NOVELTY:
Recent innovations in antibody engineering have resulted in a new generation of novel-format antibody-based products, designed to improve natural antibody properties.[1] However these non-natural antibodies can be challenging for host cell production [2,3] and expressional bottlenecks may occur at any stage of expression, [2,3] which has an impact on the production and entry of novel-format antibodies onto the clinical market. There is growing evidence that amino acid sequence can impact on protein expression [2] but the molecular mechanisms that govern this observation are still poorly understood.

EXPERIMENTAL APPROACH:
In this study we generated a panel of novel-format antibodies based around a common monoclonal antibody to which we have attached a range of binding proteins generated through phage display technology against a common antigen and with limited variation in amino acid sequence at discrete areas of the binding protein. These novel format antibodies have been screened for expression in transient HEK cells and 20 sequences were progressed into stable CHO cells to perform in-depth molecular analyses of determinants of expression of this novel antibody format.

RESULTS AND DISCUSSION:
We have observed that limited amino acid variations (between 10-16% of the binding protein) can result in up to a 10-fold difference in expression in both HEK and CHO cells. Expression in HEK cells was generally, but not always, a good predictor of expression in CHO cells. Host cell growth and viability characteristics were not adversely affected by transfection with poor expression constructs and there was no relation between poor expression and functionality of the novel-format antibody. Intracellular and extracellular western blotting has enabled categorisation of profiles that correlate with expression. These results will give insight into the relation between amino acid sequence and expression in mammalian cells and have an impact on the development of engineered antibodies for the clinical market.

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Wednesday 26th of June- 10h54
029

A QUANTITATIVE AND MECHANISTIC MODEL FOR MONOCLONAL ANTIBODY GLYCOSYLATION AS A FUNCTION OF NUTRIENT AVAILABILITY DURING CELL CULTURE

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KEY WORDS:
MONOCLONAL ANTIBODY GLYCOSYLATION / QUALITY BY DESIGN / MATHEMATICAL MODELING / BIOPROCESSING

BACKGROUND AND NOVELTY:
Monoclonal antibodies (mAbs) are one of the leading products of the pharmaceutical industry. All approved mAbs contain a consensus N-linked glycosylation site on their constant fragments, and it has been widely reported that the complex carbohydrates (glycans) bound to these sites have great influence on the safety and efficacy of these molecules. It has also been reported that numerous bioprocess conditions directly impact the composition and distribution of glycans bound to mAbs. To address these issues, we have defined a mathematical model that mechanistically and quantitatively describes the glycosylation profiles of mAbs as a function of nutrient availability during cell culture. Such a model can be used for bioprocess design, control and optimization, thus facilitating the manufacture of mAbs with built-in glycosylation-associated quality under QbD guidelines. To our knowledge, this is the first mathematical model that quantitatively relates bioprocess conditions with mAb glycosylation.

EXPERIMENTAL APPROACH:
The mathematical model links extracellular nutrient availability with nucleotide sugar (NSD) metabolism which, in turn, feeds into a previously published model for Golgi N-linked glycosylation [1]. Experimentally, murine hybridoma cells (CRL-1606, ATCC) were cultured and typical data was collected. The intracellular NSD pools were extracted using perchloric acid (PCA) and quantified using a chromatographic method (HPAEC) that allows for quantification of 8 NSDs and 4 nucleotides in under 30 minutes. Finally, the mAb glycan profiles were obtained using MALDI TOF mass spectrometry.

RESULTS AND DISCUSSION:
Time-courses for all data were produced, including the profiles for all eight NSDs and four nucleotides. The mathematical model reproduces all the experimental data accurately. For validation, simulations were performed under different conditions of nutrient availability. These simulations show that the model has the ability of reproducing previously reported phenomena, such as production of high-mannose glycans under glutamine starvation [2].

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Wednesday 26th of June- 12h05
030

ELECTRICALLY MODULATED ATTACHMENT AND DETACHMENT OF ANIMAL CELLS CULTURED ON AN ITO ELECTRODE

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KEY WORDS:
ELECTRICAL MODULATION / CELL ATTACHMENT / CELL DETACHMENT / HELA CELL / DEEP-SEA FISH CELL

BACKGROUND AND NOVELTY:
Micropatterning techniques of adhesive animal cells have been reported by numerous groups and fall into 6 major classifications. There are 1) photolithography, 2) soft lithography, 3) ink jet printing, 4) electron beam writing, 5) electrochemical desorption of self-assembled monolayers, and 6) dielectrophoresis. However, these six cell micropatterning techniques cannot modulate both the attachment and detachment of animal cells iteratively at the same positions. The purpose of this study was to develop modulation methods for the attachment and detachment of specifically positioned adhesive animal cells cultured on an electrode surface with the application of a weak electrical potential.

EXPERIMENTAL APPROACH:
A patterned indium tin oxide (ITO) optically transparent working electrode was placed on the bottom of a chamber slide with a counter- (Pt) and reference (Ag/AgCl) electrode. The ITO patterning was formed by a reticulate ITO region and arrayed square glass regions of varying size. Using the 3-electrode culture system, the author succeeded in modulation of the attachment and detachment of animal cells on the working electrode surface.

RESULTS AND DISCUSSION:
Animal cells suspended in serum or sera containing medium were drawn to and attached on a reticulate ITO electrode region to which a +0.4-V vs. Ag/AgCl-positive potential was applied. Meanwhile, the cells were successfully placed on the square glass regions by –0.3-V vs. Ag/AgCl-negative potential application. Animal cells detached not only from the ITO electrode but also from the square glass regions after the application of a ±1.0-V vs. Ag/AgCl, 9-MHz triangular wave potential in PBS(–) for 30–60 min. Triangular wave potential-induced cell detachment is almost completely noncytotoxic, and no statistical differences between trypsinization and the high frequency wave potential application was observed in HeLa cell growth.

REF-A001

PRODUCTION OF MONOCLONAL ANTIBODY, ANTI-CD3 BY HYBRIDOMA CELLS CULTIVATED IN BASKET SPINNER UNDER FREE AND IMMOBILIZED CONDITIONS

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KEY WORDS:
PRODUCTION / MONOCLONAL / BASKET SPINNER / IMMOBILIZATION / HYBRIDOMA

BACKGROUND AND NOVELTY:
Recently, packed-bed bioreactors have been used for the cultivation of a wide range of cell lines and for the production of a large variety of pharmaceuticals including MAbs. Packed-bed bioreactor depends on the immobilization of cells within a suitable stationary matrix (the bed). Packed-bed bioreactors also have the advantage of being capable of generating high cell densities having a low free-cell concentration in suspension; hence, simplifying downstream processing.

EXPERIMENTAL APPROACH:
Hybridoma cells (OKT3), producing IgG2a monoclonal antibodies against CD3 antigen of human T lymphocytes were adapted to serum free medium. The specificity of the produced MAbs was performed by indirect immunofluorescence staining of T lymphocytes from peripheral blood followed by flowcytometeric analysis. Continuous production of MAb was performed in Basket Basket through cell immobilization on Fibra-Cel disks.

RESULTS AND DISCUSSION:
The results obtained showed that upon using flow cytometry and the fluorochrome-conjugated secondary antibody attached specifically to the supernatant MAb from the cells adapted to serum free medium succeeded in sorting 76.8% of the gated cells (lymphocytes), confirming the binding of MAb of the adapted cells to CD3 positive lymphocytes. This means that stable hybridoma cells were successfully adapted to grow under serum free conditions. Upon cultivating the cells in backed spinner basket, the MAb titer increased in each successive batch to reach to 298.5 mg.L-1 after 216 h. This might be due to the protection of the cells against shear stress and air/02 sparging through their immobilization on the micro-carriers, promoting the use of serum- or protein-free medium. Moreover, the micro-carrier is designed to ensure sufficient nutrient supply and also to remove toxic metabolites. On the other hand, the rate of glucose consumption and lactate production increased for each repeated batch, which explains the decrease in batch time and reflects the better physiological state of the cells.

REF-A002

EVALUATION OF A NEW 2D ROCKING-TYPE SINGLE-USE BIOREACTOR FOR STREAMLINED CELL EXPANSION

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KEY WORDS:
CELL EXPANSION / SINGLE-USE BIOREACTOR / SINGLE-USE SENSOR

BACKGROUND AND NOVELTY:
Multi-stage seed trains are typically used to expand a cell population to seed large-scale bioreactors. Co-development of a novel 2-D rocking single-use bioreactor that has a wide range of working volumes will be presented. The rocking mechanism enables high oxygen transfer rates at low shear stress.

Conventional seed trains consist of multiple containers of different types such as spinner flasks, shake flasks, and single-use bioreactors of different sizes. Each device requires its own equipment, incubators and/or platforms contributing to the highly manual and time consuming nature of these operations. Repeated manipulations, also increase the risk of contamination.

EXPERIMENTAL APPROACH:
Test expansions were conducted using the novel bioreactor equipped with a disposable bioreactor bag designed to accommodate a range of culture volumes from 160 mL to 25 L as the cells grow. The new bag was also fitted with integrated single-use sensors to allow better control of cell culture conditions and a new concept for perfusion with an integrated membrane to achieve higher cell concentrations.

RESULTS AND DISCUSSION:
Cell growth and sensor data from the test expansions will be presented and compared with results from a more conventional multi-container seed train. The potential for using the sensor data to control cell culture conditions and to automate the expansion steps will be discussed.

REF-A003

CHARACTERIZATION OF SOY AND WHEAT GLUTEN HYDROLYSATES PROMOTING VERO CELLS GROWTH UNDER ANIMAL COMPONENT FREE CONDITIONS

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KEY WORDS:
SOY AND WHEAT HYDROLYSATES / VERO

BACKGROUND AND NOVELTY:
We developed an animal component free medium named IPT-AFM that sustains Vero cells growth and rabies virus production in stirred bioreactor. This medium contains plant hydrolysates, namely soy (Hypep 1510) and wheat gluten hydrolysates (Hypeps 4601 and 4605) (Rourou et al., 2009). These peptones were shown to promote cell attachment and growth. In a previous work, we fractionated these hydrolysates in order to identify and isolate the peptides showing a positive effect on Vero cells adhesion, attachment and growth (Rourou et al., 2011). After Hypep 4605 removal, we demonstrated that that the performances of IPT-AFM were preserved.

Recently, there has been a tremendous research interest in the production, characterization, and evaluation of bioactive ingredients present in plant-derived products. Their positive impact on body functions or conditions is highly sought.

EXPERIMENTAL APPROACH:
The aim of this work is to assess the global chemical composition such as size distribution, amino acids and carbohydrates composition; and the biological activities of the fractions previously isolated and that had shown a positive effect on Vero cell growth.

RESULTS AND DISCUSSION:
Peptides have certain bio-functionalities and may therefore fulfil therapeutic roles in body systems. For this reason, to assess the biological activities of the selected fractions of soy hydrolysates, we will particularly focus on the ACE inhibitory activity (antihypertensive), antioxidant and anticancer activities. In addition, we will apply the Tricine-SDS-PAGE to analyse the different fractions issues from both hydrolysates.

Then fractions that exhibit a biological activity will be further fractionnated to isolate the peptides. The biological activities of these peptides will be also tested.

REF-A004

RAPID MHC CLASS II PROTEIN PRODUCTION BY TRANSIENT GENE EXPRESSION OF INSECT CELLS

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KEY WORDS:
INSECT CELLS / TRANSIENT GENE EXPRESSION / MHC II MOLECULES / HIGH EFFICIENT

BACKGROUND AND NOVELTY:
Recombinant major histocompatibility complex (MHC) class II proteins have important uses in basic and clinical immunology. They are commonly expressed from stable Drosophila S2-derived cell lines with volumetric productivities of 10-15 mg/L.

EXPERIMENTAL APPROACH:
Here we describe a more rapid approach to high-level expression of MHC II molecules by large-scale transient gene expression (TGE) of suspension-adapted insect cells. The MHC II and chain genes were cloned into separate expression vectors under the control of either an inducible or a constitutive promoter, and the plasmids were co-transfected into suspension-adapted High Five™, Sf9, or Schneider S2 cells using a chemical reagent for DNA delivery.

RESULTS AND DISCUSSION:
Volumetric yields of over 200 mg/L were obtained within 4 days for some MHC class II proteins. Our results demonstrate a simple, fast and low-cost approach to express recombinant MHC class II molecules.

REF-A005

CRYOPRESERVATIVE SOLUTION USING RAKKYO FRUCTAN AS CRYOPROTECTANT

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KEY WORDS:
FRUCTAN / RAKKYO / CRYOPRESERVATION / DMSO / FBS

BACKGROUND AND NOVELTY:
Cryopreservation of the cells allows great flexible application for cell therapy, as well as industrial production of biologics such as antibody therapeutics. Conventionally, cryopreservative solution contains both of fetal bovine serum (FBS) and dimethyl sulfoxide (DMSO) as cryoprotectants. However, both of them have problems.

FBS frequently induces differentiation of stem cells and so it should not be used for cell therapy. Additionally, FBS has serious concern about zoonotic infections such as abnormal prions, pathogen of bovine spongiform encephalopathy (BSE), indicating necessity of FBS-free cryopreservative solution. DMSO has cytotoxicity and often induces stem cells to differentiate. Therefore, it is necessary to reduce the concentration of DMSO in cryoprotectant solution.

In this study, we report that rakkyo fructan, plant-derived polysaccharide, significantly improved the viability of the cells frozen in DMSO-free solution.

EXPERIMENTAL APPROACH:
Cells were collected by centrifugation, removed the culture supernatant and then suspended in PBS containing rakkyo fructan or in FBS containing DMSO as positive control. They were transferred to freezing tubes, placed in a BIOCELL container, frozen and stored at -80 °C for several days. Stored cells were defrosted at 37 °C rapidly and removed the freezing media. They were cultured in each culture media again. The defrosted cells were stained with trypan blue exclusion method and counted with hemocytometer.

RESULTS AND DISCUSSION:
The cells stored in rakkyo fructan solution successfully survived after deep freezing-store and thawing process. After frozen and thawed in rakkyo fructan solution, CHO-DP12 cells and HepG2 cells maintained the growth rate and kept high protein productivity.

We successfully developed serum-free freezing media using rakkyo fructan. The freezing media using rakkyo fructan will be extensively used to protect various animal cells against freezing stress.

REF-A006

DEVELOPMENT AND PERFORMANCE QUALIFICATION OF A NEW SINGLE USE BIOREACTOR SYSTEM

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KEY WORDS:
CELL EXPANSION / SINGLE-USE BIOREACTOR / SINGLE-USE SENSOR

BACKGROUND AND NOVELTY:
A newly developed single use bioreactor system, the Pall XRS 20 Bioreactor system, consists of a rocking platform which holds a pre-sterilised single use biocontainer. Fluid motion essential for gas transfer and liquid-liquid mixing within the biocontainer is achieved using two motors mounted on opposite axes. This enables motion on both the x and y axis (bi-axial agitation). Each bioreactor system includes a control tower to allow the operator to select the agitation rate and rock angles, as well as control the temperature, pH and dissolved oxygen content of the biocontainers liquid content.

EXPERIMENTAL APPROACH:
Our approach included design of experiment to refine agitation parameters for optimized mixing and mass transfer properties, followed by multiple cell culture batch and fed-batch experiments to compare cell culture performance in the new design versus performance in conventional rocker-style systems.

RESULTS AND DISCUSSION:
We present how the introduction of bi-axial agitation in to bench scale single-use bioreactors can significantly improve CHO cell culture performance over conventional rocker platforms. We will present examples of how the bi-axial agitation during high cell density cell culture maintained all the benefits of a low shear environment yet significantly reduced mixing time and increased O2 transfer rate with no accumulation of CO2. For fed-batch process this resulted in consistently reproducible increases in cell titre, IVCC and monoclonal antibody titre; whilst maintaining an identical product quality profile compared to results from conventional rocker systems.

REF-A007

EFFECT OF REDOX POTENTIAL ON ANTIBODY GLYCOSYLATION IN MAMMALIAN CELL CULTURES

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KEY WORDS:
CELL CULTURE / MONOCLONAL / CAMELID / REDOX / GLYCOSYLATION

BACKGROUND AND NOVELTY:
Consistent and proper glycosylation is very important in ensuring Mabs (monoclonal antibodies) efficacy and effectiveness. The consensus glycan in an immunoglobulin has often been related to its position in the interstitial space between the disulfide bonded CH2 domains of two heavy chains. This can reduce the accessibility of glycosyl-transferase enzymes particularly the galactosyltranfersase that adds the terminal galactose to form the G1 or G2 structures. Lowering culture redox potentials (CRP) may disrupt Mab interchain disulfide bonds and lead to different glycan profiles. Using CHO and NS0 cell cultures, several Mabs were analysed for their glycan profiles; including 2 humanized IgG1s and a humanized camelid Ig in the presence of a reducing agent.

EXPERIMENTAL APPROACH:
Cell cultures of CHO and NS0 were subjected to various concentrations of reducing agent (dithiothreitol;DTT) and monitored daily for growth parameters and redox potential. Using HILIC-HPLC methods, shifts in the GI (Galactosylation Index) were monitored in the presence of DTT. In addition to glycan profiling, IgG1 from NS0 cultures were radiolabelled to determine the assembly pathway and changes in pathway intermediates in the presence of the reducing agent.

RESULTS AND DISCUSSION:
The GI was decreased in NS0-IgG1 cultures by as much as 35% in those cultures with lower CRP. In contrast, CHO-IgG1 cultures had no change in GI and the camelid Ig exhibited a 16% increase in GI. The autoradiographs of the protein A purified intracellular NS0-IgG1 verified an assembly pathway of HC HC2 LCHC2 LC2HC2. Densitometry analysis of assembly intermediates showed that the ratio of heavy chain dimer to heavy chain monomer increased over time within the reducing agent cultures. A correlation between redox potential, GI shifts and assembly intermediates is suggested by the data for this particular IgG1. This could have wide ranging implications for process development activities and lead to control mechanisms that influence glycan profiles of Mabs.

REF-A008

VIRAL VECTOR PRODUCTION IN THE INTEGRITY™ ICELLIS® DISPOSABLE FIXED-BED BIOREACTOR FROM BENCH-SCALE TO INDUSTRIAL SCALE

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KEY WORDS:
VIRAL VECTORS / SINGLE-USE BIOREACTOR / TRANSIENT TRANSFECTION / RECOMBINANT VIRUSES / SCALE-UP

BACKGROUND AND NOVELTY:
Recombinant viruses (e.g. lentivirus and adeno-associated-virus) can be used as human gene therapy vectors. They are mainly produced in adherent cell cultures (e.g. HEK293T, A549, VERO) in Roller Bottles (RB) or multiple-tray-stacks using either transient transfection (e.g PEI, PO4 precipitation) or infection (e.g. recombinant viruses) strategies. Therefore, iCELLis® bioreactors offer a new production alternative with stronger process controls and ease of scale-up.

The iCELLis bioreactor from ATMI LifeSciences is designed for adherent cell culture applications. Cells grow on microfibers carriers packed in a fixed-bed providing up to 500m^2 of growth surface area in a small reactor volume. Environmental conditions, combined with the large growth surface area in the iCELLis yields high cell productivity.

EXPERIMENTAL APPROACH:
First, a mirrored approach of the previous production in RB or CF was set-up in the small scale iCELLis bioreactor. Transfection/infection efficiency through the fixed-bed of the iCELLis system is evaluated by Flow Cytometry (measuring the expression of protein marker). At harvest, extracellular viral vectors are cultivated in the cell culture medium. In the case of intracellular vectors, cell disruption is carried out directly in the bioreactor by physico-chemical methods.

RESULTS AND DISCUSSION:
Here we present results of AAV and paramyxovirus production in the iCELLis nano versus classical culture recipients. These results indicated higher titer for AAV (5.108 vg/cm2) in small scale iCELLis versus 3.108 in Cells Stack-5 plates (CS, Corning) and 0.5-1 log higher titers than control (tissue culture flask) for paramyxovirus. Results of transient transfection processes (by PEI or PO4 precipitation) showed similar transfection levels in iCELLis than in CS. Results in iCELLis demonstrated that the system allows high biomass growth, regulation, and virus productivity with a minimum space requirement. The technology can be considered an efficient tool for the production of viral vectors.

REF-A009

CRYOPRESERVATIVE SOLUTION USING RAKKYO FRUCTAN AS CRYOPROTECTANT

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KEY WORDS:
VIRUS / SINGLE-USE BIOREACTOR / SCALE-UP

BACKGROUND AND NOVELTY:
Viral vaccines are usually produced by anchorage-dependent cells in static multitray systems, roller bottles or bioreactors with microbeads. However, these technologies do not enable process intensification as they involve many manual operations. In addition, microbeads-based processes require extensive development and expertise.
To enable process intensification, ATMI developed iCELLis®, a scalable range of disposable fixed-bed bioreactors that operate in perfusion mode. The fixed-bed accommodates up to 500m^2 of growth surface area in only 25 liters reactor volume. The fixed-bed is pre-packed with microfiber carriers to avoid extensive process development related to microbeads. It can be inoculated at a very low cell density which simplifies seed train.

EXPERIMENTAL APPROACH:
Here we present the scale-up of MDBK, Vero and HEK293 processes from iCELLis benchtop bioreactor to production units. Scaling-up with iCELLis systems is quick and similar to that of chromatography columns. As the bioreactor scale increases, the fixed-bed height remains constant while the diameter increases. The cell culture data presented here illustrates the success of this scale-up approach.

RESULTS AND DISCUSSION:
Existing multitray system processes were transferred to small scale iCELLis by keeping same culture parameters (pH, DO & t°) and identical ratios for cells/surface and media/cells. Next came evaluation and optimization of cell culture conditions: compaction of carriers, the linear speed through the fixed-bed and the perfusion rate. When these conditions guaranteed homogeneous cell distribution and good viral productivity, the process was directly scaled to an industrial level. At such scale, we obtained a cell biomass of 1.75x1012 Vero cells (500m^2), 2.2x1011 MDBK cells (133m^2) and 4.5x1011 HEK293cells (133m^2).
The study demonstrated that iCELLis bioreactors simplify viral production processes and that linear scale-up in iCELLis is easily developed from 0.53m2 to 500m2 in less than one year.

REF-A010

A COMPARATIVE STUDY OF SINGLE-USE WAVE-MIXED AND STIRRED BIOREACTORS IN INSECT CELL/BEVS-BASED PROTEIN EXPRESSION AT BENCHTOP SCALE

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KEY WORDS:
INSECT CELLS / BEVS / PROTEIN EXPRESSION / SINGLE-USE BIOREACTORS

BACKGROUND AND NOVELTY:
The cultivation of insect cells in conjunction with the Baculovirus Expression Vector System (BEVS) is considered a promising source for new vaccine candidates as well as protein complexes. Wave-mixed and stirred bioreactors are currently employed for the cultivation of various insect cells and expression of their protein products. However, wave-mixed bioreactors have been reported several times as recommended cultivation systems for insect cell-based protein production processes, because of their low shear mixing principle which results in minimized foam formation. Still, no comparative study describing protein expression in wave-mixed and stirred single-use bioreactors has been published so far. This study investigates and compares the expansion of Spodoptera frugiperda-9 (Sf-9) suspension cells as well as the expression of a model protein, the secreted alkaline phosphatase (SeAP).

EXPERIMENTAL APPROACH:
Optimum infection and production conditions were determined in high-throughput screening systems such as the orbitally-shaken BioLector (m2p-labs) or TubeSpin® Bioreactor 50 (TPP). Growth and production studies were carried out in the wave-mixed BIOSTAT CultiBag RM (27 °C, 19 - 32 rpm, 0.1 vvm, pO2 50 %, pH 6.2) and stirred UniVessel SU (27 °C, 180 rpm, 0.1 vvm, pO2 50 %, pH 6.2) single-use bioreactor from Sartorius Stedim.

RESULTS AND DISCUSSION:
Best protein expression in screening experiments was obtained when using a cell count of infection (CCI) of 2 x 10E6 cells/mL, a multiplicity of infection (MOI) of 0.01 and a time of harvest (TOH) of 144 h. Whereas growth experiments in the two benchtop bioreactors resulted in high cell densities exceeding 10 x 10E6 viable cells/mL, strong foaming in the UniVessel SU required the addition of an antifoam agent, which was not required with the BIOSTAT CultiBag RM. Production experiments in the two bioreactors revealed comparable protein expression with respect to protein quality and activity. Maximum SeAP activities of up to 64 U/mL were achieved.

REF-A011

BIOPROCESS DEVELOPMENT OF DROSOPHILA S2 CELL CULTURE FOR THE EXPRESSION OF RABIES VIRUS GLYCOPROTEIN

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KEY WORDS:
DROSOPHILAS2CELLS/RABIESVIRUS/BIOPROCESS/RECOMBINANT PROTEIN

BACKGROUND AND NOVELTY:
Here we report a bioprocess development using Drosophila melanogaster Schneider 2 (S2) cells to produce the rabies virus glycoprotein (RVGP). The S2 cell system offers suitable bioprocess conditions for generating high-level expression of functional membrane proteins. It is relatively easy and safe to handle and are scalable. Acquired knowledge on biology and engineering of S2 cells opens well based conditions for production of recombinant proteins.

EXPERIMENTAL APPROACH:
We have constructed gene vectors with the hygromycin selection gene (H) in which the RVGP gene was inserted under the control of the metalothionein (Mt) promoter. After transfection, cell populations with (S2MtRVGP-H-his) and without (S2MtRVGP-H) His-tag amino acid motif were selected. The expression of RVGP was evaluated by qRT-PCR, flow cytometry, ELISA and western-blotting. Protocols for cell cultures in scalable bioreactors were developed and batches of RVGP were produced and purified. The ability of S2 cells derived RVGP to induce immune response and protect mice against an experimental rabies virus challenge were investigated.

RESULTS AND DISCUSSION:
High RVGP expression level could be detected in both S2 cell populations (~ 52 % of RVGP positive cells with ~ 4 micrograms of RVGP per 1E7 cells). RVGP mRNA kinetic analysis by qRT-PCR enlightened the relationship between S2 cell growth and specific productivity, showing a peak of RVGP mRNA and RVGP synthesis at the transition to the stationary cell growth phase. Parameters for storage and lysis of cells bearing the RVGP were studied and a protocol of RVGP purification was developed based on His-tag affinity chromatography (HisTrap FF-FPLC) after membrane preparations by ultracentrifugation and solubilizing with OG. High levels of antibodies against RVGP (~ 4 EU/mL after 21 days of immunization) were found in immunized mice (3 weekly doses of 3 micrograms of RVGP each). Preliminary data show that RVGP immunization was capable of inducing protection against rabies experimental challenge (~ 90 % of mice survived a rabies virus challenge). Our data describe bioprocess optimization steps for high-level and biological active RVGP expression in stably transfected S2 cells.

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REF-A012

EFFECT OF AMINO ACIDS ADDITION IN S2 DROSOPHILA MELANOGASTER CELLS CONTINUOUS CULTURE

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KEY WORDS:
INSECT CELLS / GLUTAMINE / CYSTEINE / CELL CONCENTRATION / BIOREACTOR

BACKGROUND AND NOVELTY:
S2 cells from Drosophila melanogaster have been used as expression systems for recombinant proteins. A major goal in these process is increase cell density in bioreactor, to obtain more product. In this study we added amino acids to a commercial medium in order to increase cell concentration in continuous cultures of S2 cells.

EXPERIMENTAL APPROACH:
A S2 cell population was used in this work, which expresses recombinant rabies virus glycoprotein (RVGP) gene under the control of constitutive actin promoter, obtained by co-transfection procedure (Yokomizo et al., 2007). The cells were grown in a Biostat B bioreactor, at 28 degrees Celsius, 90 rpm agitation frequency, and dissolved oxygen controlled at 30 % of air saturation. pH was only monitored. Medium used was SF900 II (Invitrogen) pure or supplemented with glutamine, a pool of (asparagine, proline, serine and cysteine), or cysteine only. Three different dilution rates (0.8, 0.5 and 0.2/day) were visited. In parallel, Schott shake flasks runs were performed, to test conditions.

RESULTS AND DISCUSSION:
With pure SF900 II medium at different dilution rates, cell concentration reached in steady states 26.4 E6 cell/mL, with residual concentration of glucose of circa 5.3 g/L and glutamine of 0.45 g/L. The addition of glutamine (1.7 g/L) or a pool of amino acids in the feed medium did not contribute to increase significantly cell concentration. However, addition of only cysteine (0.3 g/L) in the feed medium resulted in a 12% increase in cell concentration as compared to pure SF900 II medium. Also, specific glucose and glutamine consumption rates were reduced in this case. Thus, it can be concluded that in SF900 II the cysteine limited cell growth of S2 cells and the addition of asparagine, proline and serine have a negative effect in cell concentration.

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Acknowledgments:CNPq

REF-A013

DROSOPHILA MELANOGASTER S2 CELL CULTURES IN CLASSICAL AND DISPOSABLE FIXED BED ICELLIS BIOREACTORS

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KEY WORDS:
DROSOPHILA S2 CELLS / FIXED BED ICELLIS BIOREACTOR

BACKGROUND AND NOVELTY:
New generations of disposable bioreactors have a central place in recent developments in animal cell biotechnology. ATMI LifeSciences developed Integrity® iCELLis®, a scalable line of single-use high-cell-density bioreactors operating in perfusion mode. By combining the advantages of single-use technologies with the benefits of a fixed-bed system, iCELLis systems have been shown to be an important advance in terms of high productivity combined with ready to use bioreactor for academic and industrial use. Our aim in the present work has been to evaluate the growth of Drosophila melanogaster S2 cells in classical and disposable iCELLis bioreactors.

EXPERIMENTAL APPROACH:
An S2 cell population was selected after transfection of gene expression vectors carrying the cDNA encoding the rabies virus glycoprotein (RVGP) gene (Moraes et al., Biotechnology Advances 2012, 30:613-628). Cells were initially cultivated in 50 mL shake flasks in SF900II medium and inoculated into the bioreactors at 5x1E5 cells/mL. Cell cultures were performed in a 1L suspension bioreactor (BioFlo - New Brunswick) at 90 rpm and in a iCELLis Nano bioreactor (with fixed bed of 0.53 m^2 total available surface) (ATMI) at 400-700 rpm and with a 800 ml medium working volume. Medium exchanges were performed and comparable temperature (28ºC) and dissolved oxygen (50 %) parameters were used.

RESULTS AND DISCUSSION:
The data show the good productivity in terms of cells in both systems with a potential for further developments. S2 cell cultures attained concentrations higher than 1E7 cells/mL after 5 days, showing maximal specific cell growth of 1.056 d-1. Glucose and glutamine were regularly consumed indicating suitable metabolic state of the cells. Lactate was produced in low concentrations. The iCELLis system provides a ready to use and flexible system for cell cultivation. Beside its contribution to optimization approaches of productivity, the present study provides bioprocess conditions for further studies of recombinant proteins expressed in S2 cells.

REF-A014

SINGLE-USE TECHNOLOGIES SUPPORT CONTINUOUS PROCESSING IN BIOPRODUCTION

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KEY WORDS:
CONTINUOUS / PERFUSION / STEADY-STATE / BIOPRODUCTION

BACKGROUND AND NOVELTY:
Continuous processing (CP) in pharmaceutical production has been strongly encouraged of late by regulatory and engineering stakeholders alike (1). CP offers many advantages over such currently popular modes of animal cell culture as batch and fed-batch. It provides heightened processing consistency resulting in improved product uniformity while reducing intervention in process intermediates. Process capability can be heightened and activities or chemistries unavailable in batch presented. CP also provides advantages in facility design and build through reduced footprint and increased facility utilization. Finally it's on-line monitoring and real-time quality assurance supported makes it amenable to CQV and even parametric or real-time release initiatives (2).
(1) http://www.in-pharmatechnologist.com/Processing/Continuous-manufacturing-will-make-current-methods-obsolete-FDA-says
(2) http://www.bioresearchonline.com/doc.mvc/Continuous-Processing-In-Bioproduction-0001

EXPERIMENTAL APPROACH:
Centocor (now Janssen Biotech) has long been employing semi-continuous operations in upstream processes of approved biopharmaceutical manufacturing. Genzyme manufactures such products as Lumizyme in CHO-based perfusion culture. Their continued commitment to perfusion-based production is demonstrated by its recent expansion of such capacity at their Geel, Belgium plant. Practical implementation of the perfusion mode of culture has been facilitated of late by the increased process understanding in general, the ability to take many more real-time process measurements, as well as by appearance of improved process control technologies. A new contributor to the growing field of continuous processing is the rapid uptake of single-use technologies (SUT). Reviewed here are those specific features afforded by SUT that relate to continuous processing-specific PD and manufacturing.

RESULTS AND DISCUSSION:
Continuous processing operations are being implemented throughout the process train and these examples in pharmaceutical production can be thought of as 'building blocks' toward an untimely fully continuous manufacturing line. Equipment and systems supporting continuous processing in operations from seed-stock expansion to fill and finish are appearing. As perfusion-modified reactors are contiguously combined with such other enabling technologies as single-use mixers and storage systems, the design of closed, disposable and continuous manufacturing systems for biopharma is finally being accomplished.

BIBLIOGRAPHY, ACKNOWLEDGEMENTS:
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OTHER INFORMATION
Member, Scientific Advisory Committee: World Biopharm Forum, Robinson College, Cambridge University, June, 2013

REF-A015

PRODUCTION AND PURIFICATION OF DIFFERENT SEROTYPES OF RECOMBINANT ADENO-ASSOCIATED VIRUS AS A VECTORED VACCINE AGAINST HEPATITIS E VIRUS

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KEY WORDS:
HEPATITIS E VIRUS / INSECT CELLS / BACULOVIRUS / ADENO-ASSOCIATED VIRUS

BACKGROUND AND NOVELTY:
Hepatitis E virus (HEV) infection is the major cause of acute hepatitis in Southeast and Central Asia and the second most important cause in the Middle East and North Africa. Currently, no commercial HEV vaccine is available. In the absence of an appropriate cell culture system for HEV propagation, HEV pseudocapsids (ORF2 protein) have been produced either in Escherichia coli or in insect cells and they have been shown to protect monkeys against virus challenge and to be effective in the prevention of natural HEV infection of humans. In this work, we investigated the development of a novel candidate vaccine against hepatitis E infection using adeno-associated virus (AAV) as a vector expressing the gene of the truncated capsid protein of HEV (aa 112-aa 660). rAAV will be produced in Sf9 cells using the baculovirus expression vector system.

EXPERIMENTAL APPROACH:
For this purpose, construction of recombinant baculoviruses (BacRep, BacCap for serotypes 2, 5 & 6 and BacITRHEVORF2) were performed and viral stocks were amplified in Sf9 cells. To improve rAAV 2, 5 and 6 production in Sf9 insect cells, viral production was optimized in Erlenmeyer flask using the experimental design approach. We analyzed the effects of the following factors: initial cell density, time of infection, temperature and individual Multiplicity of infection (MOI) of the three or dual (rAAVT6 and rAAVT2) baculoviruses.

RESULTS AND DISCUSSION:
We determined optimal production conditions for all the serotypes of rAAV (2, 5 and 6). We showed that regardless of AAV serotype, cell density level had a positive effect on rAAV production. In addition for rAAV2 and rAAV5, Sf9 cell infection at 30°C resulted in a higher titer compared to 27°C. Whereas for rAAVT6 and rAAVT2, MOI of BacRep2 showed a marked effect on the production. The highest titer of rAAV was varying between 1.23E+11 vg/ml for rAAV2 to 9.68E+10 vg/ml for rAAVT6.

Currently, purification of various serotypes of rAAV using affinity and ion exchange chromatography is under investigation.

BIBLIOGRAPHY, ACKNOWLEDGEMENTS:

OTHER INFORMATION
This project is financed by the International Centre for Genetic Engineering and Biotechnology (ICGEB), Ref. No. CRP/TUN09-01

REF-A016

EFFECT OF AMINO ACIDS ADDITION IN S2 DROSOPHILA MELANOGASTER CELLS CONTINUOUS CULTURE

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KEY WORDS:
NOVA BIOMEDICAL / PAT / METABOLITE / VIRUS / ANALYSIS

BACKGROUND AND NOVELTY:
Current initiatives within the biopharmaceutical industry to adopt PAT (Process Analytical Technology) approaches in manufacturing, include activities in early process development. With adherent cell lines, online sampling for real-time process monitoring and process adjustment is challenging due to the heterogenity of the cell culture and potential blockages to automated sampling systems caused by microcarriers. In this study, we have tested the BioProfile® FLEX automated sampling and analysis system with OPC (Open Productivity & Connectivity) for continuous feedback control of metabolite concentrations in bioreactors.

EXPERIMENTAL APPROACH:
The first part of the study demonstrated that glutamine, glucose and glutamate consumption was dependent on cell growth phase. In the second part of the study, metabolite concentrations were analysed and controlled in the bioreactor using the OPC enabled BioProfile® FLEX analyser. Sampling was performed via a sterile online sampler, allowing automated nutrient feed control. Once established on non-infected cells, the system was further applied to a model viral (rabies) production process for 14 days. Cell culture experiments were carried out with automated sampling and analysis performed every hour to control and monitor the viral production process.

RESULTS AND DISCUSSION:
This study demonstrates that it is possible to effectively control and adjust, in real-time, the required concentration of metabolites to sustain controlled cell metabolism, as viral infection and production progresses. The BioProfile® FLEX online sampler and analyser, in conjuntion with an OPC enabled bioreactor controller, provided reliable results and improved process control for development activities.

REF-A017

MODIFICATION OF MONOCLONAL ANTIBODY GLYCANS USING GLYCOSYLATION INHIBITORS: EFFECTS ON PRODUCTION, ACTIVITY AND STABILITY

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KEY WORDS:
MONOCLONAL ANTIBODY / GLYCOSYLATION / INHIBITOR

BACKGROUND AND NOVELTY:
Monoclonal antibodies (Mabs) are now commonly used as biotherapeutic treatments in cancer, autoimmune diseases and other conditions. Commercialization requires high productivity of Mabs in recombinant cell lines, but increasingly product quality with respect to posttranslational modification has been recognized as equally important. N-linked glycosylation of the Fc region (Asn297) in Mabs plays a critical role in their ability to elicit effector functions. Oligosaccharides with low fucosylation allow the Fc region to interact more efficiently with Fc receptors allowing greater effector activation, such as antibody-dependent cell-mediated cytotoxicity (ADCC). Reduction of core fucosylation and an increase in bisecting GlcNAc of Asn297 oligosaccharides has been the focus of several strategies using siRNA and genetic engineering. An alternative method is to use media additives that modify glycans at discrete points during the glycoprotein processing reactions in the Golgi. Mannosidase inhibitors, such as kifunensine, have been shown to generate Mabs with increased effector function.
The objective of this study was to produce mabs with a wide range of glycan microheterogeneity to study the effect of glycosylation on stability and function.

EXPERIMENTAL APPROACH:
We have used several glycoprotein processing inhibitors (kifunensine, swainsonine, castanopermine) to alter glycosylation in recombinant CHO cell lines that produces a chimeric camelid-human Mab (CHO-EG2) and a humanized murine IgG (CHO-DP12).

RESULTS AND DISCUSSION:
Mabs can be efficiently produced in the presence of these inhibitors with little effect on growth of the cells. Glycosylation of the mabs was determined with HILIC (hydrophilic interaction liquid chromatography) analysis showing the modified glycan chains. We have analyzed effects of modifications of the mab glycans on stability, melting temperature, and aggregation using DLS (dynamic light scattering). We have also produced mabs with variable core fucosylation using a new fucosylation inhibitor, a fluorinated analogue of fucose (Rillahan et al 2012), which is incorporated as GDP-F-fucose and inhibits the FUT 8 fucosyltransferase, and are investigating the effects of different levels of fucosylation.

BIBLIOGRAPHY, ACKNOWLEDGEMENTS:
Rillahan, C., Antonopoulos, A., Lefort, C., Sonon, R., Azadi, P., Ley, K., Dell, A., Haslam, S., and Paulson, J.C. 2012 Nature Chemical Biology 8: 661-668.

REF-A018

OPTIMIZATION OF CELLVENTOTM CHO-200 PRODUCTION MEDIA AND FEED PERFORMANCE THROUGH EXPERIMENTAL EVALUATION OF MULTIPLE FED-BATCH PROCESSES

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KEY WORDS:
CHEMICALLY-DEFINED MEDIA / FED-BATCH PROCESS / PROCESS OPTIMIZATION / DESIGN OF EXPERIMENTS / CELL CULTURE PERFORMANCE

BACKGROUND AND NOVELTY:
Successful bio-therapeutic production of monoclonal antibodies and recombinant proteins is highly dependent on the utilization of effective cell culture media that have been optimally designed using quality raw materials, well-defined manufacturing processes, and formulations that are all devised from sound experimentation. Merck Millipore has recently developed and launched Cellvento™ CHO-200, a chemically-defined, animal origin-free production media and companion feed supplement using a rational design of experiments (DOE) approach to optimize their formulations; and qualified raw materials to produce reproducibly consistent dry powder media lots in a cGMP-compliant facility.

EXPERIMENTAL APPROACH:
In order to provide adequate applications support for this media and feed to the upstream biomanufacturing market, we employed a DOE approach to optimize fed-batch media feeding protocols. Specifically, a monoclonal antibody-expressing CHO-S cell line was used to evaluate the impact of media and feeds on cell growth and productivity in small-scale shaker flask and spin tube cultures. The impact of various feed components, volumes, and frequency of administration on overall CHO cell-based monoclonal antibody productivity was investigated. The optimal frequency of feed addition determined in the small-scale fed-batch cultures was confirmed in 3L Cellready bioreactors. In addition to growth curves and titer determinations, JMP statistical DOE software was leveraged to more quantitatively analyze and characterize the contribution of feed components, volume, and frequency on overall CHO cell productivity.

RESULTS AND DISCUSSION:
Our results demonstrated that Cellvento CHO-200 media and feed, when used in an optimized upstream process, yielded superior performance when compared to other commercially-available media and feed offerings, and our experimental findings have been made available to process development scientists interested in optimizing their upstream processes.

REF-A019

DEVELOPMENT OF A DROSOPHILA S2 INSECT-CELL BASED PLACENTAL MALARIA VACCINE PRODUCTION PROCESS

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KEY WORDS:
MALARIA VACCINE / NON-VIRAL INSECT / DROSOPHILA

BACKGROUND AND NOVELTY:
Malaria during pregnancy is the cause of 1500 neonatal deaths a day. Moreover, 40% of all low weight births are caused by pregnancy associated malaria. Researchers at Copenhagen University have identified the VAR2CSA protein as a potential protective recombinant placental malaria vaccine. ExpreS2ion Biotechnologies is responsible for developing the protein production process based on VAR2CSA. This talk will focus on the technology and process development aspects of developing a high-yielding, cost effective, phase I clinical production process based on the ExpreS2 insect cell expression system.

EXPERIMENTAL APPROACH:
This is a novel, non-viral, insect-cell based expression technology applied to the development of a critically needed vaccine. The VAR2CSA protein which the vaccine is based on is hard to express and comparison studies between insect, bacteria and yeast have shown that an insect cell system is the only one leading to a clinically useful immune response. Process optimization is also critically important as the cost of manufacture must be as low as possible to allow the vaccine to be used in the countries where it is most needed.

RESULTS AND DISCUSSION:
S2 cells have proved to be very effective for the production of a broad variety of protein classes, such as viral proteins, toxins, membrane proteins, virus like particles (VLPs), and enzymes. Insect cell-based expression platforms (S2 cells and Baculovirus Expression Vector Systems – BEVS) have now been established as versatile and robust vaccine manufacturing platforms. An efficient manufacturing platform such as the Drosophila S2 cell-based ExpreS2 platform, which is robust and easily scalable, may become critical in enabling the development of some types of vaccines. In addition to the cell line itself; several other components are required to constitute a high performing, recombinant protein production platform.

BIBLIOGRAPHY, ACKNOWLEDGEMENTS:
Charlotte Dyring, MSc PhD, CEO, Founder, ExpreS2ion Biotechnologies ApS, Denmark.

Dr. Dyring (Danish) graduated in Chemical Engineering from the Technical University of Copenhagen (DTU) in 1992 and subsequently received a doctoral degree in combination with an industrial research degree from DTU and the Danish Academy of Technical Sciences, respectively. Dr. Dyring has been working in several scientific positions in the pharmaceutical and the biotechnology industry at Kabi Pharmacia (later Pharmacia & Upjohn) and Pharmexa A/S (Affitech A/S) since 1995. Dr. Dyring has an extensive track record in the field of protein expression in animal cells, mastering a wide array of expression systems, tools and techniques, and she has substantial practical experience with upstream process development according to industry standards, including the process transfer to cGMP manufacturing. Dr. Dyring is a recognized world leading expert of the Drosophila S2 expression technology and was instrumental in the development of the technology to the current level of sophistication and robustness.

REF-A020

ASSESSMENT OF PROCESS PERFORMANCE AND PRODUCT QUALITY IN HIGH PERFORMING FED-BATCH CULTURES

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KEY WORDS:
PRODUCT QUALITY / FED-BATCH / CHO CELLS / IGG PRODUCTION

BACKGROUND AND NOVELTY:
Higher yields, more potent compounds, smaller batch sizes and the cost pressure on R&D budgets push the production of biopharmaceuticals towards single-use bioreactors. Simple cultivation systems can be an attractive platform for the production of e.g. monoclonal antibodies by high cell density fed-batch processes. We have developed a high cell density fed-batch process based on an IgG1 producing CHO cell line and the ActiCHO Media System platform in stirred tank bioreactors as well as in WAVE Bioreactor system. Furthermore, the product quality for key product quality attributes, i.e. glycan distribution, molecular size distribution and charge heterogeneity was analyzed.

EXPERIMENTAL APPROACH:
The development of this high cell density fed-batch process was first established in conventional stainless steel bioreactors up to 100-L cultivation volume. The process yielded maximum viable cell densities in the range of 20 MVC/ml and product titers in the range of 5 g/L. The process was then transferred to a WAVE Bioreactor system and the process performance was evaluated in 5-L, 10-L, 25-L and 100-L scale. The glycan distribution, molecular size distribution and charge heterogeneity was analyzed using LC-MS, SEC and IEX chromatography.

RESULTS AND DISCUSSION:
The process performed comparable across scales and bioreactor systems with respect to viable cell density profiles, viability, metabolite profiles and titers.

In conclusion, WAVE Bioreactors are a functional alternative for the production of monoclonal antibodies in high cell density cell culture processes.

REF-A021

AUTOMATION OF TRANSIENT PROTEIN EXPRESSION IN MAMMALIAN CELLS

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KEY WORDS:
MONOCLONAL ANTIBODY / GLYCOSYLATION / INHIBITOR

BACKGROUND AND NOVELTY:
The availability of sufficient amounts of proteins, like therapeutic monoclonal antibodies or Fc-fusion proteins, is a prerequisite for their further characterization and optimization within biopharmaceutical research and development. An automated platform for the transient expression of proteins in a mammalian cell line has been established successfully in order to increase throughput and reduce development time. In addition, documentation and traceability is improved.

EXPERIMENTAL APPROACH:
The concept of the automation for the transient expression of proteins is based on an automated platform combining the transfection of a mammalian cell line with a feeding and analysis tool (viable cell density/ml, cell viability, productivity mg/L) and a unique harvesting step of expressed proteins via centrifugation and filtration for downstream purification and further characterization. The cell line used for transient expression is a human embryonic kidney cell line (HEK293-6E) grown in suspension, using 35 ml tube spins with vented lids. The automation concept was developed in cooperation with the company Synchron Lab Automation & Engineering, Netherlands (NL). All main process parts, like transfection, feeding, analysis and harvesting, are programmed as distinct methods and can be selected individually.

RESULTS AND DISCUSSION:
The automated transient expression of proteins is in good correlation to manually performed transient transfections in terms of quality and quantity using HEK293-6E as host cell line. The workstation is able to provide 192 x 35 ml transient transfections in one campaign yielding 250 to 400 mg/L of secreted proteins. In addition to reproducibility and robustness, the incidence of human errors can be reduced. The automation platform contains an integrated data management system connected to our Biologic Data Platform (BDP), thus enabling the documentation and traceability of a large number of different projects at different stages of research and development.

REF-A022

EVALUATION OF THE ADVANCED MICRO-SCALE BIOREACTOR (AMBRTM) AS A HIGHTHROUGHPUT TOOL FOR CULTURE PROCESS DEVELOPMENT

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KEY WORDS:
MICRO-BIOREACTORS / HIGHTHROUGHPUT / CELL CULTURE / PROCESS DEVELOPMENT

BACKGROUND AND NOVELTY:
Bio-pharmaceutical industries face an increasing demand to accelerate process development and reduce its costs. This challenge necessitates highthroughput tools to replace the traditional combination of shake flasks and small-scale stirred tank bioreactor. Here we evaluated and implemented the advanced micro-scale bioreactor (ambrTM) system that has the capabilities for automated sampling, feed addition and pH, dissolved oxygen, gassing, agitation and temperature controls.

EXPERIMENTAL APPROACH:
We evaluated parameters including overall system performance (cell growth and viability, production titer and product quality), reproducibility, comparison to traditional stirred tank bioreactor and tightness of pH dissolved oxygen and temperature control.

RESULTS AND DISCUSSION:
The direct comparison of the ambrTM system with stirred tank bioreactor on the same fed batch process allowed us to fully evaluate the capabilities of this high-throughput system. Based on these results, we are confident to use the ambrTM system to accelerate early stage process development and limit its costs.

DIFFERENTIAL AFFECTS OF LOW GLUCOSE ON THE MACROHETEROGENEITY AND MICROHETEROGENEITY OF GLYCOSYLATION IN CHO-EG2 CAMELID MONOCLONAL ANTIBODIES

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KEY WORDS:
MONOCLONAL ANTIBODY / GLYCOSYLATION / GLUCOSE CONCENTRATION

BACKGROUND AND NOVELTY:
The demand for high yield recombinant protein production systems has focused industry on culture media and feed strategies that optimize productivity, yet maintain product quality attributes such as glycosylation. Minimizing media components such as glucose and glutamine, reduces the production of lactate and ammonia, but may also affect glycosylation. The first steps in the glycosylation pathway involve the synthesis of lipid-linked oligosaccharides (LLOs) via addition of sugars through nucleotide sugar donors. Glycan macroheterogeneity is introduced by variation in site-specific glycosylation with the transfer of the oligosaccharide to the protein. Further modification of the oligosaccharide can occur through processing reactions, where some sugars are removed and additional sugars added through nucleotide sugar donors. This produces microheterogeneity of the glycan pool. Both macroheterogeneity and microheterogeneity may be affected by the availability of precursors.

EXPERIMENTAL APPROACH:
The objective of this study was to determine how variable concentrations of glucose affect the glycosylation patterns of a camelid monoclonal antibody (Mab) produced in CHO cells and to further evaluate their effect on components of the N-glycosylation pathway, such as nucleotide sugars and LLOs.

RESULTS AND DISCUSSION:
Glucose starvation resulted in a reduction in the amount of full length LLO (GlcNac2Man9Glc3), with a concomitant increase in the production of smaller mannosyl-glycans (GlcNAc2Man2-5). Changes in macroheterogeneity of glycosylation were evident by the appearance of a lower molecular weight protein band identified by mass spectrometry as a non-glycosylated species. Overall N-glycosylation was reduced from 100% to 45% in cells subjected to 24 hours glucose starvation. Glucose deprivation also led to changes in microheterogeneity with a decrease in galactosylation and sialylation.

ENGINEERING CHARACTERISATION OF A SINGLE-USE BIOREACTOR AND THE BIOLOGICAL RESPONSE OF CHO CELLS TO THEIR HYDRODYNAMIC ENVIRONMENT

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KEY WORDS:
SINGLE-USE / PARTICLE IMAGE VELOCIMETRY / CHO CELLS / HYDRODYNAMICS

BACKGROUND AND NOVELTY:
The need to provide cells with reduced hydrodynamic stresses during the upstream processing of mammalian cell cultures, has fostered the production of a number of single-use bioreactors (SUBs) that exhibit novel mixing regimes. With such disparate mixing environments between SUBs currently on the market, the traditional scale-up procedures applied to stirred tank reactors (STRs) are not adequate. The aim of this work is to conduct a fundamental investigation into the hydrodynamics of a single-use bioreactor at laboratory scale to understand its impact upon the growth, metabolic activity and protein productivity of an antibody-producing mammalian cell culture.

EXPERIMENTAL APPROACH:
The SUB to be investigated is the 3L CellReady STR (Merck Millipore) which consists of an upward-pumping marine scoping impeller. This work presents a study characterising the macro-mixing, fluid flow pattern, energy dissipation rates, and shear stresses within the CellReady carried out using 2-dimensional Particle Image Velocimetry (PIV), along with a biological study into the impact of these fluid dynamic characteristics on mammalian cell culture performance and behaviour. The impeller speed and working volume are used to vary the hydrodynamic environment.

RESULTS AND DISCUSSION:
Disparity in cellular growth and viability between the different fluid dynamic environments was not substantial, although a significant reduction in cell specific productivity was found at the most stressful hydrodynamic condition tested. Cells grown at these conditions also displayed net lactate consumption, without a reduction in glucose uptake. A possible reason for these observations is discussed. Given the shifts seen in metabolic behaviour and cell specific productivity, it can be concluded that the fluid dynamic environment will impact upon cellular behaviour. Therefore determining the critical hydrodynamic parameters within the different flow regimes found in SUBs, will enable greater cross-compatibility and scalability across the range of SUBs.

REF-A025

INCLUSION OF MONOCLONAL ANTIBODY CRITICAL QUALITY ATTRIBUTES AS CRITERIA FOR CHEMICALLY-DEFINED CELL CULTURE MEDIA FORMULATION DEVELOPMENT

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KEY WORDS:
CIRITICAL QUALITY ATTRIBUTES / CHEMICALLY-DEFINED MEDIA / CHO / DESIGN OF EXPERIMENTS / GLYCOSYLATION

BACKGROUND AND NOVELTY:
As the biosimilar market emerges and regulation of these therapeutics is considered, the characterization and maintenance of monoclonal antibody critical quality attributes (CQAs) in particular, is drawing scrutiny. Although much effort has focused on understanding the effects of process changes on efficacy-modulating CQAs, cell culture media components can also greatly influence these antibody attributes.

EXPERIMENTAL APPROACH:
Through a series of designed studies we demonstrate the effects of common media components on antibody CQAs. In addition, we demonstrate consistency of these attributes between multiple distinct production lots of CellventoTM CHO-200 dry powder media and feeds, recently developed by Merck Millipore for CHO-S fed-batch processes. Antibody aggregation, charge heterogeneity and Gal(0-2) glycosylation patterns were evaluated, as well as host cell DNA and protein levels. JMP-based data analysis aided the de-coupling of confounding CQA-impacting factors such as viability and day-of-harvest from the effects of media components, including buffer system, on these characteristics.

RESULTS AND DISCUSSION:
The results of this study demonstrate that CQAs are indeed influenced by media formulation modifications and our observations provide the basis for including formulation adjustment as a legitimate area of focus for CQA optimization. In addition to commonly monitored experimental outputs, such as cellular growth and antibody productivity, we include CQAs parameters as criteria for formulation decisions and inputs into predicative formulation modeling. Moreover, we demonstrate the reproducibility of these biotherapeutic characteristics afforded by Merck Millipore's high quality raw materials and manufacturing processes.

REF-A026

POWERFUL EXPRESSION IN CHINESE HAMSTER OVARY CELLS USING BACTERIAL ARTIFICIAL CHROMOSOMES: FINE-TUNING OF PARAMETERS INFLUENCING PRODUCTIVITY

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KEY WORDS:
BIOTECHNOLOGY / CELL CULTURE / CHO / BACTERIAL ARTIFICIAL CHROMOSOME

BACKGROUND AND NOVELTY:
CHO (Chinese Hamster Ovary) cells are the cell line of choice for recombinant protein production and widely used for the production of biopharmaceuticals. Despite the achieved volumetric titers have increased more than 100-fold over the past two decades the establishment of well-producing cell lines remains difficult and is not always successful. Different parameters like the host cell line, the genetic construct, the cell culture medium as well as the applied cultivation strategy are the main factors considered to influence productivity. Recently, Bacterial Artificial Chromosomes (BACs) harbouring the Rosa26 locus showed promising improvements concerning transcriptional efficiency when used as shuttle vector for transgene delivery.

EXPERIMENTAL APPROACH:
In this work a stable and reproducible protocol to generate well-performing cell lines by the use of the BAC expression system in a defined host cell line was established. In a second step the optimal cultivation strategy was evaluated by testing different expression media. The model proteins in this study are IgG1 antibodies and the HIV-1 gp140 envelope protein. The choice of these model proteins provides highly complex molecules with already existing scientific data.

RESULTS AND DISCUSSION:
CHO-K1, -S, -DG44, -DUKX-B11 host cells were compared in different cell culture media. After these preliminary experiments CHO-K1 was chosen for establishment of optimized BAC transfection protocols. Subsequently, stable recombinant cell lines were generated and various process conditions and media compositions are evaluated according to growth rates, maximum cell concentrations and volumetric product titers in batch and fed-batch experiments.

BIBLIOGRAPHY, ACKNOWLEDGEMENTS:
BAC constructs were kindly provided by Dr. Emilio Casanova (Ludwig Boltzmann Institute for Cancer Research, Vienna)

OTHER INFORMATION
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THE EFFECT OF LOW GLUCOSE AND GLUTAMINE CONCENTRATIONS ON THE GLYCOSYLATION PROFILE OF CAMELID MONOCLONAL ANTIBODY EG2 PRODUCED IN CHO CULTURES

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KEY WORDS:
MONOCLONAL ANTIBODY / GLYCOSYLATION / GLUCOSE / GLUTAMINE

BACKGROUND AND NOVELTY:
Biological systems are capable of reacting to minimal changes in the environment. These changes can perturb normal cell function and metabolism, which in turn may affect pathways involved in glycosylation. Thus, different strategies in media and feed development have been implemented not only to maximize production rates from available nutrients and minimal byproduct formation, but also to improve product quality. The objective of this study was to determine whether low concentrations of glucose and glutamine had an effect on the glycosylation of a human-camelid monoclonal antibody (CHO-EG2) synthesized during batch culture.

EXPERIMENTAL APPROACH:
For this purpose, cells were cultured for 18 hours in a starvation media and then were split in different shaker flasks with a matrix of various ratios of glucose (3mM to 25mM) and glutamine (0mM to 4mM). Cell growth, substrate consumption, by-product formation and antibody concentration were measured. Glycosylation was analyzed using hydrophillic interaction liquid chromatography (HILIC).

RESULTS AND DISCUSSION:
The chosen range of glutamine did not have an effect on cell growth. However, in the absence of glutamine and at reduced glucose concentrations there was a decrease in cell growth, which in turn affected product formation. Using HILIC we showed that Mabs produced under glutamine deprivation combined with low levels of glucose contained a higher percentage of truncated oligosaccharides, with a reduced sialylation compared to the Mabs produced under normal conditions (4mM Gln, 25mM Glc). However, the galactosylation index was not changed. Isolated Mabs produced under low glucose conditions produced two bands by SDS-PAGE chromatography. The appearance of a lower molecular weight band was confirmed to correspond to a non-glycosylated Mab by mass spectrometry analysis, indicating a change in the macroheterogeneity.

ENHANCED INSIGHT INTO THE CAP AND CAP-T EXPRESSION SYSTEMS AND THEIR PROPERTIES

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KEY WORDS:
CAP / CAP-T CELLS / RECOMBINANT PROTEIN PRODUCTION

BACKGROUND AND NOVELTY:
The CAP cell line has recently been established from primary human amniocytes by immortalization as a novel cell line for recombinant protein production. Complementary to this one clone of the cell line was stably transfected to express the SV40 Large T Antigen gene. This gave rise to the CAP-T cell line claimed to enable enhanced transient gene expression in combination with expression vectors carrying the SV40 Origin of Replication. We tested both the CAP and the CAP-T cell lines in the context of protein production for research purposes and explored a variety of transfection process parameters aiming at increased protein titers.

EXPERIMENTAL APPROACH:
For transient transfection of CAP-T cells we investigated the influence of medium, transfection reagent, starting cell density and addition of known expression enhancers to the growth medium. After developing a robust protocol for polyethyleneimine (PEI)-mediated transient transfection at high cell density, this protocol has successfully been applied to the generation of antigens and selection of antibody candidates on small to medium scale. In addition we evaluated the potential of CAP cells by establishing probably episomally stable and stably integrated, MTX amplified cell pools by nucleofection and selection.

RESULTS AND DISCUSSION:
Our comparative results which will be presented show that the CAP/CAP-T cell system is an attractive alternative to the repertoire of existing host cell lines such as HEK293 and CHO cells for transient and stable recombinant protein production in research.

REF-A029

ANTIVIRAL ACTIVITY AGAINST INFLUENZA, MEASLES AND PICORNAVIRUS OBSERVED IN THE HEMOLYMPH OF PODALIA SP (LEPIDOPTERA: MEGALOPYGIDAE)

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KEY WORDS:
ANTIVIRAL / LEPIDOPTERA / INFLUENZA / MEASLES / BIOPROSPECTION

BACKGROUND AND NOVELTY:
The control of human viruses is of high interest in human and animal health. Despite the frequent appearance of drug resistant viruses, the development of new antiviral agents is needed to support medicine. Several works have demonstrated the presence of bioactive peptides and their potential use as therapeutic agents in insect hemolymph. However, relatively little data are available on molecules from insects with antiviral activities.

EXPERIMENTAL APPROACH:
In this study, the effects of supplementation of infected culture with hemolymph from larvae of Podalia sp (Lepidoptera: Megalopygidae) were investigated. Cytotoxicity and genotoxicity was evaluated, and no adverse effects were observed in culture after hemolymph addition (up to 5%). The effect of hemolymph on virus growth was measured on confluent monolayers of infected cells with measles virus, influenza virus (H1N1) (enveloped virus) and picornavirus (non enveloped virus). The cultures were observed daily for evidence of cytopathic effect. The analyses of the viral titer demonstrated that the addition of 1% of Megalopygidae hemolymph decreased significantly (p=0.002) the virus titer. The antiviral protein responsible for this activity was isolated and purified by gel filtration chromatography using a gel filtration column system (Superdex 75) and further fractionated using a Resource-Q ion exchange column system.

RESULTS AND DISCUSSION:
Experiments with the purified protein led to a 32-fold reduction in influenza virus production, 64-fold reduction in measles virus production and a 256-fold reduction in picornavirus production. Heating and freezing seem to have no influence over its antiviral activity. The protein does not display virucidal activity and does not act on receptors on the cell membrane. The observations suggest an intracellular mechanism of action where the protein may act as a constitutive agent that affects the innate antiviral immune response.

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REF-A030

OPTIMIZED FERMENTATION CONDITIONS FOR IMPROVED ANTIBODY YIELD IN HYBRIDOMA CELLS

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KEY WORDS:
HYBRIDOMA CELLS / DESIGN OF EXPERIMENT / FERMENTATION / HUMAN INSULIN-LIKE GROWTH FACTOR / MEDIA OPTIMIZATION

BACKGROUND AND NOVELTY:
Ever since new suspension cell lines as adapted HEK293, CHO or Per.C6 have captured the biopharmaceutical production market, traditionally antibody producing cells like Hybridoma cells sank into oblivion. However, they are still of particular interest in academic and industrial diagnostic research for fast and sufficient antibody production needed as proof of concept, for toxicology and in vivo studies. Although, Hybridoma cultivation in fetal bovine serum (FBS) containing animal derived ingredients, like contaminating IgG, is undesirable and leads to difficulties in purification. When reducing the serum to a minimum other key components of the FBS have to be replaced. Therefore, human insulin-like growth factor (IGF) and the surfactant Pluronic F68 were supplemented to improve overall cell performance and to reduce shear forces during shaking respectively employing Design of Experiment (DoE).

EXPERIMENTAL APPROACH:
DoE was used to lower FBS concentration, combined with supplementation of IGF and Pluronic F68. Cells were cultivated for five days in shaker flask. Cell concentration and viability were quantified every day and were defined as response factors for DoE analysis. The response factors were used from exponential growth phase and analyzed with the DoE software Modde, Umetrics. Cultures grew with optimized conditions were used as inoculum for subsequent bioreactor fermentations.

RESULTS AND DISCUSSION:
Reduction of FBS without supplementation resulted in decreased viability and cell concentration. However, FBS can be decreased from 10% to 6% by adding 100 µg/L human IGF and 0.2 g/L Pluronic F68. Compared to the original basal medium an improvement in cell growth and viability was achieved. For entirely serum-free Hybridoma culture further critical ingredients like transferrin and albumin have to be replaced. However, serum-free media leads to higher production costs and can result in a reduction in antibody yield. Nevertheless DoE is a powerful and effective tool saving time in process optimization.

REF-A031

EFFICIENT CLONING OF SINGLE CHO-S CELLS USING A NOVEL ANIMAL COMPONENT-FREE CULTURE MEDIUM SUPPLEMENT

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KEY WORDS:
CHO CELLS / BIOPROCESSING / MONOCLONAL CELL LINES / CLONING EFFICIENCY / MONOCLONAL ANTIBODIES

BACKGROUND AND NOVELTY:
Over the past decade, the number of biotherapeutic drugs produced in Chinese hamster ovary (CHO) cells has increased dramatically. To achieve consistently high expression of a protein product, monoclonal cultures of transfected CHO cells are generated by single-cell cloning. Efficient expansion of single CHO cells typically requires the use of medium containing fetal bovine serum (FBS) or alternatively, use of conditioned medium or co-culture with feeder cells. However, these systems are not defined, and batch variation and risk of contamination from adventitious agents make use of FBS undesirable. To address these issues, we have developed a defined, animal component-free (ACF) culture supplement containing only recombinant proteins and synthetic components that significantly increases CHO cell cloning efficiency when added to protein-free media.

EXPERIMENTAL APPROACH:
The cloning efficiency of CHO-S cells plated at limiting dilution in 96-well plates (average 1 cell/well) was compared in different commercially available, protein-free culture media supplemented with L-glutamine with or without ACF supplement. Cells grown in Dulbecco's Modified Eagle Medium (DMEM) plus 10% FBS were used as a positive control. Cultures were incubated for 10-14 days and screened to identify wells containing >100 cells/well.

RESULTS AND DISCUSSION:
Growth of CHO-S cells in DMEM plus 10% FBS resulted in ~50% of wells containing >100 cells. No significant cell growth was observed in any of the tested media in the absence of serum. The addition of ACF CHO supplement rescued cell proliferation in all four media without serum, resulting in cloning efficiencies that ranged from 50-100% of that observed in DMEM plus 10% FBS. These results show that a new ACF CHO supplement supports high cloning efficiencies for single CHO-S cells cultured in commonly used, commercially available, protein-free media. This completely defined ACF culture system should increase the reproducibility and productivity of biological drug manufacturing.

REF-A032

OBTAINING OF A PROPRIETARY CHO CELL LINE, EVALUATION OF GLUTAMINE SYNTHETASE GENE KNOCK-OUT AND PRODUCTION OF ANTI-RABIES MAB USING AUTOMATED ANALYSIS AND AMBR MINI BIOREACTORS

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KEY WORDS:
CHO CELL LINE / AUTOMATION / SCALE-UP / AMBR / KNOCK-OUT

BACKGROUND AND NOVELTY:
Sanofi developed an in-house expression platform incorporating a fully documented CHO cell. First a CHO bank was thawed in a serum free medium then the cell line was adapted to grow individually in suspension in a chemically defined medium. The Premaster Cell Bank generated was extensively tested against contaminating agents.

EXPERIMENTAL APPROACH:
The selection of producers was based on the glutamine synthetase enzyme produced at a basal level by the CHO cell. This basal expression level was inhibited by the addition of Methionine Sulfo Ximine (MSX) in the selection medium. The gene of this essential enzyme was brought with the gene of interest in order to select the highest producers. However, this chemical selection may have an impact on cell growth and is not absolute.

The knock-out (KO) of the glutamin synthetase gene (GS) was performed on the Sanofi cell line. In the absence of an endogenous GS gene, no selection escape is possible and as MSX is not required, cell growth can be improved. In this experiment, we evaluated the benefit of KO GS clones combined with the need to produce SO57 (an anti-rabies mAb).

After transfection of both the parental and KO GS CHO cell lines, a selection was performed on 96 well plates with an automated Homogeneous Time Resolved Fluorescence HTRF assay. The highest producers were amplified and banked. In order to choose the cell line and the process for the production of the mAb, both were jointly tested in an Ambr mini-bioreactor automate.

The knocking out of the GS gene allowed enrichment with high producers of the transfected pool. The evaluation in the Ambr platform led to the selection of an improved couple cell line / process in one step.

RESULTS AND DISCUSSION:
These results were applied on a 30 L single use bioreactor resulting in a harvesting of 30 g of antibody. The new expression platform coupled with the automation of the analytical and process tools reduced the time from transfection to batch production by up to 3 months.

REF-A033

PROCESS DEVELOPMENT STRATEGIES TO ENABLE LARGE SCALE EXPANSION OF MESENCHYMAL STEM CELLS FOR CELLULAR THERAPY

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KEY WORDS:
MESENCHYMAL STEM CELLS / CELL THERPAY / PROCESS DEVELOPMENT / MICROCARRIER / SERUM-FREE MEDIA

BACKGROUND AND NOVELTY:
The potential demand for clinical and commercial scale human mesenchymal stem cells (MSC) for cellular therapies requires a large-scale and well characterized culture system for MSC production. Currently, the majority of processes to produce MSCs rely on 2-dimensional, planar technologies that are expensive, labor intensive, and limited in scale potential. Strategies for process development have been used successfully in other industrial therapeutic markets such as the monoclonal antibody and vaccine industries to increase product yield. Many of these approaches such as Design of Experiment-based optimization (DOE) strategies can be used to develop cell culture media, reagents, and scale-up methods that may make cost effective and efficient manufacturing processes possible for cell therapy-relevant cells.

EXPERIMENTAL APPROACH:
Here we report the results of DOE-based optimization studies to develop a microcarrier-based expansion system for human MSCs. Spinner flask studies demonstrated the ability of a xeno-free system to support expansion of MSC from bone marrow (BM MSC) and adipose tissue (ADSC) while maintaining the expected phenotype and differentiation potential. After 14 days of culture, BM MSC reached a maximum cell density of 200,000 cells/ml (fold-increase of 18) while ADSC expanded to 140,000 cells/ml (fold-increase of 14). Medium and process optimization strategies and the incorporation of fed-batch and perfusion approaches were used to increase the efficiency of the system. Human MSCs were expanded to a cell density of greater than 500,000 cells/ml in DASGIP bench-top bioreactors. The cells maintained tri-lineage differentiation potential and retained the MSC immunophenotypic profile.

RESULTS AND DISCUSSION:
This work demonstrates the ability of a serum-free and xeno-free medium to support large-scale expansion of human MSC. This system can produce large numbers of high quality MSC, representing an efficient alternative to the traditional cell expansion protocol for clinical-scale manufacture of MSC.

REF-A034

SCALABILITY STUDIES OF THE UPSTREAM MANUFACTURING PROCESS DURING THE DEVELOPMENT OF ANTIBODY MIXTURES

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KEY WORDS:
ANTIBODY / MIXTURES / CELL CULTURE / SCALABILITY

BACKGROUND AND NOVELTY:
The synergistic effects of combinations of antibodies reported in particular for cancer treatment demonstrate that antibody mixtures represent a promising new class of therapeutics. Therefore, recombinant antibody combinations or mixtures are receiving more and more attention as a strategy to improve therapeutic efficacy.

Symphogen is using the SympressTM platform process for single-batch manufacture of antibody mixtures. Using this manufacturing platform, it is possible to produce several antibodies in a mixture with suitable quality characteristics and high batch-to-batch consistency.

EXPERIMENTAL APPROACH:
During the development of antibody mixture products, it is important to have scalable models of the upstream process that can be used as a tool for efficient early development as well as be used as an important predictive tool for later-stage investigations of the process.

RESULTS AND DISCUSSION:
We here present data from several upstream models that we have developed for manufacturing of antibody mixtures ranging from deep-well plates run in a robotized format through micro-bioreactors to traditional shakers and bioreactors. The combination of high-throughput analytical tools and upstream models at several scales has allowed us to systematically develop and refine the Sympress platform technology so it delivers robust high-quality antibody mixtures.

STEM CELLS CULTURE ON MICROCARRIERS INSIDE SHAKE FLASKS: 2 - KINETIC STUDY AND COMPARISON WITH CULTURE IN SPINNER FLASKS

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KEY WORDS:
STEM CELLS / SHAKING FLASK / KINETICS

BACKGROUND AND NOVELTY:
Mesenchymal stem cells (MSC) are promising tools for tissue engineering and will be required in sufficient amounts. However, the choice of bioreactor technology as well as aeration and agitation conditions is still challenging for their mass expansion. Spinner flasks were already proved to be suitable for MSC culture on microcarriers. While orbitally shaken bioreactors were used for continuous cell lines and present some advantages such as reduced cost, sufficient oxygen transfer capacity and good scale-up ability, they have not been yet validated for MSCs culture. In this work, kinetics of MSCs cultures were studied in orbitally shaken flasks and compared with results obtained in spinner flasks.

EXPERIMENTAL APPROACH:
Porcine MSC were seeded at 0.3 x 10^5 cells/mL on 1.2 g/L Cytodex 1 microcarriers in -MEM supplemented with 10 % SVF and FGF2. Shake flasks (250 and 500 mL) equipped with O2 and pH optical sensors (Presens), were placed into an incubator (Kühner). Concentration of cells, glucose, glutamine, lactate and ammonium ions was daily determined. Cell multipotency was assayed by using differentiation kits. Additional cultures were performed in 250 mL spinner flasks. Two working volumes were used for each culture system and medium was renewed every 1 or 2 days.

RESULTS AND DISCUSSION:
Whatever operating parameters, MSC growth, nutrient consumption and metabolite production were observed to be very similar in shake and spinner flasks, leading to a 40-fold increase of cell density after 10 days. Yet, cell aggregates formed quicker and reached a larger size in spinner flask as revealed by microscopic observations. Despite smaller mean power dissipations estimated in spinner flask, shake flask offered a non-turbulent flow and a more homogeneous distribution of hydromechanical stresses, which seemed favourable to MSC growth. Moreover, the oxygen profiles observed in shake flasks revealed that low pO2 conditions could be obtained by a judicious choice of the shaking frequency and medium volume.

STEM CELLS CULTURE ON MICROCARRIERS IN SHAKING FLASKS : 1 - EXPERIMENTAL DETERMINATION OF MINIMAL SHAKING FREQUENCY AND DIMENSIONAL ANALYSIS TO GET MICROCARRIERS INTO SUSPENSION

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KEY WORDS:
SHAKE FLASK / ORBITAL SHAKING / MICROCARRIER / DIMENSIONNAL ANALYSIS / MAMMALIAN CELL CULTURE

BACKGROUND AND NOVELTY:
Shake flasks are widely used as lab-scale bioreactors for culturing mammalian cells, these systems being able to support culture processes in terms of mixing and hydro-mechanical forces characteristics [1, 2, 3]. For adherent cell lines cultured on microcarriers, such as mesenchymal stem cells, defining the impact of the shaking frequency on the process has to include the actual setting up of the microcarriers into suspension. While correlations are available in literature to predict the minimum agitation speed, Nc (1/s), necessary to get microcarriers in suspension in mechanically agitated bioreactors [4], such correlation does not yet exist for orbital shaking culture systems. Our study offers to fill this gap by using a rigorous dimensional analysis of physics.

EXPERIMENTAL APPROACH:
Experimental measurements of Nc have been made by observing shake flasks on an orbital shaking platform (Kuhner) and visually checking the suspension state of stained Cytodex 1 carriers within the flasks. About 200 various experimental conditions, were tested: shake flask diameter, d, flask filling volume, orbital shaking diameter, d0, liquid viscosity, volume fraction of particles, particle diameter, and particle mass density. The values of these parameters were based on classical adherent-cell culture parameters.

RESULTS AND DISCUSSION:
Regarding the dimensional analysis of the process, two correlations were established to evaluate a critical Froude number, used to determine the resistance of submerged carriers moving through liquid medium. The first one directly used Nc, while the second one was based on an intermediate variable: the critical shaking velocity Vc (m/s), depending of Nc, d0 and d values. The identification of model parameters leads to a final average error less than 5 %. Thus, our results allowed to establish robust correlations that characterize the influence of diverse operating parameters on the minimal shaking frequency (Nc) to get microcarriers into suspension.

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REF-A037

BIOLOGICS DATA PLATFORM FOR TAILORED SUPPORT OF AUTOMATION IN TRANSIENT PROTEIN EXPRESSION AND CELL LINE DEVELOPMENT

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KEY WORDS:
BIOLOGICS DATA PLATFORM / TRANSIENT TRANSFECTION / CELL LINE DEVELOPMENT / AUTOMATION WORKSTATIONS / ENTERPRISE-IT SOLUTION

BACKGROUND AND NOVELTY:
The biologics drug R&D process in pharmaceutical and biotech companies is characterized by division of labor across sites and high-throughput approaches. The integrated management of data from molecules, clones, materials and analytical experiments is a key challenge in this context. We have successfully implemented the Biologics Data Platform (BDP), a novel enterprise-IT solution based on Genedata Biologics software, for tailored support of our screening and protein production processes. Here, we describe the integration of BDP with our automation workstations in transient transfection for mg-scale protein expression and cell line development.

EXPERIMENTAL APPROACH:
We implemented an automated workstation for parallelized transient transfection, feeding, sampling and harvesting of 35 ml-spin tube cultures. We implemented vector batch and expression batch registration processes in BDP to guide the automated workstation and to receive analytics data from the workstation.

Additionally, we implemented an automated cell line development workstation for seeding, selection, incubation, passaging, analyzing, and cryo-conservation of cells. Also here we implemented functionalities in BDP to support the cell line development process.

RESULTS AND DISCUSSION:
We demonstrate the advantages of comprehensive management in one IT system of transient expression batches, cell line clones and fed-batch experiments together with molecule information such as primary sequences and experimental data. The system facilitates correlation analyses which will be discussed with examples.

REF-A038

IMPACT OF MIRNAS ON THE CHO PROTEOME

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KEY WORDS:
CHO / MIRNA / LABEL-FREE LC-MS / BIOINFORMATICS / MIR-7

BACKGROUND AND NOVELTY:
MicroRNAs (miRNAs) are small non-coding RNAs (~22 nucleotides in length) that can post-transcriptionally regulate gene expression through inhibition of protein translation or degradation of target mRNAs. MiRNAs play critical roles in the regulation of biological processes such as growth, apoptosis, productivity and secretion thus representing a potential route toward enhancing desirable characteristics of mammalian cells for biopharmaceutical production. Relatively few studies to date explore the potential of miRNAs as cell line engineering tools for bioprocessing. This is vitally important if they are to be exploited for cell line engineering for process improvements.

EXPERIMENTAL APPROACH:
We have previously found that miR-7 over-expression significantly inhibits the growth of recombinant Chinese hamster ovary (CHO) cells without impacting cellular viability, with an associated increase in normalised productivity. In this study we have carried out a quantitative label-free Liquid Chromatography – Mass Spectrometry (LC-MS) proteomic profiling study of proteins exhibiting altered levels following over-expression of miR-7 to gain insights into the potential mechanisms involved in the observed phenotype.

RESULTS AND DISCUSSION:
In total 93 proteins showing decreased levels and 74 proteins with increased levels following over-expression of miR-7 were found. Pathway analysis suggests that proteins involved in protein translation (e.g. ribosomal proteins), RNA and DNA processing (including histones) are enriched in the list showing decreased expression. Protein folding and secretion proteins were found to be up-regulated following miR-7 over-expression. Bioinformatic analysis with miRWalk (combines the output of 6 selected miRNA target prediction algorithms) was used to evaluate potential direct targets of miR-7. Two genes, stathmin and catalase, overlapped in a number of the predictive target databases for mouse and rat, and are likely to be possible direct targets of miR-7 in CHO cells

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REF-A039

FACILITATING MULTISITE BIOPROCESS TRANSFER: MULTI-INSTRUMENT AND MULTI-PLATFORM COMPARABILITY AND LONG-TERM NOVA BIOMEDICAL'S BIOPROFILE® CHEMISTRY AND GAS ANALYZERS

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KEY WORDS:
PROCESS TRANSFER / COMPARABILITY / ANALYZER / NOVA BIOMEDICAL / PROCESS MONITORING

BACKGROUND AND NOVELTY:
The Biopharmaceutical industry has grown exponentially and more companies are now operating in a global market with sites thousands of miles apart. The need is now even greater for robust bioprocess monitoring solutions that can provide consistent instrument-to-instrument results. The seamless transfer of information across multiple sites relies heavily on the comparability of process data from various technologies, ensuring effective monitoring and control of critical process parameters.

EXPERIMENTAL APPROACH:
This study provides data supporting comparability of the BioProfile® (Nova Biomedical, Waltham, MA) chemistry and gas analyzers across several development and manufacturing sites in the United States. In addition, the long-term performance stability of the BioProfile systems was also tested. Five BioProfile FLEX and four BioProfile 100 Plus analyzers were used to determine linearity, precision, accuracy, and instrument-to-instrument comparability. The age of the instruments used for this study ranged from new to over 8 years old, with several hundred samples to over 20,000 samples run on a given analyzer.

RESULTS AND DISCUSSION:
The results of this study show a high level of comparability between the BioProfile analyzers. In addition, comparability was also demonstrated between both the new and aged analyzers, providing evidence of the long-term robustness and the quality of data that can be generated from the BioProfile analyzers. Nova Biomedical's BioProfile analyzers provide the tools to facilitate multisite bioprocess transfer in the Biopharmaceutical industry.

REF-A040

ON-LINE MONITORING OF ADHERENT CELLS CULTIVATED ON MICROCARRIERS IN PERFUSION REACTOR

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KEY WORDS:
MULTI-FREQUENCY PERMITTIVITY / PERFUSION BIOREACTOR / MICROCARRIERS / VERO CELLS / BIOPROCESS MONITORING

BACKGROUND AND NOVELTY:
Animal cell density is a critical parameter to on-line monitor culture processes. Some industrial processes currently cultivate anchorage-dependent cells on microcarriers (MCs), such as Vero cells for vaccine production. Expansion of stem cells could also require the use of microbeads. Perfusion reactors allow to reach high cell densities which may result in carrier saturation. So, monitoring high densities of viable anchorage-dependent cells by using a capacitance probe remains challenging. Our aim was to deeply consider the effect of Vero cell confluency on MCs on the on-line monitoring of high cell densities by permittivity measurements.

EXPERIMENTAL APPROACH:
Cells were cultivated in a serum-free medium on spherical Cytodex 1 microcarriers inside a 2 L perfusion reactor using a settling retention system and a flow rate of 0.5 L/d. Various microcarrier concentrations (1.5, 3 and 6 g/L) were used. Off-line cell quantification was performed by Crystal violet method, while cell diameter and viability were assessed by Vi-CELLTM system. On-line permittivity was measured by using Fogale Biomass system® over a frequency range of 0.3 to 10 MHz.

RESULTS AND DISCUSSION:
Depending on the operating conditions, various maximal cell concentrations between 2 and 4.5 x 10E6 cells/mL (100 to 500 cells/MC) were reached and maintained during more than 8 days. Results showed a linear correlation between on-line permittivities and off-line volumetric cell densities, even for the highest volumetric cell concentrations. This correlation was not affected by MCs concentration but was no longer linear when cell number per MC exceeded 150. This behavior was attributed to the diameter decrease of Vero cells on microcarrier surface, resulting from high cell density in perfusion reactor. For the first time, our results demonstrate that permittivity sensor can be considered as a reliable tool to monitor high adherent cell densities, provided they do not exceed cell confluency on adhesion surface, and to detect cell confluency occurrence.

REF-A041

BENCHMARKING OF COMMERCIALLY AVAILABLE CHO CELL CULTURE MEDIA FOR ANTIBODY PRODUCTION

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KEY WORDS:
ANTIBODY, MEDIUM, CHO CELLS, RECOMBINANT PROTEIN PRODUCTION

BACKGROUND AND NOVELTY:
Chinese hamster ovary (CHO) cells have become the preferred expression system for recombinant proteins. A key factor for a high yield process is the cultivation medium. We investigated 8 commercially available CHO cell culture media to examine their impact on cell growth, recombinant protein production and cell metabolism.

EXPERIMENTAL APPROACH:
A recombinant CHO DG44 cell line expressing a human IgG1 antibody was cultivated in shake flasks to compare the following media:
- ActiCHO P (GE Healthcare)
- CD Opti-CHOTM (Life Technologies)
- CD Opti-CHOTM (Life Technologies)
- CD Forti-CHOTM (Life Technologies)
- Ex-CellR CD CHO (Sigma Aldrich)
- ProCHOTM 5 (Lonza)
- BalanCDTM CHO Growth A (Irvine Scientific)
- CellventoTM CHO-100 (EMD Millipore)

For ActiCHO two feed strategies, initial spiking or daily addition of supplements were investigated. Also in BalanCD medium one culture was run as fedbatch adding Feed 1 on days 1, 3, 5.

The cultures were sampled daily to determine cell growth, viability, IgG and metabolite concentrations including amino acid analysis.

RESULTS AND DISCUSSION:
Selection of an appropriate medium was crucial for high antibody production. The cell concentration was increased up to threefold from 2.59x106 c/mL to 7.73x106 c/mL. Similarly the final product concentration rose from 384 mg/L to 876 mg/L.

An adequate feeding strategy further boosted cell concentrations to 1.99x107 c/mL and titers to >5 g/L. The specific productivities (qP) were similar for all non-fed cultures. There the qP gradually declined from 50-70 pg/(c*d) (pcd) to 8 pcd during the cultivation. In feed-spiked ActiCHO P a similar trend was observed but the qP was always 30-80% higher. Contrastingly, a daily feed stabilized the qP at 50 pcd for several days.

Spent medium analysis revealed that high concentrations as well as a balanced proportion of amino acids were important for a good productivity. Besides glutamine and glutamate, critical amino acids for this cell line turned out to be tyrosine, asparagine and serine.

REF-A042

WHEN QUALITY COMES FIRST- CHALLENGES FOR MAMMALIAN CELL LINE DEVELOPMENT OF "DIFFICULT TO EXPRESS" PROTEINS

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KEY WORDS:
NGNA / AGGREGRATES / CELL LINE DEVELOPMENT / NOVEL ASSAY / PRODUCT QUALITY

BACKGROUND AND NOVELTY:
Productivity has always been a primary criterion for clone selection during cell line development since it is directly associated with the cost of goods manufactured. While high productivity is desired, product quality issues, such as immunogenic factors, sometimes become unpleasant surprises at the later stage of the development program. Product aggregation and the presence of N-glycolyneuraminic acid (NGNA or Neu5Gc) have been linked to increased product immunogenicity. Addressing these risk factors early can potentially reduce the overall immunogenicity risk profile of the product.

EXPERIMENTAL APPROACH:
Production of BMN-X molecule in CHOK1 cells results in two activity peaks separated by anion exchange chromatography. Size exclusion chromatography detected various HMW forms in the later peak. A simple charge heterogeneity method for the quantification of the HMW forms can be used to rapidly screen clones, production conditions and new designs which favor the formation of the correct form of BMN-X.

CHO cells produce glycosylation patterns that are close to human, however, these cells do express NGNA and this expression seems to be cell type and clone specific. We implemented a 96-well plate based ELISA assay using an antibody specific to NGNA to identify and eliminate clones with high levels of Neu5Gc glycosylation early in development.

RESULTS AND DISCUSSION:
In conclusion, the ability to identify and eliminate clones with high levels of NGNA or a high percentage of HMW forms early in development allowed us to focus only on clones with better quality attributes. Traditionally, these assays were performed with purified protein samples less conducive to high throughput screening methods. Implementing these new screening assays enabled us to assess preliminary product quality with µL to mL quantity of cell culture harvest fluid. In addition, we discuss the impact of cell culture media and culture process on the levels of HMW material and NGNA, and report strategies aiming to reduce these undesired forms.

RAMAN SPECTROSCOPY AS A TOOL FOR THE MONITORING AND CONTROL OF MAMMALIAN CELL CULTURE BIOREACTORS

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KEY WORDS:
RAMAN SPECTROSCOPY / GLUCOSE CONTROL / MODEL PREDICTIVE CONTROL / PAT

BACKGROUND AND NOVELTY:
The philosophy behind the FDA's process analytical technology initiative and the move from quality by inspection to quality by design (QbD) is that identifying, monitoring and controlling the critical process parameters (CPPs) positively impacts the critical quality attributes (CQAs) of a product as a result of better process understanding and control, earlier fault detection and continuous process improvementTo achieve this higher degree of process control, development of online process monitoring is essential. Raman spectroscopy has great potential as an analytical tool for bioprocesses: it is non-invasive, non-destructive, does not require sampling, can quantify multiple analytes simultaneously and provide continuous real-time measurements.

EXPERIMENTAL APPROACH:
Raman spectroscopy was used for the in situ real-time quantitative determination of glucose, glutamine, lactate, ammonia, glutamate, total cell density (TCD) and viable cell density (VCD) in a CHO cell fed-batch process. Chemometric data analysis was used to correlate the spectral data with off-line reference methods. The effect of the number of calibration samples and variation within the calibration sample set was investigated as well as the transferability of the calibration models from a 3 L reactor to 15 L system.

RESULTS AND DISCUSSION:
Calibration models were successfully built using data acquired over the course of three bioprocess runs. The standard error of prediction was 1.82 mM for glucose, 0.44 mM for glutamine, 0.22 mM for glutamate, 0.13 mM for ammonia, 9.77 mM for lactate and 0.43 x 106 cells/ml for TCD. The Raman-determined glucose concentration was used as part of a closed-loop feedback control strategy which maintained glucose at a setpoint. A form of advanced control called model predictive control (MPC) was implemented to successfully maintain glucose at a fixed concentration of 11 mM. A 1.5 fold increase in maximum viable cell density was observed when the MPC-controlled bioprocess was compared with a bolus fed-batch process.

BIBLIOGRAPHY, ACKNOWLEDGEMENTS:
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ENGINEERING CHARACTERISATION OF A ROCKED BAG BIOREACTOR TO EVALUATE KEY EFFECTORS OF SUCCESSFUL MAMMALIAN CELL CULTURE

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KEY WORDS:
ROCKED BAGS / MAMMALIAN CELL CULTURE / ENGINEERING CHARACTERISATION / OXYGEN TRANSFER / CO2 STRIPPING

BACKGROUND AND NOVELTY:
Engineering characterisation is essential for efficient and knowledge-led process development in biomanufacturing. Despite diverse applications of rocked bag bioreactors, there is currently little understanding of the fundamental determinants of fluid mixing and mass transfer, which are important for mammalian cell culture.

EXPERIMENTAL APPROACH:
In this work, a flexible single-use rocked bag bioreactor system has been fully evaluated in terms of volumetric oxygen mass transfer coefficient (kLa), CO2 stripping rate and liquid phase mixing time at 10L and 20L scale. Five inputs were identified as potentially affecting gas transfer and mixing characteristics: rocking rate, rocking angle, fill volume, rocking acceleration and air flow rate. Using these findings, industrially relevant fed-batch GS-CHO cell cultures were then conducted to demonstrate the effects of these parameters on cell growth, productivity and metabolite profile.

RESULTS AND DISCUSSION:
It was found that oxygen transfer could be an issue for mammalian cell culture at the cell densities reached in industrial fed-batch processes. Within sensible operating ranges for cell culture, the oxygen mass transfer coefficient, kLa, was most sensitive to rocking rate and fill volume (5-fold higher at 25rpm compared to 15rpm). Bubble formation and the presence of a dispersed gas phase was observed at moderate rocking rates and was prevalent at high rocking rate or low fill volume. In terms of scale-up, kLa did not change significantly with a doubling in scale. In contrast, the liquid phase mixing time, approximately doubled. CO2 stripping was largely determined by air flow rate at both 10 and 20L scales but was proportionately slower at 20L scale. Cell culture at 10L scale confirms the importance of kLa evaluation with regards to cell growth and antibody production and CO2 stripping in terms of process control, but these results suggest that the relative importance of different engineering parameters will alter upon scale-up.

REF-A045

MULTIDIMENSION CULTIVATION ANALYSIS BY STANDARD AND OMICS METHODS FOR OPTIMIZATION OF THERAPEUTICS PRODUCTION

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KEY WORDS:
CHO / CELL CULTURE MEDIUM / TRANSCRIPTOMICS / METABOLOMICS

BACKGROUND AND NOVELTY:
During the last decades Chinese Hamster Ovary (CHO) cells have been extensively used for research and biotechnological applications. About 40% of newly approved glycosylated protein pharmaceuticals are produced in these cells today [1]. Despite the increasing relevance of CHO cells for biopharmaceutical production little is known about effects of intracellular processes on productivity and product quality.

EXPERIMENTAL APPROACH:
In the last years insulin was more and more replaced by IGF in cell culture media. To compare the intracellular effects of these two supplements an antibody producing CHO cell line was cultivated in batch mode using insulin, IGF-1 or no growth factor. Subsequently, different omics-techniques were applied to analyze medium and cell samples. Metabolome and glycan analysis was performed using a HILIC-ESI-MS and a HPAEC-PAD method, respectively. Furthermore, an in house developed customized cDNA microarray with 41,304 probes based on sequence data from different CHO cell lines was applied for transcriptome analysis.

RESULTS AND DISCUSSION:
Cultivation data illustrated that maximal cell density was higher in cultivations with insulin and IGF-1 compared to those without growth factor. Additionally, glucose consumption and lactate production was slightly higher in cultivations with these supplements. In contrast to that product quantity and product quality was equal in all cultivations. The most abundant glycoforms were G0F and G1F with about 50% and 40%, respectively. Transcriptome data showed that IGF supplementation result in the highest significant transcription change, e. g. on day three of cultivation 3,187 probes in IGF samples and 1,214 probes in insulin samples indicated different transcription levels.

Data on cell growth and productivity as well as omics results were brought together to achieve a deeper insight into cellular processes and their influence on productivity and product quality.

BIBLIOGRAPHY, ACKNOWLEDGEMENTS:
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REF-A046

QUALITY BY DESIGN IN RAW MATERIALS TESTING: CONSIDERATIONS, STRATEGIES AND EXPERIENCE OF A TESTING LABORATORY

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KEY WORDS:
RAW MATERIALS / QBD / QUALITY BY DESIGN / TESTING

BACKGROUND AND NOVELTY:
Testing of raw materials is an essential step in the production cycle of biological therapeutics and vaccines. The implementation of Quality by Design (ICH Q8) in manufacturing processes is required across the pharmaceutical industry to ensure the consistent production of a product to the required level of quality.

EXPERIMENTAL APPROACH:
The mapping of extraneous agents in raw materials, to high resolution, is an essential step in the Quality by Design process, and requires appropriate testing design and the inclusion of molecular techniques capable of detection of known and novel contaminants. Results from the evaluation of novel testing technologies will be presented, highlighting the most appropriate technologies for evaluation of raw materials, and the drawbacks of comparable technologies, specifically for the identification of extraneous viruses.

RESULTS AND DISCUSSION:
Case studies regarding the discovery of several viruses in animal sera and cell lines will be presented, and will be considered in the context of current regulatory recommendation and guidelines for testing. Strategies for routine testing to mitigate risk of extraneous agents in raw materials will also be presented.

REF-A047

A NEW PLATFORM OF MILLI-BIOREACTORS FOR ANIMAL CELL CULTURE

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KEY WORDS:
MILLI-BIOREACTORS / SCALE-DOWN / CELL CULTURE

BACKGROUND AND NOVELTY:
Today, there is an increasing demand for small-scale bioreactors for animal cell culture as useful tool for scale-down systems. By reducing culture medium volumes, more experiments can be managed in parallel, culture costs are reduced, especially for screening or hydrodynamics effects study purpose. Moreover, these systems are especially needed when cell quantities are limiting, for example in the case of stem cells. However, most of the small scale bioreactors such as shaken micro-well plates or shaking flasks do not exhibit a geometric homothety with pilot or production scales. Thus, the hydrodynamic environment may sensibly vary from the lab scale to production scale entailing production variability. To fill this gap, we have designed and built a platform of six 250 mL milli-mechanically stirred bioreactors for animal cell culture with a minimal liquid volume of 50 mL.

EXPERIMENTAL APPROACH:
The milli-bioreactors can be equipped simultaneously with three standard sterilizable probes such as oxygen, pH, CO2 or biomass probes. Marine propellers, Rushton turbines and ear-elephant impellers are available to ensure liquid mixing and mass transfer. A porous sintered cylinder is used to sparge air or pure oxygen bubbles in the liquid. A dedicated software allows the control of the 6 bioreactors independently.

RESULTS AND DISCUSSION:
The design of the milli-bioreactors was based on the scale-down of a standard 2-L bioreactor on the basis of various criteria. First a geometric homothety was imposed so that agitation and aeration criteria could be relevant. The volumetric oxygen transfer coefficient kLa was maintained by keeping constant the power dissipation per unit of volume and the superficial gas velocity. Consequently, averaged and maximal dissipation rate and thus hydrodynamic stresses range are supposed to be similar between the two lab-scale bioreactors. The first kinetics studies performed in the milli-bioreactors have confirmed their ability as scale-down bioreactor for animal cell culture.

REF-A048

APPROACHES TO DEVELOP AND CHARACTERIZE AN IMPROVED COMMERCIAL CHO CELL CULTURE PROCESS WITH NOVEL PROCESSING CONDITIONS TO ACHIEVE COMPARABILITY

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KEY WORDS:
CHO / GLYCOSYLATION / SIALIC ACID / PROCESS CHARACTERIZATION

BACKGROUND AND NOVELTY:
Understanding cell culture parameters that affect protein glycosylation has been a challenging area of investigation for decades. Results continue to be relevant because cell culture engineers must ensure product comparability and demonstrate manufacturing consistency. These goals are especially important when improving commercial processes with previously established specifications for product quality attributes.

EXPERIMENTAL APPROACH:
An improved CHO cell culture process was developed from an existing commercial process. Titer was improved while removing animal-derived raw materials and providing better facility fit for future process transfer. Although most product quality attributes were comparable, a higher than typical sialic acid content was observed. Experiments were conducted to understand which parameters were contributing to the high sialic acid content. Specific conditions were chosen based on knowledge of biosynthetic and degradative glycosylation pathways. After conditions were finalized, the process was transferred to various sites and scales. Small-scale experiments were also conducted to characterize parameter ranges.

RESULTS AND DISCUSSION:
Media components and process parameters were identified that consistently affected sialic acid content while monitoring other product quality attributes, such as deamidation and mannose-phoshate content. Comparable sialic acid content was achieved using low pH conditions that releases and activates sialidase, an endogenous CHO enzyme. At small-scale, multivariate and univariate experiments were combined to design an overall process characterization strategy. Process parameters were chosen using a risk based approach, and test ranges were proposed by stochastic simulations utilizing effect estimates from process development data. Ranges were meant to be sufficiently broad without risking excessive number of results outside established specifications. The new, characterized process has been successfully demonstrated at multiple sites and scales.

REF-A049

NEXT GENERATION HUMAN ALPHA INTERFERONS OBTAINED IN CHO CELLS BY O-GLYCOENGINEERING

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KEY WORDS:
O-GLYCOENGINEERING / INTERFERON / ANIMAL CELLS / GLYCOSYLATION

BACKGROUND AND NOVELTY:
Multiple biological activities of human alpha interferons (hIFN-) including antiviral, antiproliferative and immunomodulating properties have motivated the development of their recombinant forms to be used for treatment of numerous viral and tumor diseases. Unfortunately, one major issue regarding the clinical use of rhIFN- is its short half-life in circulation after injection of patients. For that reason, administration of high and frequent doses of the cytokine is required, leading to many adverse side effects.

Our goal was to exploit the ability of O-glycans to extend the half-lives of proteins to create O-glycosylated rhIFN- variants with lower in vivo clearance and preserved bioactivity.

EXPERIMENTAL APPROACH:
For this purpose, we fused a peptide containing four potential O-glycosylation sites derived from the carboxi-terminal sequence of the hCG -subunit (CTP) to the N-terminal and the C-terminal ends of rhIFN- 2b. CHO-K1 cells were employed as hosts to express CTP-IFN and IFN-CTP for physicochemical and biological characterization.

RESULTS AND DISCUSSION:
For both molecules SDS-PAGE followed by Western blot evidenced the presence of a broad band between 28 and 37 kDa, indicating a great microheterogeneity of glycoforms with higher molecular mass with respect to wild-type IFN. This result was confirmed by isoelectric focusing/Western blot, which clearly showed a great number of isoforms with highly acidic pI due to the incorporation of the CTP and, consequently, of new carbohydrates containing terminal sialic acids. In vitro antiviral specific bioactivity of CTP-IFN and IFN-CTP ranged between 66 and 74%, respectively, compared with that of native rhIFN- 2b, while antiproliferative specific bioactivity remained practically invariable (92 and 112%, respectively).

These preliminary results suggest that O-glycoengineering could be an attractive approach in order to increase charge and mass of IFN with the aim of improving its pharmacokinetic properties while preserving high in vitro bioactivity.

REF-A050

FEEDING STRATEGY OPTIMIZATION IN INTERACTION WITH TARGET SEEDING DENSITY OF A FED-BATCH PROCESS FOR MONOCLONAL ANTIBODY PRODUCTION

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KEY WORDS:
FED-BATCH / QBD / MONOCLONAL ANTIBODY / FEEDING STRATEGY OPTIMIZATION

BACKGROUND AND NOVELTY:
Current trend towards Quality by Design (QbD) leads the process development exercise towards systematic experimentation, rational development, process understanding, characterization and control. In this poster, an example of the application of QbD approach is given to enhance monoclonal antibody (mAb) titer and to ensure quality consistency of the product.

EXPERIMENTAL APPROACH:
A fed-batch process in 2L scale was run with different daily fixed volume feed additions. In this experiment, a correlation between feeding strategy and specific mAb productivity was observed. A custom Design of Experiment (DoE) with a statistical software was then performed on feeding strategy in growth phase, feeding strategy in production phase and day of change of feeding strategy. DoE allowed to reduce the number of required experiments to a reasonable number while maintaining statistically significant results. A second custom DoE was performed to further optimize the feed strategy and study the impact of an additional parameter the target seeding density (TSD).

RESULTS AND DISCUSSION:
In the process runs performed with different daily fixed volume feed additions, it was observed that a significant decrease in the specific mAb productivity occurred if the feed ratio per the projection of a subset of process performance attributes was below a specific threshold. A feed addition strategy based on this projected subset of process performance attributes was therefore developed. The feed ratio and the feed volume before day of changing strategy were two parameters significantly impacting mAb titer at harvest. It was shown that the higher they were, the higher was the titer. Thus it was decided to investigate the impact of greater feed ratio in interaction with the TSD. Feed ratio and the interaction feed ratio*TSD had a significant impact on mAb titer at harvest. The higher they were, the higher was the titer. ANOVA analysis showed that a 36% increase in the mAb titer was obtained in the optimized conditions compared to control condition.

REF-A051

PROCESS DEVELOPMENT AND OPTIMIZATION OF FED-BATCH PRODUCTION PROCESSES FOR THERAPEUTIC PROTEINS BY CHO CELLS

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KEY WORDS:
PROCESS DEVELOPMENT

BACKGROUND AND NOVELTY:
In the biopharmaceutical industry, process development and optimization is key to produce high quality recombinant proteins at high yields. As technologies mature, pressure on cost and timelines is greater for delivering scalable and robust processes.
Overall, process development should be viewed as a continuum from the early stages up to process validation. Here we outline a lean approach on upstream development during the initial phases to optimize yields while maintaining the desired product quality profiles.

EXPERIMENTAL APPROACH:
Early-stage process development was designed to lead to the establishment of a baseline process and to systematically include experiments with input parameters that have a high impact on performance and quality. At this stage, potential for pre-harvest titer and yield increases as well as product quality challenges were identified. Feed adjustments and systematic experiments with top, high and medium impact parameters have then been performed to develop a robust and scalable process. This approach was applied to two early stage upstream processes.

RESULTS AND DISCUSSION:
A baseline process was established and optimization of the feeding strategy was then performed. The feed regime was further optimized in combinatorial studies with an additional parameter: the target seeding density. In parallel, the impact of the process duration was also assessed.Then, the design of the feeding strategy was simplified in order to facilitate the process transfer to larger scale facility. Feeding, pH and temperature ranging studies and mode of feed addition were also performed in small scale due to facility fit considerations in the context of scale up. Consolidation runs were carried out and the robustness of the processes was assessed by performing over- and underfeeding experiments. For both projects, high titers and quality consistency were achieved with a feeding strategy designed to be robust and scalable.

REF-A052

PRODUCT QUALITY LESSONS LEARNED FROM DEVELOPING AND IMPLEMENTING A CHEMICALLY-DEFINED CHO PLATFORM CELL CULTURE PROCESS

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KEY WORDS:
CELL CULTURE PLATFORM / CHEMICALLY DEFINED MEDIA / PRODUCT QUALITY

BACKGROUND AND NOVELTY:
Many biopharmaceutical companies have developed cell culture platform processes for the production of recombinant monoclonal antibodies in mammalian cells. The use of platform processes for the production of clinical material has several advantages including lower costs for process development and faster generation of clinical material, thus enabling a reduced timeline to entry into clinical studies. Considerable efforts in the industry have been directed towards the development of chemically defined media for industrial cell culture processes. The use of such media reduces or eliminates some of the risks associated with the use of hydrolysate containing media, including inconsistent performance due to lot to lot variability, potential to introduce adventitious agents, raw material sourcing. During the development and implementation of our first chemically defined CHO cell culture process platform we observed some changes in mAb product quality profiles compared to results obtained in our older platform process using hydrolysate containing media.

EXPERIMENTAL APPROACH:
Cell culture experiments aimed at identifying media components that could be responsible for the observed changes in product quality (charge variants, drug substance color) were conducted using several cell lines expressing different mAbs. Different concentrations of certain media components or component groups were evaluated and product quality was assessed.

RESULTS AND DISCUSSION:
Our studies led to the identification of several media components that can have a significant effect on several product quality attributes. Copper and zinc were identified as key components that can affect the presence of basic variants (proline-amidation, C-terminal lysine). Certain vitamins and iron were identified as levers that can be used to manipulate drug substance color. This presentation highlights that the use of chemically defined media enables us to better understand our processes (and observed challenges) and will ultimately lead to better process control.

REF-A053

SCALE-UP CONSIDERATIONS FOR MONOCLONAL ANTIBODY PRODUCTION PROCESSES

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KEY WORDS:
SCALE-UP / STIRRED TANK BIOREACTOR / OXYGEN TRANSFER FLUX

BACKGROUND AND NOVELTY:
When scaling up a monoclonal antibody production process in stirred tank bioreactor, oxygen transfer is probably one of the most challenging parameters to consider. Approaches such as keeping constant specific power input or tip speed across the scales are widely described in the literature and are often based on the assumption that mammalian cells are sensitive to shear stress.
However, with the high cell densities reached in modern processes, such scale-up strategies can lead to relatively high gas flow rate to compensate low agitation speed which could be detrimental to cells in its own right.
As an alternative, we explored a scale-up strategy based on the overall oxygen transfer flux (OTF) required by the cell culture process. OTF was defined as directly proportional to oxygen transfer coefficient (kLA) and oxygen enrichment in the gas mix. This way the overall gas flow can be kept at low values, while satisfying the oxygen requirements of a high cell density culture.

EXPERIMENTAL APPROACH:
The maximum OTF associated with a model process was calculated according to the following equation:
OTF = kLA x (% of oxygen in gas mix) / 20
Aim was to scale-up a fed-batch process developed at in 10L glass bioreactors to a 80L stainless steel bioreactor.
kLA measurements were performed on the 2 systems and were considered together with the capability of the equipments to enrich gas with oxygen.

RESULTS AND DISCUSSION:
From the kLA mapping, different combinations of agitation speed / gas flow rates / oxygen enrichment were determined in order to achieve the desired OTF. Cell culture results showed that a wide range of agitation speeds was well tolerated by the cells, However, high gas flow rates negatively impacted cell culture performance.
Using constant maximum OTF as a main criteria, scale-up from 10L to 80L bioreactor of our process was performed. Relatively high agitation speed was applied while maintaining gas flow rates low, but sufficient to ensure CO2 removal, and using a gas mix highly enriched in oxygen.

REF-A054

DIFFERENCES IN THE PRODUCTION OF HYPERGLYCOSYLATED IFN ALPHA IN CHO AND HEK 293 CELLS

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KEY WORDS:
CHO CELLS / HEK CELLS / INTERFERON / N-GLYCOSYLATION / PROTEIN EXPRESSION

BACKGROUND AND NOVELTY:
IFN4N is an IFN-alpha2b mutein developed in our laboratory using glycoengineering. This molecule contains 4 potential N-glycosylation sites, resulting in higher apparent molecular mass and longer plasmatic half-life compared to non glycosylated IFN-alpha used for clinical applications. CHO cells are widespread used for the large-scale production of therapeutic recombinant proteins and HEK 293 cells are an interesting system for the generation of recombinant cell lines because they are easy to transfect and, consequently, they allow the production of high levels of the protein of interest.

EXPERIMENTAL APPROACH:
In this work, lentiviral vectors containing the sequence of IFN4N were assembled and used for transduction of CHO and HEK 293 cells. The recombinant cell lines were subjected to a process of selective pressure using increasing concentrations of puromycin. After cloning, 6 clones were selected for the study of their growth, production parameters and characterization of the secreted IFN4N.

RESULTS AND DISCUSSION:
The CHO and HEK IFN4N producing cell lines resistant to the highest concentration of puromycin showed an 8-fold and 15-fold increment in IFN4N specific productivity, respectively, compared to the parental line. HEK clones exhibited lower μ and maximum cell density than CHO clones, but higher IFN4N cumulative production was achieved. Significant differences in the glycosylation pattern of IFN4N produced in CHO (CHO-IFN4N) and HEK293 (HEK-IFN4N) were observed by isoelectric focusing followed by western blot. Specifically, IFN4N produced in CHO cells showed more acidic isoforms than the one produced in HEK. Furthermore, this result was consistent with a lower in vitro antiviral and antiproliferative specific biological activity evidenced by the CHO-IFN4N compared to the HEK-IFN4N.

Considering that glycosylation affects protein stability, solubility, pharmacokinetics and immunogenicity, differences between CHO and HEK cells should be capitalized to select the proper system for the cytokine´s production.

REF-A055

KEY ASPECTS FOR A SMOOTH TRANSITION FROM DEVELOPMENT TO COMMERCIALIZATION OF A MONOCLONAL ANTIBODY PRODUCTION PROCESS

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KEY WORDS:
MONOCLONAL ANTIBODY PRODUCTION / PROCESS CHARACTERIZATION / CRITICALITY ASSESSMENT

BACKGROUND AND NOVELTY:
For the production of therapeutic monoclonal antibodies it is mandatory to demonstrate proper control of a manufacturing process, thus ensuring that the biological product consistently meets its desired quality attributes and specifications. For process developers this means rational and systematic process development and process definition with thorough understanding of the impact of process parameters on product quality attributes. However in a commercial environment the Time to Market is also key, therefore lean but high quality process development is required and should be viewed as a continuum from early stage development to process validation. Process characterization is the final stage linking development and commercial manufacturing. Here we outline a roadmap for an upstream process and give examples for specific stages of the pre-validation phase.

EXPERIMENTAL APPROACH:
In parallel with scale down model establishment, a failure mode and effects analysis (FMEA) was performed in collaboration with subject matter experts from development, technology transfer, quality assurance and manufacturing. This powerful risk prioritization tool involved three orthogonal risk evaluations: severity, occurrence and detectability. This enabled the identification of potentially key and/or critical parameters and prioritization of follow-up activities.

RESULTS AND DISCUSSION:
Based on the outlined approach we identified the parameters to be studied at laboratory scale. Once the experimental work was completed, process performance and product quality data were used to achieve four goals:

1. Enhance in-house understanding of the process
2. Re-assess severity of the individual parameter failure mode
3. Establish PARs (Proven Acceptable Range) and NORs (Normal Operating Range)
4. Define criticality of the given parameter using an in-house criticality tree

We reduced the NOR of parameters that showed a direct and significant impact on product quality within the tested ranges to reduce the risk of falling outside of the PAR.

REF-A056

ADDRESSING PRODUCT QUALITY CHANGES DURING LATE STAGE CELL CULTURE PROCESS DEVELOPMENT

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KEY WORDS:
CHO / THERAPEUTIC PROTEIN / SEQUENCE VARIANTS / PROCESS CHARACTERIZATION

BACKGROUND AND NOVELTY:
The cell line and cell culture process used for late stage projects may differ from those used early in clinical development. Decisions to change the cell line, process or both are based on several considerations. One common reason for making such change between early and late stage projects is that a more productive cell line may be warranted based on forecasted commercial demand. Another reason for change between early and late stage is that the production platform used for initial development has evolved or has been optimized from the previous state. These changes may lead to variation in product quality attributes from early development experience.

EXPERIMENTAL APPROACH:
Experiments were carried out using Genentech's current production platform and bioreactor scale down model.

RESULTS AND DISCUSSION:
The case study presented here describes the challenges associated with observed product quality differences between the new process which implemented a higher productivity cell line and chemically-defined media. Studies were completed to minimize the product quality changes initially observed including the prevention of amino acid substitutions (sequence variants) in the antibody product.

REF-A057

IMPLEMENTING PFIZER'S BIONET DELTA V BIOREACTOR CONTROL SYSTEM

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KEY WORDS:
PROCESS DEVELOPMENT / BIOTHERAPEUTICS / BIOREACTOR / EFFICIENCY / PROCESS UNDERSTANDING

BACKGROUND AND NOVELTY:
In this oral presentation, we describe the systematic approach taken by Pfizer's BioTherapeutics Pharmaceutical Sciences Bioprocess R&D to identify, evaluate, and implement the company's next generation bioreactor control system. A five year commitment in resource investment (capital and FTE) and strategic partnership with key bioprocess technology vendors was necessary to achieve this goal.

EXPERIMENTAL APPROACH:
The endeavor will be described in a time-line of events and three distinct phases will be highlighted: Technology Evaluation (Phase 1), Technology Maturation (Phase 2), and Technology Deployment (Phase 3). Key team challenges/learnings and solutions to work through for each phase will be shared.

RESULTS AND DISCUSSION:
The result of this effort is a bioreactor control system that drives efficiency in the PD laboratories, increases data capture capability reinforcing process development towards QbD (Quality by Design), and provides a flexible structure to integrate new emerging bioprocess technologies to support process understanding.

REF-A059

TECHNOLOGY TRANSFER AND SCALE DOWN MODEL DEVELOPMENT STRATEGY FOR BIOTHERAPEUTICS PRODUCED IN MAMMALIAN CELLS

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KEY WORDS:
CELL CULTURE / BIOPROCESS / SCALE DOWN MODEL / TECHNOLOGY TRANSFER

BACKGROUND AND NOVELTY:
The goal of a manufacturing process development for drug substance is to establish a commercial manufacturing process capable of consistency producing drug substance of the intended quality. Based on the regulatory requirements, the manufacturing process has to be characterized prior to process validation. Since performing the characterization study at the manufacturing scale is not practically feasible, development of a scale down model that represents the performance of the commercial process is essential to achieve reliable process characterization. This study describes the methodology applied to ensure a successful scale down development and the associated technology transfer required prior to the establishment of the small scale process.

EXPERIMENTAL APPROACH:
The scale down model is developed by identifying volume-dependent parameters and volume-independent parameters. A systematic method to prioritize operating parameters and to evaluate their potential impact to the process is the Failure Mode and Effect Analysis (FMEA). An enhanced approach to manufacturing process development will then include a process characterization study (PCS), a systematic investigation to understand the commercial scale process in detail including relationship between key operating parameters and the cell culture performance for growth, productivity and the product quality attributes.

RESULTS AND DISCUSSION:
The information and knowledge gained from process development studies and manufacturing experience provide scientific understanding to support the establishment of the design space, product specifications and manufacturing control. To ensure a successful scale down model establishment, the technology transfer has to elucidate necessary information and detailed documentation to be transferred by focusing on the handling variations of raw materials, raw material lead time and storage, sampling and testing, equipment scale differences, process flow diagram, process controls, data to be recorded.

REF-A060

TOWARDS UNDERSTANDING THE COMPLEXITY OF HYDROLYSATES

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KEY WORDS:
HYDROLYSATES / QBD / STATISTICS

BACKGROUND AND NOVELTY:
Hydrolysates are complex media supplements composed of many as well as different types of compounds. FrieslandCampina Domo's Quality by Design project has generated detailed information of these compounds (annotation and quantification) and cell culture performance data of many lots of Proyield soy SE50MAF-UF.
Using different statistical approaches, key compounds present in hydrolysates are identified that significantly correlate with cell culture performance. These compounds are demonstrated to interact with several other compounds in a complex biochemical network.
This network of compounds is a unique and native feature of hydrolysates and non-existent in chemically defined media. Addition of these individual key compounds to media in some cases slightly improves titer, but the effect is still much smaller than the effect of the complete hydrolysate. This suggests that the effect of a hydrolysate cannot be mimicked by adding key metabolites.
Working in close collaboration with our customers, we gain understanding about the relation between the complex composition of hydrolysates and their effect on cell growth and titer in the application.

EXPERIMENTAL APPROACH:
The Biochemical composition of soy hydrolysates (Proyield Soy SE50MAF-UF, Frieslandcampina Domo) has been identified by Metabolomics Biochemical profiling.
Biochemical profiling, together with peptide profiling and analysis of inorganic compounds, provides a complete characterization of this hydrolysate product.
By applying statistical tools which include Two-mode cluster analysis, Bootstrapped stepwise regression and 2D correlation analysis, a series of compounds in the hydrolysate were identified that correlate with cell growth or Production of IgG in a CHO cell line (key compounds).

RESULTS AND DISCUSSION:
The enhancing effect of some of these key components on specific production could be proven, but is still much lower than the enhancing effect of a hydrolysates supplement. 2D correlation analysis reveals a complex network of positive and negative.

REF-A061

DESIGN OF A MINI-BIOREACTORS PLATFORM BASED ON A SCALE-DOWN CHEMICAL ENGINEERING APPROACH

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KEY WORDS:
SCALE-DOWN / MINI-BIOREACTORS / HYDRODYNAMICS

BACKGROUND AND NOVELTY:
There is an increasing demand for small-scale bioreactors as tools for scale-down processes. By reducing culture volumes, more experiments can be managed in parallel and costs are decreased, especially for media screening or hydrodynamics effects study. Moreover, small systems are especially pertinent when cell availability is limited, as in the case of stem cells. However, most of the small-scale bioreactors (shaken well plates or shaking flasks) do not exhibit a geometric homothety with production scale. Thus, hydrodynamics may significantly vary entailing productivity variability. To fill this gap, our objective was to design and built a platform of six 250 mL mini-mechanically stirred bioreactors by using a chemical engineering scale-down approach.

EXPERIMENTAL APPROACH:
Specifications required that mini-bioreactors could be equipped simultaneously with three sterilizable probes (dissolved O2, pH, CO2 or biomass) and were usable with a minimal liquid volume of 70 mL. Marine propellers, Rushton turbines and ear-elephant impellers were provided to ensure various liquid mixing and mass transfers. Aeration could be done by a porous sintered cylinder, an open pipe or a perforated ring. A software allowed the control of the 6 bioreactors independently.

RESULTS AND DISCUSSION:
The bioreactors design was based on the scale-down of a standard 2-L bioreactor using various criteria. First, a geometric homothety was imposed so that agitation and aeration criteria could be relevant. Furthermore, the same volumetric oxygen transfer coefficient kLa was maintained by keeping constant the power dissipation per unit of volume and the superficial gas velocity. This was done by a theoretical approach based on a flow similarity hypothesis. Consequently, averaged and maximal turbulent dissipation rate and thus hydrodynamic stresses range are expected to be similar between the two bioreactors. The first kinetics studies performed in the mini-bioreactors have confirmed their ability as scale-down bioreactors for animal cell culture.

REF-A062

AN INTEGRATED SYNCHRONIZATION APPROACH FOR STUDYING CELL-CYCLE DEPENDENT PROCESSES OF MAMMALIAN CELLS UNDER PHYSIOLOGICAL CONDITIONS

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KEY WORDS:
SYNCHRONIZATION / MAMMALIAN CELL CULTURE / ELUTRIATION / DIALYSIS CULTURE

BACKGROUND AND NOVELTY:
The study of central metabolism and the interactions of its dynamics with growth, product formation and cell division is a key issue for decoding the complex metabolic network of eukaryotic 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. The enrichment of cells within a specific cell cycle phase, or cell synchronization, should in this way allow for the generation of a cell population with characteristics required for cell cycle related studies.

EXPERIMENTAL APPROACH:
Using a combined approach, centrifugal elutriation was employed for synchronization in different cell cycle phases of two production cell lines. Cells were afterwards cultivated in benchtop bioreactors with culture volumes ranging between 200 mL and 1 L. A dialysis bioreactor (Bioengineering AG, Switzerland) with a total volume of 3.8 L was used for the cultivation of one cell line in order to allow for a higher number of synchronous cell divisions.

RESULTS AND DISCUSSION:
Our first results demonstrated the successful separation of a heterogeneous AGE1.HN® cell population into synchronous subpopulations [1]. These subpopulations showed a high degree of synchrony independently of the targeted cell cycle phase. Bioreactor culture showed no noticeable perturbation in the doubling time of the population. With these result, one of the most important requirements for Omics research was fulfilled. The dynamic behaviour of the synchronous growing cells was systematically studied not only based on cell growth, but also on the distribution of the cell size and the DNA content of the cells. Furthermore, dialysis culture allowed for a higher number of synchronous cell divisions without noticeable perturbations. With this contribution, we present an integrated approach for cell synchronization and further unperturbed cultivation which is useful for studying cell-cycle dependent processes under physiological conditions.

BIBLIOGRAPHY, ACKNOWLEDGEMENTS:
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REF-A063

MODEL-BASED DESIGN OF THE FIRST STEPS OF A SEED TRAIN FOR CELL CULTURE PROCESSES

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KEY WORDS:
PROCESS DEVELOPMENT / SEED TRAIN / MODEL-BASED DESIGN

BACKGROUND AND NOVELTY:
The production of biopharmaceuticals for therapeutic and diagnostic applications with suspension cells in bioreactors requires a seed train up to production scale. For the final process steps in pilot and production scale, the scale-up steps are usually de-fined (e.g. a factor of 5 - 10), so that the respective cell concentrations required for seeding and harvesting can be considered as similar. More difficult in this respect are the first steps, the transitions to T-flasks, spinner tubes, roller bottles, shake flasks, stirred bioreactors or single-use reactors, because here often the same scale-up steps cannot be realized. The experimental effort to lay-out these steps is corre-spondingly high. At the same time it is known that the first cultivation steps have a significant impact on the success or failure on production scale.

EXPERIMENTAL APPROACH:
In the present work, for a suspendable cell line (AGE1.HN, ProBioGen AG) basic kinetic information for cell growth and death, substrate uptake and metabolite production were generated in four well directed shake flask batches using different initial substrate and metabolite concentrations. Based on data of two batches, a Nelder-Mead-algorithm has been applied to determine the model parameters of an unstructured kinetic model. Using a MATLAB / Simulink simulation based on this model, optimal points of time or viable cell concentrations respectively for harvest of a seed train from spinner tubes over shake flasks up to a stirred bioreactor (5 L) were determined and subsequently verified experimentally. Model prediction for optimization and experiment agreed very well. To be able to judge the optimization, the optimization results have been compared to a formerly manually optimized seed train of the same cell line. Without such time consuming lab work, the tool has delivered the same optimized seed train only based on data of two batches.

RESULTS AND DISCUSSION:
The concept offers a simple and inexpensive strategy for design of the first scale-up steps.

BIBLIOGRAPHY, ACKNOWLEDGEMENTS:
Acknowledgement: The bioreactor (Labfors 5 Cell) was kindly provided by the company Infors AG, the cell line AGE1.HN by ProBioGen AG.

SHAKE FLASK CONDITIONS AND OPTIMISATION FOR CONSISTENT CHO CELL CULTURE GROWTH

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KEY WORDS:
SHAKE FLASK / SCALE-UP / DOE / CHO

BACKGROUND AND NOVELTY:
Shake flasks (SFs) are the most commonly used culture system on a millilitre scale. Shake flask are used during the development of a mammalian cell lines for a range of tasks including expansion of seed cultures and screening and evaluation of potential production cell lines using platform methods. In order to develop a robust cell line development programme or seed expansion process, the SF culture parameters that impact on cell line performance should be evaluated. Whilst optimisation of SF culture such that culture performance is representative of performance in bioreactors conditions is a focus of much research , other experimental aims are also important. For example, establishing conditions that result in robust, consistent and predictable growth across the working volume of a range of different shake flask sizes and configurations would improve scale up and technical transfer into cGMP manufacturing.

EXPERIMENTAL APPROACH:
Statistical-based factorial design methods were used optimise culture conditions which would provide robust and consistent growth of CHO cultures across the working volume of a range of different SF sizes. The impact of flask venting, agitation speed, SF working volume and total volume and flask design were evaluated. Culture growth, Monoclonal antibody and metabolite productivity and substrate utilisation were used as responses. These responses were optimised to maximise growth and monoclonal antibody productivity while maintaining the performance robustness and consistency across SF volumes.

RESULTS AND DISCUSSION:
Total and working volume were shown to have a large impact on growth and productivity. Differences in maximum IVC (Time Integral of Viable Cell Concentration) were observed initially in the range of ~ 280 to 380 x 106 cells/mL.h across the conditions evaluated. Conditions were identified which improved growth robustness and consistency across SF volumes. The use of vented flasks was shown to have a positive impact on growth consistency across SF volumes. The impact and exploitation of these improvements will be discussed.

CHARACTERIZING HETEROGENEITIES OF ENVIRONMENTAL CONDITIONS IN VARIOUS BIOREACTOR SCALES USED FOR MAMMALIAN CELL CULTURES

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KEY WORDS:
BIOREACTOR CHARACTERIZATION / BIOREACTOR UP- AND DOWN-SCALING / MAMMALIAN CELL CULTURES

BACKGROUND AND NOVELTY:
Large scale mammalian cell cultures up to 20000L have been established as an industrial standard over the last few years. Process up scaling to those volumes is a fundamental necessity to guarantee process performance and in particular product quality. Therefore, it is of up-most importance to be aware of scale dependent variables, i.e. reactor geometry, shear stress distribution, dissolved O2 and CO2 distribution, gas mass transfer rates and pH perturbations due to feeding policy and position of feeding ports, which can be very different in between scales and might result to non-desired process variations.

EXPERIMENTAL APPROACH:
Computational Fluid Dynamic (CFD) simulations were used to study scale dependent parameters (e.g. shear stress, kLa, DO, pCO2, pH perturbations and mixing time) covering a volume range from 3L up to 15000L., Good agreement of CFD simulations with experimentally measured data obtained at each scale supports applied approach. Consequently, the trajectory analysis was used to characterize heterogeneities of environmental conditions among various vessel scales.

RESULTS AND DISCUSSION:
The results were implemented into a rational scale-down model to mimic conditions present in large scale bioreactors. Excellent agreement between scale-down cultivation data and those obtained from large scale bioreactors support the proposed methodology. An indirect implication of this work was to identify physical limits of various environmental parameters to which cells respond in terms of growth, product quantity and quality. This new approach allows generation of a rational engineering design space as well as can be used for further process optimization and control of the process robustness.

REF-A066

IMPLEMENTATION OF A MICRO BIOREACTOR SYSTEM IN A PLATFORM FOR CELL LINE AND PROCESS DEVELOPMENT

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KEY WORDS:
MICRO BIOREACTOR / AUTOMATION / FED BATCH / PROCESS DEVELOPMENT

BACKGROUND AND NOVELTY:
Requirements for rapid cell line and process development have pushed more and more process runs to a higher throughput in smaller scale. Tight time lines are very important when developing a platform approach for an antibody program from cell line development to production of clinical material. Evaluation of cell culture media and additives can be very time consuming as well as process parameter optimisation. The aim of the implementation of a micro bioreactor system was therefore to find an approach where cell culture media and additives can be tested and at the same time have control over bioreactor process parameters to facilitate the scale up of the bioreactor process.

EXPERIMENTAL APPROACH:
The micro bioreactor system (Ambr) from TAP Biosystems was selected for rapid process development and cell line development. Cell culture performance will be compared to glass bioreactors as well as 250L SUB. Titer, protein quality and timeline for cell line selection will be evaluated.

RESULTS AND DISCUSSION:
Data will be shown
(1) where the feed strategy for a CHO-S cell culture process was optimised and the antibody concentration could be increased from 0.4 g/L to 2.7 g/L after three runs of optimisation in the Ambr system in less than two months.
(2) where evaluation of media and additives were performed in order to improve product potency by altering the glycosylation pattern.
(3) where the micro bioreactor system was useful during cell line development in evaluation of clone stability and protein expression. Feed strategy and clone screen could be evaluated simultaneously and thereby base the clone selection on bioreactor data where parameters such as pH and dissolved oxygen (DO) can be controlled instead of using traditional shake flask experiments.
The process performance in the micro bioreactor system was comparable to 250L scale which is very useful for a platform approach where an early estimation of process performance in production of clinical material is advantageous.

REF-A067

NEW IMPROVED AUTOMATION OF THE AMBRTM MICROBIOREACTOR IMPLEMENTED IN A CLONE SELECTION WORKFLOW

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KEY WORDS:
SCALE-UP MODEL / CLONE SELECTION / BIOPROCESSING / PH REGULATION / CELL LINE DEVELOPMENT

BACKGROUND AND NOVELTY:
Within the biopharmaceutical industry, it is increasingly recognised that the shake flask has significant limitations as a model for predicting large scale bioreactor performance of clones, largely due to lack of pH and DO control in the shake flask. In addition, the shake flask is unsuitable for high throughput screening and process development programs due to the high level of manual labour required to take samples and maintain cultures on a day-to-day basis. While bioreactor models are better, some manual interventions are required such as sampling for offline pH measurement and recalibration.

EXPERIMENTAL APPROACH:
A recent development in microbioreactor technology, the ambr™ system, has enabled high throughput bioreactor studies at the 10-15mL culture scale, with multiple reports of positive culture performance (1, 2, 3). However, some aspects of the culture maintenance have not been fully automated, such as offline pH recalibration and external calculation of feed volumes. In the high throughput ambr system, these tasks can become manually intensive. Here, for the first time, we investigate the capability of novel improvements in the ambr system, including both a new integrated device for automated pH recalibration and new software capability allowing complex feedback calculations.

RESULTS AND DISCUSSION:
These new elements of the ambr technology will be implemented in a candidate monoclonal antibody clone screening program, and the associated benefits will be analysed and discussed. It is anticipated that the study will demonstrate improvements in frequency and consistency of pH recalibration, improvements in pH control, and reductions in hands on staff time required to maintain the parallel microbioreactor cultures. Automated calculation and addition of feed volumes will also increase efficiency of the laboratory workflow, further reducing staff time requirements and so enabling larger high throughput studies to be carried out with existing laboratory and staff resources.

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REF-A068

SABIN-IPV PROCESS OPTIMIZATION FOR AN AFFORDABLE IPV IN THE POST POLIO-ERADICATION ERA

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KEY WORDS:
VERO CELLS / SCALE-DOWN / POLIOVIRUS / INACTIVATED POLIO VACCINE / ANIMAL-COMPONENT-FREE

BACKGROUND AND NOVELTY:
A production process for inactivated polio vaccine (IPV) using attenuated Sabin poliovirus strains was developed based on the current large-scale Salk-IPV production technology [1]. This activity for the WHO plays an important role in their polio eradication strategy since the use of attenuated Sabin poliovirus strains, instead of wild-type Salk strains, provides additional safety during vaccine manufacturing. Development of a new Sabin-IPV opens opportunities to implement improvements in the process. In this way, a more affordable IPV for the post-eradication era is strived for.

EXPERIMENTAL APPROACH:
To achieve these objectives, a scale-down – scale-up strategy was followed using historical manufacturing data. Based on this, a 2.3-L scale-down model of the current 750-L bioreactors has been setup. This lab-scale process, both USP (cell and virus culture) and DSP (clarification, concentration, purification and inactivation) unit-operations approximate the large-scale [2]. Subsequently, using this scale-down model, a modified process using attenuated Sabin poliovirus strains, was developed. This process was used to produce Sabin-IPV batches under cGMP for the currently ongoing phase I/IIa safety and dose-finding clinical trial in naïve infants [1].

RESULTS AND DISCUSSION:
In parallel, using the scale-down model, the process is being optimized to further reduce the cost per dose. The effect of three different Vero cell culture strategies, using animal-component-free cell- and virus culture media, on subsequent poliovirus production was investigated. Increased cell densities allowed up to 3 times higher D-antigen levels when compared with that obtained from batch-wise Vero cell culture. The increased product yields showed opportunities to reduce vaccine cost per dose by efficient use of bioreactor capacity. Further, the use of animal-component-free cell- and virus culture media showed opportunities for modernization of human viral vaccine manufacturing.

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OTHER INFORMATION
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REF-A069

WHY COMPLICATE, WHEN SIMPLE THINGS CAN MAKE BIG DIFFERENCES TO YOUR PROCESS

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KEY WORDS:
FED BATCH / PROCESS OPTIMIZATION / PROCESS DILUTION / INOCULUM MAINTENANCE / CHO

BACKGROUND AND NOVELTY:
Process improvement strategies for most fed batch culture fall under 3 categories: a. Cell line development methodologies, b. basal medium and feeds formulations, and c. Online process analytical techniques. Further process optimization is done by manipulation of typical process parameters like DO, pH, temperature etc. However there are certain small parameters that are rarely discussed during process optimization. In this poster, we will discuss 3 such parameters: Inoculum conditions, Common salts concentration in process and the total process dilution.

EXPERIMENTAL APPROACH:
Fed batch process was run with the following conditions in Shake flask and Bioreactor:
Case 1: Inoculum maintained at different pH, lactate, and osmolality.
Case 2: Common salts concentration in the process.
Case 3: Different levels of dilutions by feed addition. For all cases, multiple cell lines were studied and analyzed for cell conc., viability, glucose, lactate, osmolality.

RESULTS AND DISCUSSION:
No significant difference was observed during the inoculum stage when cells were maintained at different levels of pH, lactate and osmolality. However, about two fold increase in the cell concentration was seen in the production process favoring cell growth and maximum culture longevity across multiple CHO cell lines.
Common salts are largely used as an osmolality correction agent in medium formulation. In our studies we observed that common salts' concentration plays other roles that can significantly affect process performance.
Feed addition causes varying levels of dilutions in the process depending on feeding strategy. It is expected that higher dilution decreases total cell concentration and titer. However, within a certain range, we observed an increase in total cell concentration, productivity and better product quality profile with increase in dilution.
Thus simple factors as illustrated by the above cases can impact process performance in a big way. Paying attention to these simple factors can help in developing an efficient process.

REF-A070

PLATFORM PROCESS WILL GIVE PLATFORM PRODUCT – CAN WE AFFORD IT?

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KEY WORDS:
PLATFORM FED BATCH PROCESS / CLONE SELECTION / PRODUCT QUALITY / MABS / CHO

BACKGROUND AND NOVELTY:
As cell culture technology is maturing, there is a drive towards developing platform processes. The typical components of a platform process consist of cell line development, basal medium and feed strategy, process parameters and scale up approach. Some prominent benefits are shorter development timelines, facility fit, use of historical data, inventory management and easier integration with downstream process.

In our experience, the platform approach works well when target mAbs can have any levels of product quality (PQ) attributes like galactosylation and charge variant but may not work for mAbs with specific target for PQ.

EXPERIMENTAL APPROACH:
Following 3 cases will be discussed: 1. Multiple Cell lines expressing different mAbs developed using same technology 2. Difference in lead clone selection criteria – growth versus specific productivity and 3. Cell lines expressing same mAb developed using different technology.

For these cases, fed batch runs were performed using platform process in the shake flasks and lab bioreactors. Samples were measured for growth, productivity, metabolites and PQ attributes.

RESULTS AND DISCUSSION:
Case1: All cell lines showed similar growth and PQ profiles. Case2: The cell lines selected using different criteria showed two distinct cell growth patterns. Significant higher cell growth was observed for clones selected using the growth-based selection criteria in comparison to productivity based selection. However, PQ from these cell lines was comparable. Case3: The cell lines as expected showed differences in cell growth and metabolite profiles. These differences significantly impacted PQ attributes.
In all cases, the platform process had to be modified to achieve growth and PQ targets.
Our studies suggest that to produce mAbs with specific growth and PQ targets, platform process approach may not be appropriate as significant process changes may be needed to achieve these goals. We developed multiple platform processes to overcome illustrated disadvantages.

REF-A071

APPLICATIONS OF BIOMASS PROBE AS A PAT TOOL

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KEY WORDS:
PROCESS ANALYTICAL TECHNIQUES (PAT) / BIOMASS PROBE / FED BATCH / PERFUSION / CHO

BACKGROUND AND NOVELTY:
Cell culture process requires continuous monitoring of cell conc. and viability. Common techniques used apart from manual cell counting are automated cell counter and biomass probes. Unlike automated cell counters, biomass probe offer continuous monitoring of cell growth in the process. However, a major disadvantage of this technology is its correlation with cell size and morphology as it changes during the process. In this poster we will present usefulness of biomass probe in spite of above disadvantages.

EXPERIMENTAL APPROACH:
Cell conc. was estimated by automated cell counter (Vi-cell and Cedex) and compared with the readings of a biomass probe (Aber) to establish a correlation. This was done for both fed-batch and perfusion process in seed and production stages. For the perfusion process, an additional probe was inserted in the perfusate line to monitor the cell retention of the perfusion device.

RESULTS AND DISCUSSION:
The correlation between biomass probe readings and actual cell conc. was observed to decrease with increased process duration and drop in cell viability. Two cases are presented where biomass probe has advantages over traditional offline sampling and can be used as an effective PAT tool.
Case 1:Process consistency- Transfer of cells during seed stages is done based on cell conc. to maintain the log growth phase. For cell lines having high growth rate, biomass probe was found to be a better technique for such transfers leading to process consistency. Similarly in the production run, process consistency was improved by using biomass probe to trigger process events like feeding.
Case 2:Improvement in perfusion process- In our process, loss in retention in the perfusion device led to decrease in cell conc. and productivity. By monitoring retention continuously, corrective actions could be taken to reduce these losses. Introducing a biomass probe in the perfusate line overcame operational constraints of sampling continuously to monitor retention efficiency which led to improved process performance.

CHEMICAL MODIFICATIONS OF RAW MATERIALS IN DRY POWDER MEDIA FORMULATIONS TO SIMPLIFY CHO FED-BATCH PROCESSES

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KEY WORDS:
CHEMICALLY DEFINED MEDIA / SOLUBILITY / FED-REGIME / TYROSINE / CYSTEIN

BACKGROUND AND NOVELTY:
The solubility of e.g. L-Tyrosine and L-Cystein in chemically defined cell culture media is a limiting factor at neutral pH. This requires the usage of an additional feed at basic pH with all bioprocess related limitations like local pH spots resulting in cell death followed by cytoplasmic protein release supporting CHO cell aggregation and cell clumping.

EXPERIMENTAL APPROACH:
This can be circumvented by using modified molecules allowing for solubility at neutral pH in one main feed at concentrations with more than 50 g per liter of e.g. L-Tyrosine. We demonstrate that these novel moieties are metabolized by CHO cells and show no effect on the NBE level as this highly conserved t-RNA coordinated translation process uses unprocessed L-Tyrosine only.

RESULTS AND DISCUSSION:
This novel amino acid allows in combination with L-Cystein for a simplification of the fed batch regime without the necessity to add extreme pH solutions to the bioreactor.

BIBLIOGRAPHY, ACKNOWLEDGEMENTS:
Dr. Jörg von Hagen is head of Merck Millipore Process Development R&D in Darmstadt (Germany). Having studied biotechnology and signal transduction in Giessen and Darmstadt, he received his academic degree with an award-winning thesis in 2001. Dr. von Hagen has more than 20 years of practical expertise in biotechnology, especially in molecular cell biology and proteomics.

BI-HEX®: INNOVATIONS IN BOEHRINGER INGELHEIM'S INTEGRATED CELL CULTURE PROCESS DEVELOPMENT

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KEY WORDS:
CELL CULTURE / PROCESS DEVELOPMENT / NEW MOLECULAR FORMAT / CELLULAR ENGINEERING INNOVATION

BACKGROUND AND NOVELTY:
BI-HEX® is Boehringer Ingelheim's integrated cell culture platform used for developing and manufacturing processes. The CHO-based BI-HEX platform combines in one concept state-of-the-art technologies within vector design, cell line generation, process and media optimization. Strategies are in place for introduction of a new generation of media, shortening of seed expansion times, improved process feeding, and introduction of process formats and cellular modifications to control product quality. Innovations to accomodate newer molecule formats, higher performance benchmarks in process time and resources, and more stringent product quality requirements will be highlighted.

EXPERIMENTAL APPROACH:
Multiple tools were used ranging from systems biotechnology involving metabolic flux analysis and controlled feeding to statistical experimental design and multivariate analysis. Vector and cell line engineering have been other focus of activities. Newer advancements include exploration of controlled varying feeding based on online monitoring of cell growth.

RESULTS AND DISCUSSION:
Standardized workpackages and lean project structures have been implemented that allow reduced development and production cycle times. Process modifications including a balanced chemically defined medium and an advanced feeding approach have led to significantly higher growth and titers. Product quality matching has been enhanced via development of a standardized work package involving high throughput exploration of the design space. Cellular and process levers implemented allow for control on product quality attributes like ADCC activity.

REF-A074

SCALE TRANSLATION OF A 24-WELL MINIATURE BIOREACTOR AND SUBSEQUENT IMPACT ON PRODUCT AND BROTH QUALITY

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KEY WORDS:
MINIATURE SHAKEN BIOREACTOR / CHO CELL CULTURE / ANTIBODY PRODUCTION / PRODUCT QUALITY / ULTRA SCALE-DOWN (USD) PRIMARY RECOVERY

BACKGROUND AND NOVELTY:
To accelerate cell culture process development, most companies have validated scale-down models of their pilot and manufacturing scale bioreactors. Advancing such mimics to even smaller scales requires the large scale engineering environment to be accurately recreated. Here we describe a shaken microwell methodology that accurately reproduces not only cell growth kinetics but also key attributes related to product quality and broth processability.

EXPERIMENTAL APPROACH:
The micro24 bioreactor system enables system level control of agitation, with individual well control of pH, DO and temperature. Two distinct plate types are investigated, allowing for either headspace or direct gas sparging. An engineering characterisation was performed evaluating fluid mixing, gas transfer capacity and the dispersed gas phase. Cell culture is investigated using a model CHO cell line expressing a whole IgG1 mAb.

In addition, this work describes scale-up of micro24 results to laboratory scale stirred tank bioreactors (2L) and use of the device for selection of robust and scaleable cell lines through evaluation of product quality; and broth quality as evaluated by primary clarification efficiency using a USD depth filtration rig.

RESULTS AND DISCUSSION:
Apparent kLa values ranged between 3–22 hr^-1 and 4–53 hr^-1 for headspace aeration and direct gas sparging respectively. Mixing times (tm) were generally in the range 1–13 seconds and decreased with increasing shaking frequency (500–800 rpm). Direct gas sparging also helped to reduce tm values.

Cultures performed with headspace aeration showed the highest VCD and antibody titres, whereas those operated with direct gas sparging showed cell growth kinetics and product titres that were more comparable to those found in a conventional 2L stirred bioreactor. Initial results also indicate that key product and broth processability attributes are maintained making the combination of micro24 and USD technologies useful tools in 'Quality by Design' driven cell culture process development.

REF-A075

COMPARATIVE STUDY OF CHEMICALLY DEFINED CELL CULTURE SUPPLEMENTS FOR BIOPROCESS APPLICATIONS

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KEY WORDS:
SCALE-UP / CHEMICALLY DEFINED (CD) / FEED / SUPPLEMENT / TITER

BACKGROUND AND NOVELTY:
Throughout biopharmaceutical process development, many decisions are made in the production of biological therapies. Each biopharmaceutical process requires a unique cell culture environment for optimized performance. One bioprocess challenge is the selection and optimization of a cell culture supplement or feed that will give desired titer, growth characteristics, and protein quality. Therefore, the availability of a family of chemically defined (CD) supplements offering a diverse performance profile can significantly improve process development timelines. BD has developed a CD supplement pack to address this issue. The current studies were performed to determine the comparability of these supplements against other commercially available CD supplements or feeds, as well as their scalability in larger-scale processes.

EXPERIMENTAL APPROACH:
Three in-house mAb-producing CHO cell lines were used in these experiments. The comparative study was executed using five BD CD supplements and multiple commercially available CD supplements to evaluate their effect on production, growth, and viability. Shaking deep wells and conventional shaker flasks were used in these studies. The scalability of the five BD CD supplements was tested in small scale 1L bioreactors.

RESULTS AND DISCUSSION:
In testing on three CHO cell lines, protein yields of BD CD supplements were superior to results obtained with commercially available supplements. In addition, the BD supplements were seen to be suitable for use in small-scale bioreactors. The five BD supplements produced a 2-3-fold increase in protein production over media control in the 1L bioreactors. Equivalent results were obtained in shake flask studies during the development of these five BD CD supplements, hence demonstrating successful scalability. The growth characteristics were comparable or slightly better in 1L bioreactors compared to shake flasks.

DEVELOPMENT AND OPTIMIZATION OF A SET OF CHEMICALLY DEFINED MEDIA SUPPLEMENTS FOR MULTIPLE CHO CELL CULTURE SYSTEMS

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KEY WORDS:
CHEMICALLY DEFINED (CD) / FEED / SUPPLEMENT / PROCESS DEVELOPMENT / ANIMAL-FREE

BACKGROUND AND NOVELTY:
In biopharmaceutical production, optimization of cell culture parameters is a central component of process development. Every cell line can offer unique challenges for devising processes that yield desired growth profiles, high titers, and suitable product quality. Usually many different growth conditions must be tried before process goals are met. During the optimization process, the use of culture supplementation is often attempted for boosting performance, and this aspect of development can also benefit from having a diverse set of formulations to choose from.

EXPERIMENTAL APPROACH:
Three monoclonal antibody-expressing CHO cell lines were used for development and testing of animal-component-free, chemically defined (CD) formulation candidates, using CD basal media in all studies. Shaking deep well and conventional shaker flask formats were used during development. Formulations were generated using knowledge and analysis of cell line nutritional requirements, and design-of-experiments studies were carried out to optimize component levels. Formulation screening was accomplished using both batch and fed-batch modes.

RESULTS AND DISCUSSION:
The set of top formulations was selected during development for further commercialization. This set enhanced recombinant protein production in the test cell lines while retaining suitable product quality. As no single supplement was ideal for enhancing performance across all conditions, the need to test several supplements to cover a wider range of formulation space during process development was evident. In addition to being chemically defined, the supplement formulations are fully animal-free and protein-free, simplifying compliance with regulatory requirements. These results suggest that this collection of BD supplements can serve to enhance the quality and performance of bioprocesses across a diverse set of cell lines and growth conditions.

RELEVANCE OF YEAST EXTRACT FRACTIONATION BY CROSS-FLOW NANOFILTRATION TO SUPPLEMENT CHO CELL CULTURE MEDIUM

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KEY WORDS:
YEAST EXTRACT / NANOFILTRATION / CULTURE MEDIUM / CHO CELLS

BACKGROUND AND NOVELTY:
Yeast extract (YE), which is a soluble fractions of yeast autolysates, is known to greatly enhance mammalian cell culture performances, but their undefined composition decreases process reliability. Accordingly, it appears necessary to simplify YE composition and to better investigate the properties of molecules presenting a positive stimulating effect. This study aims to implement a process of YE fractionation by cross-flow nanofiltration, then to analyze the fractions composition and to evaluate their influence on CHO cell growth.

EXPERIMENTAL APPROACH:
The nanofiltration process was performed in a ProScale system (Millipore), using a spiral-wound Nanomax 50 membrane (500 Da, 0.4 m2). YE and fractions were characterized by their composition in amino acids, carbohydrates, nucleic acids and by their peptides molecular size distribution [1]. Trehalose was assayed by an enzymatic kit (Megazyme) and r-IgG by ELISA. CHO-AMW were cultivated inside 125 mL shake flasks in reference BDM serum-free medium supplemented with various YE fraction levels (0.5 to 4 g/L). The concentrations of cells and of metabolites were followed throughout cultures.

RESULTS AND DISCUSSION:
The nanofiltration process balance revealed that retentate molecules represented only 26 % of total YE, the majority of YE molecules exhibiting a molecular weight under 300 to 500 Da. Consequently, permeate contained molecules already present in reference medium, plus di-tri peptides and trehalose, while retentate contained molecules lacking in reference medium. Permeate exhibited similar stimulating effect than YE on maximal cell density and IgG production, highlighting the interest of nanofiltration process to refine YE and simplify dowstream processing. Furthermore, permeate reconstitution in amino acids and trehalose underlined that di and tri-peptides was used as source of nitrogenous substrate. On the other hand, the activity of the retentate, which increased the specific cell growth rate, was shown to be mainly due to cationic oligopeptides.

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REF-A078

PROCESS EVALUATION FOR THE PRODUCTION OF A LABILE RECOMBINANT PROTEIN

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KEY WORDS:
PERFUSION PROCESS / CHO CELLS

BACKGROUND AND NOVELTY:
Growing worldwide competition and patent expirations in the biopharmaceutical arena are increasingly turning process productivity into a central issue in the development of animal cell-based technologies. Perfusion is an important tool in this context, because high-cell-density processes operating continuously can be established, which are of advantage for the production of unstable molecules due to the low residence time of the product in the bioreactor. In this work, medium supplementation, operational conditions and two cell retention devices were investigated with the aim of developing an efficient perfusion process for the production of a labile therapeutic protein.

EXPERIMENTAL APPROACH:
A recombinant CHO cell line was cultivated in the customized chemically defined, animal-component-free medium "TC-LECC", derived from a commercial culture media platform (TeutoCell AG). The effect of supplementation of this medium with a recombinant hormone on cell growth and productivity was investigated. Precultures and batch runs were carried out in shake flasks at 37oC, 5% CO2, 180 rpm and 5-cm shaker orbit. Perfusion cultures were carried out in stirred bioreactors at 30% air saturation, and temperature and pH shifts were investigated. Two different cell retention devices (the ATF system and a gravity settler) were evaluated.

RESULTS AND DISCUSSION:
Regarding the hormone supplement, its absence in perfusion led to higher cell density, but less active product. Regarding pH, a shift down to 6.7 resulted in increased product activity, but a decreasing temperature shift did not enhance active product concentration. Among the two different cell retention devices, limitations regarding the retention of the product inside the bioreactor were experienced with the ATF system. The reasons for this are currently under further study. Using the gravity settler, product activity in the bioreactor and in the harvest were comparable, and a process stably operating for over 1 month at high cell concentrations could be established.

REF-A079

THE CHALLENGE TO SCALE UP A STIRRED SINGLE-USE BIOREACTOR FROM 50 TO 2000 L

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KEY WORDS:
SINGLE-USE / BIOREACTOR / SCALE UP / CHARACTERIZATION / QUALITY BY DESIGN

BACKGROUND AND NOVELTY:
Single-use bioreactors are an attractive technology for biopharmaceutical industry. They are excessively used for mammalian cell cultivations e.g. for the expression of vaccines or monoclonal antibodies. This is motivated by several advantages of these bioreactors like reduced risk of cross contaminations or short lead times. Commonly, single-use bioreactors differ in terms of shape, agitation principle and gassing strategy. Hence, a direct process transfer or scale up can be challenging. Consequently, re-usable bioreactors are still regarded as gold standard due to their well-known and defined geometrical properties.

EXPERIMENTAL APPROACH:
Based on this knowledge a stirred single-use bioreactor family was developed with similar geometrical ratios.
To follow a Quality by Design (QbD) approach the single-use bioreactor family evaluated here was characterized by using process engineering methods. For definition of a control space specific power input per volume, mixing times and kLa were determined for scales from 50 to 2000 L.

RESULTS AND DISCUSSION:
The process engineering characterization indicates that these systems are suitable for cultivations of mammalian cells. Based on the data scale up and process transfer are possible between this bioreactor family as well as re-usable systems. Therefore, the presented stirred bioreactors are a major progress for single-use technology.

EVALUATION OF FED BATCH AND PERFUSION CULTURE CONDITIONS FOR PRODUCTION OF A MONOCLONAL ANTIBODY

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KEY WORDS:
CELL CULTURE / FED BATCH / PERFUSION

BACKGROUND AND NOVELTY:
To reduce production costs for new pharmaceutical ingredients speeding up of the process development phase is of importance. Fed batch is the preferred cultivation technique in the pharmaceutical industry. It is simple and robust. However, it has been shown that perfusion cultivation can achieve 5 – 10 times higher productivity. The major weakness of perfusion is its higher technical complexity.

Today, most manufacturers apply platform technologies in Upstream and Downstream processes. However, obtaining high performance requires adaptation of the platform process to different cell lines and/or media. It is therefore advantageous to use flexible equipment and to develop protocols for rapid estimation of cultivation conditions.

EXPERIMENTAL APPROACH:
In our study we used a model CHO cell line producing the antibody G8.8 against Ep-CAM (Epithelial Cell Adhesion Molecule).
For developing a perfusion process we compared five different cell retention systems (SpinFilter, Cell Settler, Centritech Lab III, Biosep and ATF) with regard to achieve high viable cell densities. In addition, we compared the ATF system with two different membranes (2 µm for retention of cells and 50 kDa for retention of cells as well as antibodies).

Beside the feed medium composition itself the most important process conditions for establishing a fed batch process are amount of feed and feed time course. In addition a fed batch process might be improved by lowering the temperature or changing osmolality at a certain time of cultivation. In shake flask cultivations we used Design of Experiments (DoE) to examine feed volume, time of feed start, time of temperature reduction and time of osmolality increase in a single DoE-run.

RESULTS AND DISCUSSION:
The best results were achieved for perfusion culture with the ATF system with cell densities up to 1.3 x 108 cells mL-1. The next best were the Centrifuge and the Cell Settler with cell densities approximately at 3 x 107 cells mL-1. Using BioSep and Spinfilter, cell densities up to 2 x 107 cells mL-1 were obtained.

A bolus feed applied once a day was used for the fed batch shake flask cultivations. Maximum antibody titer was achieved for a feed of approximately 15 mL per day. Time of feed start has almost no influence. Reduction of temperature and increase of osmolality have a negative influence for the used cell line and medium.

DEVELOPING A SCALABLE, HIGH PERFORMANCE BIO-PRODUCTION PROCESS IN MINIMAL TIME

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KEY WORDS:
CELL CULTURE MEDIA / MICROBIOREACTORS / CHEMICALLY DEFINED / CHO / FEED

BACKGROUND AND NOVELTY:
With the tight timelines associated with the biotherapeutic product development and launch process, process development groups are challenged with developing robust, scalable processes in minimal time. Cell culture media and feeding strategies are critical factors, so it is essential to quickly identify a high performance medium that is easily adaptable and scalable. One strategy is to perform media optimization and feed studies to design a process specific for a particular cell line, but this can be costly and time consuming. An alternative approach is to screen commercially available production media and feeds to quickly identify a high performance medium and feed supplement that can be used as part of a platform process. This strategy speeds time to market, which is critical in launching any new product.

EXPERIMENTAL APPROACH:
A series of studies were performed using scale-down models to quickly identify a commercially available base medium that demonstrated high performance across a panel of CHO lines. The evaluation included the use of a microbioreactor system to model the conditions that would be observed in a scaled up bioreactor system. BD Select™ CD CHO Medium 1, a new, novel CHO medium developed specifically for use as a high performance growth and production medium, demonstrated favorable results across the panel of CHO lines. Feed studies were also conducted using the microbioreactor system and the performance of the selected base medium was further increased with the use of the BD Select™ CD CHO Feed Medium A.

RESULTS AND DISCUSSION:
By using predictive scaled down screening methods, a large number of conditions were screened in minimal time using minimal resources. As a result, BD Select™ CD CHO Medium 1 and BD Select™ CD CHO Feed Medium A were quickly identified as high performing candidates for use in a scaled up process.

REF-A082

PERFUSION SEED CULTURES FOR MORE EFFICIENT PRODUCTION BIOREACTOR UTILIZATION

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KEY WORDS:
PERFUSION / SEED TRAIN / PLANT UTILIZATION / VOLUMETRIC PRODUCTIVITY / ATF

BACKGROUND AND NOVELTY:
The production bioreactor is one of the bottlenecks in GMP biologic manufacturing. Traditional fed-batch production processes consist of an unproductive growth phase where cell mass accumulates followed by a more productive stationary phase where the majority of the drug product is generated. That unproductive growth phase lengthens run duration and lowers volumetric productivity, which leads to inefficient production bioreactor utilization and reduces the output rate. We can improve volumetric productivity and debottleneck production bioreactor usage by shifting the growth phase from the production stage into the N-1 seed train stage. Since it is difficult to sustain high cell densities using traditional batch seed train cultures, we propose the use of perfusion in the N-1 seed train bioreactor.

EXPERIMENTAL APPROACH:
Using alternating tangential flow (ATF) technology, we performed perfusion N-1 seed cultures with two different cell lines. Exponential growth was observed throughout the N-1 duration, with Cell Line A reaching 2x its peak fed-batch VCD and Cell Line B reaching 4x its peak fed-batch VCD. Fouling was not observed for either cell line. At the end of perfusion N-1, the cultures were split into high seed fed-batch production cultures.

RESULTS AND DISCUSSION:
Cell Line A's high seed fed-batch production culture tracked the performance of its low seed process, reaching the same harvest titer as the low seed process in 60% of the time. Perfusion N-1 also enabled Cell Line B's high seed fed-batch production culture to reach 2x the VCD of its low seed process, yielding the same titer as the low seed process in 50% of the time. Thus, it is feasible to double the output in the same amount of time.
We have demonstrated proof-of-concept at the bench scale for using perfusion N-1 to inoculate high seed fed-batch production cultures for increasing output and optimizing production bioreactor utilization. Process and media optimizations for perfusion N-1 and high seed production will also be discussed.

REF-A083

USING DESIGN OF EXPERIMENTS TO DEVELOP A BIPHASIC CULTIVATION STRATEGY FOR OPTIMAL PROTEIN EXPRESSION AND PRODUCT QUALITY

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KEY WORDS:
CHO / FC FUSION PROTEIN / BIPHASIC CULTIVATION / DOE

BACKGROUND AND NOVELTY:
In biphasic cultivations the culture conditions are changed to allow maximum recombinant protein expression after accumulating biomass during an initial phase. However, the influence of specific culture parameters and their optimal setpoint are cell line dependent. Additionally their impact on product quality needs to be investigated. In this study a full factorial design was used to evaluate the influence of two key process parameters, culture pH and temperature, on the process performance. A CHO cell line expressing a Fc fusion protein was grown in a biphasic process. A synergistic effect of both process parameters was observed and compared to the previous standard conditions, a drastic improvement of recombinant protein production as well as protein quality was achieved.

EXPERIMENTAL APPROACH:
CHO cells were grown in batch culture. During late exponential phase pH and temperature were shifted according to a full factorial design. The investigated temperature range was 28.5 to 38.5°C and the pH range was 6.75 to 7.05. A central composite design was used and appropriate parameter combinations to cope with the non-linearity of the response were selected.

The cultures were sampled daily to determine cell growth and viability, metabolite concentrations and recombinant protein production. The cultures were terminated once the viability decreased below 70%. At harvest, the product quality was assessed by determining the fraction of aggregated recombinant protein using size exclusion chromatography.

RESULTS AND DISCUSSION:
The parameter shift had a drastic effect on culture performance. Low temperatures reduced cell growth and nutrient consumption, thereby substantially extending the process duration. At the same time the recombinant protein production was stabilized. Depending on the cultivation temperature, an acidic pH reduced protein aggregation. The optimal parameter combination resulted in a 2.5 fold increase of the final product concentration and reduced protein aggregation from 75% to 2%.

REF-A084

HIGHLY EFFICIENT INOCULUM PROPAGATION IN PERFUSION CULTURE USING WAVE BIOREACTOR™ SYSTEMS

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KEY WORDS:
SEED TRAIN / PROCESS INTENSIFICATION / PERFUSION / WAVE BIOREACTOR

BACKGROUND AND NOVELTY:
Inoculum propagation in animal cell culture is typically done in a series of batch cultures with increasing cultivation volume, until a sufficient cell number to seed the production reactor is obtained. Perfusion cultures can be used to obtain high cell concentrations and drastically increase the split ratio. Especially in combination with disposable bioreactors, the turnover time can be shortened and the process flexibilty improved. We developed such a process in a single use WAVE Bioreactor and compared it to traditional batch cultures for inoculum propagation.

EXPERIMENTAL APPROACH:
CHO-S cells were grown in batch and perfusion cultures in single use WAVE Bioreactor systems. In the perfusion process, the cells were retained by a filter integrated into the reactor. The medium renewal rate was increased according to the cell growth. Cells were removed from both cultures in late exponential phase and used to seed fedbatch cultures.

All processes were sampled daily to determine cell growth and viability as well as metabolite concentrations. The fedbatch cultures were terminated once the viability decreased below 60%.

RESULTS AND DISCUSSION:
The maximum cell concentration in batch culture reached 5.1E+06 c/mL while in perfusion a tenfold higher concentration of 4.8E+07 c/mL was achieved. This allowed to increase the split ratio more than 6 fold to about 1:30 for inoculum propagated in perfusion culture. The subsequent fedbatch cultures gave similar results regarding cell growth, viability and cell metabolism.

The method described can reduce the number of expansion steps and eliminate one or two bioreactors in the seed train. Disposable bioreactors at benchtop scale have the potential to directly inoculate volumes of up to 1000 liters. Alternatively, the high biomass concentrations achieved in perfusion culture can be used to seed production bioreactors at increased cell concentrations, thereby shortening the process time in these vessels.

REF-A085

STRATEGY OF RAPID CHO CELL LINE GENERATION FOR THERAPEUTIC ANTIBODY PRODUCTION

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KEY WORDS:
CELL LINE DEVELOPMENT / CHINESE HAMSTER OVARY / ANTIBODY PRODUCTION / DEEPWELL PLATES

BACKGROUND AND NOVELTY:
Development of a platform process for generating Chinese hamster ovary (CHO) cell lines is critical for successful clinical and commercial campaign of target biologics. Although Agensys is a small biotech company, our goal for cell line development remains same as many big pharmaceutical companies. The primary goal of cell line development at Agensys is to create cell lines with high productivity, stable expression, and desirable product quality.

EXPERIMENTAL APPROACH:
We employed ClonePix FL for a primary screening post transfection and also introduced micro-well plates for intermediate screening and expansion. Initially we operated 24 deepwell plates in a batch mode but screening of the clones in the batch mode would ignore the response of clones to the nutrient feeding applied in fed-batch production, therefore, may lead to the miss of the best potential clones. Therefore we developed a feeding strategy for the 24 deepwell plate fed-batch cultures. Recently we also brought in a micro-bioreactor system for the clone evaluation.

RESULTS AND DISCUSSION:
ClonePix enables us to screen more clones after transfection while introduction of the 24 deepwell plates allows us to screen clones in suspension culture at the early stage. Screening of more clones at the early stage increases probability of identifying high antibody producers. Screening of the clones in suspension culture is more predictive than those in static culture. The micro-bioreactor system mimics the culture conditions of large-scale bioreactor and leads us to evaluate more clones in the bioreactor environment. As a result, our cell line development platform process enables us to make a rapid identification of cell lines to be used in clinical and commercial manufacturing.

REF-A086

EVALUATING AND MINIMIZING SEQUENCE VARIANTS DURING RECOMBINANT PROTEIN PRODUCTION

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KEY WORDS:
SEQUENCE VARIANTS / RECOMBINANT

BACKGROUND AND NOVELTY:
Amino acid sequence variants are defined as unintended amino acid sequence changes that contribute to product variation with potential impact to product safety, immunogenicity and efficacy. Therefore, it is important to understand the propensity for sequence variant (SV) formation during the production of recombinant proteins for therapeutic use. Coupling increasingly sensitive analytical techniques with the natural rate of spontaneous mutations and translational infidelity rates in the production of endogenous proteins, it is not surprising to find low levels of sequence variants in recombinant proteins.

EXPERIMENTAL APPROACH:
Experiments to identify strategies to prevent SVs were performed in Chinese Hamster Ovary (CHO) and E. coli production systems in bioreactor scale-down models. Amino acid feeding strategies were employed to prevent misincorporations of specific amino acids that were depleted during the production process. Codon replacement was also utilized to prevent known codon mistranslations.

RESULTS AND DISCUSSION:
This work describes strategies to prevent sequence variant formation during recombinant protein production in CHO and E. coli cells. These strategies include amino acid feeding and codon replacement. Other sequence variants which cannot be mitigated by these strategies may be managed through manufacturing process controls.

REF-A087

ULTRA SCALE-DOWN DISCOVERY OF LOW SHEAR STRESS PROCESSING FOR SELECTIVE RECOVERY OF NEXT GENERATION FUSION PROTEINS

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KEY WORDS:
FUSION PROTEIN / ULTRA SCALE-DOWN / SHEAR STRESS (PRIMARY RECOVERY) / CENTRIFUGATION / FILTRATION

BACKGROUND AND NOVELTY:
Fusion proteins offer the prospect of next generation biopharmaceuticals with multiple functions. We investigated the primary recovery of a novel fusion protein consisting of modified E2 protein from hepatitis C virus fused to human IgG1 Fc and expressed in a Chinese Hamster Ovary cell line. Fusion protein products pose increased challenges in preparation and purification. Issues of concern include the impact of process stress on cell integrity resulting in increased presence of product-related contaminants. This presentation addresses the use of low cost microwell-based ultra scale-down (USD) methods for characterising the integration of cell culture and cell removal operations to develop a bioprocess strategy.

EXPERIMENTAL APPROACH:
All cell culture broth was produced using 5 L stirred bioreactors. USD studies were used to predict removal of contaminants such as lipids, nucleic acids and cell debris as well as fusion protein recovery. A USD shear device was used to mimic the shear stress that occurs in the feed zones of non-hermetic and hydrohermetic centrifuges. For a low shear stress processing alternative, USD depth filtration technique based on the use of a robotic handling platform was used to investigate the filtration performance.

RESULTS AND DISCUSSION:
Based on the results, depth filtration delivered greater solids removal than centrifugation but a small (~10%) decrease in yield of the fusion protein was observed. Both centrifugation and filtration demonstrated little to no cell breakage. USD observations of product recovery and carryover of contaminants were also confirmed at pilot-scale, as was also the capacity or throughput achievable for continuous centrifugation or for depth filtration. The advantages are discussed of operating a lower yield cell culture and a low shear stress recovery process in return for a considerably less challenging purification demand.

BIBLIOGRAPHY, ACKNOWLEDGEMENTS:
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RAPID GENERATION OF CHEMICALLY DEFINED CELL CULTURE MEDIA AND FEED FOR IMPROVED PROCESS CONSISTENCY IN MONOCLONAL ANTIBODY PRODUCTION

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KEY WORDS:
MONOCLONAL ANTIBODY / RAW MATERIALS / PRODUCTION MEDIA / FEED SUPPLEMENT / PROCESS CONSISTENCY

BACKGROUND AND NOVELTY:
Achieving process consistency is a constant challenge for large scale manufacture of biotherapeutics using CHO cell based systems. Cell growth, productivity, and product quality in upstream processing have historically been impacted by the quality of the raw materials used in the manufacture of commercial media and feed supplements. EMD Millipore recently introduced CellventoTM CHO-200, a chemically defined (CD) animal origin free (AOF) dry powder media and companion feed designed for use with CHO cells in fed batch culture.

EXPERIMENTAL APPROACH:
In the following work, we have used a model IgG1 expressing recombinant CHO cell line, and statistical design of experiments (DOE), to formulate a production media and feed supplement with superior purity and performance consistency. Specifically, we will show data comparing growth, productivity, and product quality using multiple lots of Cellvento CHO-200. In addition, we have used a nominal fed batch process to compare the performance of Cellvento CHO-200 against other commercially available media and feeds.

RESULTS AND DISCUSSION:
The results of these studies suggest that Cellvento CHO-200 supports high density cell growth, provides improved production relative to other commercially available media, and is scalable. This work further demonstrates that superior consistency can be achieved for cell growth, titers, and product quality when Cellvento CHO-200 is used with an appropriate fed batch process. In summary, Cellvento CHO-200 provides improved process consistency in fed batch cultures relative to other commercially available media and feed.

GENERATION OF STABLE POLYCLONAL POOLS FOR LARGE-SCALE RAPID PROTEIN EXPRESSION

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KEY WORDS:
PROTEIN EXPRESSION / STABLE POOLS / CHO / POLYCLONAL POOLS

BACKGROUND AND NOVELTY:
The ability to generate suitable amounts of recombinant protein products at scale, but under short time lines, is one of the major limitations in early biopharmaceutical development. Improvements to large scale transient expression methods are one solution, however the limited production lifetime of the cells means that resources (DNA, transfection reagents) can become limiting at scale. Stably transfected cell lines have the ability to continuously produce high levels of protein but typically require long development timelines to identify a high producing, stable clone. Here, we present a process to generate stably transfected polyclonal pools suitable for use in large scale manufacture.

EXPERIMENTAL APPROACH:
The traditional method for generating stable transfectant pools involves transfecting cells with the gene(s) of interest and then splitting the contents of the cuvette into two populations in static T175 culture flasks. In comparison the proposed method divides the transfected cells into 96-well plates. The idea is to isolate transfectant populations to minimise outgrowth of non-expressing cells. The pools are screened using a high-throughput product assay and then the top producing wells are recombined to form a “Superpool.”

RESULTS AND DISCUSSION:
Antibody expression from pools generated using both methods was evaluated using a fed-batch shake flask model. A product concentration of 2 g/L was achieved using the Superpool method compared to 0.4 g/L with the T175 method. The product concentration increased to nearly 3 g/L when the Superpool was generated using the GS Xceed™ cell line. Repeating the fed-batch assessment 50 generations later showed the Superpools in GS Xceed™ maintained high levels of product expression whereas T175 pools and CHOK1SV Superpools lost almost all expression. The increase in the length of the manufacturing window makes this approach an efficient cost-effective solution for early material supply and is capable of producing over 500 g of monoclonal antibody in less than 6 weeks.

REF-A090

EXPANDING THE MOLECULAR TOOLBOX FOR CELL LINE DEVELOPMENT

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KEY WORDS:
VECTOR / XCEED / GS GENE EXPRESSION SYSTEM / CELL LINE / CHO

BACKGROUND AND NOVELTY:
The GS Gene Expression System™ and the new GS Xceed™ Gene Expression system are powerful tools for the generation of cell lines suitable for the production of recombinant therapeutic proteins. The host cells and production processes employed with the system contribute to the ability to rapidly generate highly productive cell lines; however this is underpinned by the vector architecture in the GS vectors themselves.

EXPERIMENTAL APPROACH:
In order to meet the challenge posed by evolving molecular design and the demand for shorter timelines, a flexible toolbox approach for vector generation has been employed which comprises a number of different vector and coding sequence designs. In order to eliminate issues with mRNA structure, coding sequences can be gene-optimised and a choice of signal peptide can also be incorporated into the molecule design. Further modifications have led to the development of GS vectors that are suitable for the production of more complex molecules, such as bispecific antibodies, where the concurrent production of three polypeptides is required. As these vectors contain the ability to produce recombinant protein from a third expression cassette, they also allow for the co-expression of any gene of interest alongside a two-chain therapeutic recombinant protein. Potential applications could be to achieve post-translational modifications not typically possible in the CHO host cell, or to allow for cell line engineering by co-expression of additional protein species.

RESULTS AND DISCUSSION:
The development of the toolbox is not limited to the generation of vectors for non-standard molecules as vector design can also contribute to a reduction in the time taken for cell line development. Robust cloning procedures that enable the generation of vectors with minimal subcloning efforts have been developed that allow for the generation of final expression vectors for monoclonal antibody production in the shortest timeframe.

REF-A091

DISPOSABLE ORBITALLY-SHAKEN TUBESPIN® BIOREACTOR 600 FOR INSECT CELL CULTIVATION IN SUSPENSION

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KEY WORDS:
ORBITAL SHAKING / DISPOSABLE BIOREACTOR / INSECT CELLS / GAS TRANSFER / CO2 STRIPPING

BACKGROUND AND NOVELTY:
Insect cells are a major host for recombinant protein production and are typically grown in suspension culture in spinner flasks or Erlenmeyer shake flasks. As an alternative vessel for medium-scale cultures, disposable conical tubes with ventilated caps having a nominal volume of 600 mL (TubeSpin® bioreactor 600, TPP) have recently been introduced.

EXPERIMENTAL APPROACH:
In this study, we compared cultivation of three widely-used insect cell lines (Sf9, S2, and High-fiveTM) in the TubeSpin® bioreactor 600, spinner flask, and shake flask. We measured cell growth and environmental culture conditions (pH, pO2, pCO2) in batch cultures.

RESULTS AND DISCUSSION:
For all three cell lines, a higher cell density was achieved more rapidly in the TubeSpin® bioreactor 600 than in the other two vessels. In the spinner flask and shake flask, but not in the TubeSpin® bioreactor 600, we observed oxygen limited conditions for all three cell lines under conventional culture conditions. This study validates the TubeSpin® bioreactor 600 for cell culture applications with suspension-adapted insect cells.

IMPROVING ADENOVIRUS PURIFICATION BY USING MEMBRANE CHROMATOGRAPHY. FROM A BIACORE CHIP TO A CONTINUOUS CHROMATOGRAPHY

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KEY WORDS:
MEMBRANE CHROMATOGRAPHY / DOWN SCALE / HIGH THROUGHPUT SCREENING (HTS) / SIMULATING MOVING BED (SMB) / BIACORE

BACKGROUND AND NOVELTY:
Membrane adsorbers are used in the biopharmaceutical industry almost exclusively in flow through mode for the mAb industry. More recently membrane adsorption chromatography has been applied for the purification of viral vectors in a bind elute mode yielding good overall recovery rates. However some issues still remain. For instance, the impact of the ligand density on virus purification, virus adsorption/desorption as well as the effect of competitive binding between impurities such as proteins and DNA with the target virus have to be better understood.

EXPERIMENTAL APPROACH:
In the present study the impact of new ligands (both quaternary and primary ammine), and their density on an anionic exchange membrane were assessed; also matrix structure namely grafted and not grafted were investigated. In addition, the optimal ligand density was selected for implementation of Simulating Moving Bed (SMB) chromatography.

The experiments were conducted by using a Biacore on chip technology for ligand evaluation; subsequently, 96 well plates was used to asses best ligand density and matrix structure suitable for adenovirus purification.

RESULTS AND DISCUSSION:
Our results indicate that increasing in ligand density creates more binding sites for DNA with an increase of 10-fold in the dynamic binding capacity (DBC), but not for viruses or HCP. The membrane with medium ligand concentration showed the best results in terms of purity, and recovery yield for the virus. However the low ligand density was implemented for SMB purpose due to their low irreversible binding. Also, primary amine ligands showed a suitable approach for a novel flow through purification strategy.

This work contributes to the understanding of the physico-chemical interactions between viruses and chromatographic membranes. Furthermore it shows the feasibility of Biacore sensor chip and 96 well plate as High Throughput Screening (HTS) tools. Moreover the selection of suitable membrane of membrane for SMB will pave the way for a robust continuous chromatography.

RAPID GENERATION OF RECOMBINANT PROTEINS BY TRANSIENT GENE EXPRESSION IN HIGH FIVETM INSECT CELLS

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KEY WORDS:
HIGH FIVE CELLS / TRANSIENT GENE EXPRESSION / RECOMBINANT PROTEINS / TNFR-FC

BACKGROUND AND NOVELTY:
Insect cells are frequently used for the production of recombinant proteins following gene delivery with the baculovirus vector- expression system (BVES). However, the BVES has several disadvantages, mainly due to the cytolytic activity of the virus. As an alternative, we established an efficient transient transfection process for rapid recombinant protein production with High FiveTM insect cells using a chemical reagent for transfection. This cell line has been proven to be suitable for the efficient generation of complex, glycosylated proteins using the BVES.

EXPERIMENTAL APPROACH:
Various process parameters, including the culture medium, cell viability, cell density, expression vector, and plasmid DNA and reagent amounts, were optimized.

RESULTS AND DISCUSSION:
With an optimized process, we obtained a transfection efficiency of 90% using enhanced green fluorescent protein (EGFP) as a reporter gene. Transfection with the tumor necrosis factor receptor (TNFR)-Fc fusion protein gene resulted in a volumetric yield of 150 mg/L by 4 days post-transfection. Research is ongoing to reduce the plasmid DNA amount needed for transfection and to scale up the process.

REF-A094

EVALUATION OF PROCESS PARAMETERS IN SHAKE FLASKS FOR MAMMALIAN CELL CULTURE

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KEY WORDS:
MAMMALIAN CELL CULTURE / SHAKE FLASK / PROCESS PARAMETERS / COMPARABILITY

BACKGROUND AND NOVELTY:
Shake flask cultivation is nowadays a routine technique during process development for mammalian cell lines. Here unbaffled and baffled shake flasks are applied. During shaken culture, changes in agitation velocity, shaking diameter or shake flask size affect the hydrodynamics in the shake flask. This might be reflected in the growth of the cultured cells.

Process parameters such as power input, mixing time, fluid velocity etc. have been determined and described mathematically for shake flasks used for microbial cultivation, but only to some extend for mammalian cell culture. Especially the relationship between these parameters and growth characteristics of mammalian cells is still a relatively uncovered issue.

EXPERIMENTAL APPROACH:
In this work, process parameters like specific power input, mixing time, maximum fluid velocity and Reynolds number were determined for four different shake flasks in a range of shaking velocities on a shaking machine. The specific growth rate (μmax) of the human production cell line AGE1.HN® (ProBioGen AG, Berlin, Germany) was compared to the respective process parameters.

RESULTS AND DISCUSSION:
Our results point to regions of the studied parameters, where common operation windows can be identified for μmax. In these process windows the cells show a similar μmax in different shake flask, making cell growth comparable. These process windows are common for the flasks, independently of their size and their number of baffles.

The data obtained in this work can be used for process standardization and comparability of results obtained in shaken systems i.e. to guarantee consistency of results generated during research tasks using mammalian cells.

REF-A096

SCALE-UP PRODUCTION OF PHARMACEUTICAL PROTEINS IN PLANT CELL SUSPENSIONS WITH ORBITALLY SHAKEN DISPOSABLE BIOREACTORS

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KEY WORDS:
BY-2 / CELL CULTIVATION / RECOMBINANT ANTIBODIES / SINGLE-USE BIOREACTORS / TOBACCO

BACKGROUND AND NOVELTY:
Plant cells are well suited for the production of pharmaceutical and industrial proteins either as whole-plant systems or cell suspension cultures. The latter have the advantage of being cultivated in containment under defined conditions that allow rigid process control. Cylindrical orbitally shaken single-use bioreactors are potentially favourable types of cultivation vessels for plant suspension cells because they combine reduced cell stress and contamination risk, that are characteristic for surface aerated reactors, with the flexibility and cost efficiency of disposables.

EXPERIMENTAL APPROACH:
We investigated the suitability of 50-mL TubeSpin Bioreactors, 10-L to 50-L Nalgene vessels and the 200-L OrbShake (SB-200X, Kuhner) orbital shaker for the cultivation of transgenic tobacco BY-2 cells secreting a human IgG antibody to the medium. For all scales, the oxygen consumption of the cells was online monitored during cultivation. A downstream process strategy for efficient antibody capture from the spent medium was developed and the production process including aspects of scalability, downstream processing and production costs was evaluated.

RESULTS AND DISCUSSION:
Aiming at the demonstration of process scalability, antibody producing BY-2 suspension cells were cultivated in cylindrical orbitally shaken bioreactors with nominal working volumes ranging from 5 mL to 100 L. The biomass accumulation and product formation obtained during cultivation in the 200-L bioreactor matched the performance of shake flask cultured cells. Thus, a 20-fold scale-up in culture volume did not adversely affect the productivity of the plant cells. The dissolved oxygen tension was measured online as a reliable indicator for cell growth. After 130-h cultivation almost one gram of antibody was harvested from the spent medium and purified resulting in a final product recovery of 90%. Our results prove the suitability of orbitally shaken bioreactors for the scale-up production of pharmaceutical proteins in tobacco cell suspensions.

REF-A097

A GFP-BASED APPROACH FOR THE OVEREXPRESSION AND PURIFICATION OF MAMMALIAN MEMBRANE PROTEINS

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KEY WORDS:
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BACKGROUND AND NOVELTY:
Production and purification of integral membrane proteins (IMP) for structural studies is difficult mostly due to the very low yields obtained and to protein instability. To date fewer than 300 structures of IMPs are known, less than 0.5% of all the known structures. For eukaryotes the story is even starker, with less than 50 mammalian IMPs solved. Membrane protein production and purification is therefore one of the most important remaining frontiers for structural biology research. One way to improve the throughput of eukaryotic IMP structures is to develop methods that reliably facilitate the identification of well expressing constructs that are stable and functional. In this work, we used the multi-spanning membrane MAP3K kinase TAO2 as a model to setup a GFP-based approach for the fast and cost effective multi-host expression screening towards the purification of eukaryotic IMP.

EXPERIMENTAL APPROACH:
TAO2 was cloned fused to GFP and a polyhistine tag into pOPIN-F, a vector designed to enable high-level target gene expression in multiple systems (1). A GFP-based expression screening and optimization was carried out using different E. coli strains, mammalian and insect cells to determine the host best suited for TAO2 production. GFP fluorescence was further used to facilitate detergent screening for downstream processing.

RESULTS AND DISCUSSION:
GFP was found to be a fast measure ofor protein expression and localization. By monitoring GFP fluorescence, we show that the TAO2 fusion protein only localizes correctly throughout the cell membrane when expressed in mammalian cells and only when GFP is present at the N-terminus. We indicate the practical steps that constitute our GFP-based pipeline for expression evaluation and to speed up membrane extraction detergent screening for protein purification. In short, although membrane-integrated expression is no guarantee of function, the GFP-tag speeds the empirical process towards obtaining stable and homogeneous material for functional and structural work.

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REF-A098

IMPROVEMENT OF CELL-FREEZING TECHNOLOGIES AND DISPOSABLE BIOREACTORS: TOWARD A FULLY CLOSED USP

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KEY WORDS:
DISPOSABLE / BIOREACTOR / CLOSED / COMPARABILITY

BACKGROUND AND NOVELTY:
Biotech processes still contain a number of open and at risk transfers steps. Cell culture steps from one frozen vial to containers such as shake flasks is one of these open phases. This critical expansion step may take several days or weeks and delay development or production timeline

due to contamination. By coupling traditional technologies and new technologies (disposable bags, cases and bioreactor), a study on bags freezing conditions and scalability of single-use bioreactors (SUB) has been performed in order to define a fully closed USP process.

EXPERIMENTAL APPROACH:
We have evaluated the freezing/thawing of cells in bags for fully closed operations from thawing to 1250L Bioreactor inoculation. A first trial on 7 different CHO cell lines was performed and demonstrated the feasibility of this approach. With this technique, cell amplification timelines were reduced and the risk of cross contamination eliminated. This first improvement was combined to SUB that are now commonly used for process development and as seeding or production bioreactors. If the benefits associated to these equipments have been well demonstrated on more than a decade, only a few data on their scalability are published.

During the period 2010-2012, we performed a study in order to evaluate the performances of SUB at various scale from 3L to 200L. The evaluation was performed both for seeding application and for clinical material production. Several clinical runs at 200L and 1250L scale were performed to ensure a meaningful comparison. These performances were also compared to glass and stainless steel bioreactors of different sizes ranging from 3.6L and 1250L.

RESULTS AND DISCUSSION:
Coupling cell freezing in bag and disposable bioreactors up to production scale allowed us to develop a fully closed USP process. We will extensively discuss the final set up from a technical, financial and organizational point of view emphasizing the various savings associated (labour, expense, training…) with single use systems and closed processes.