



POSTER PRESENTATIONS

PO001

UNDERSTANDING THE IMPACT OF CELL CULTURE PROCESS MANIPULATIONS ON MONOCLONAL ANTIBODY CRITICAL QUALITY ATTRIBUTES AND FUNCTIONAL RESPONSE

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Background and novelty: The uncertainty of early clinical trial results often led to sub-optimal manufacturing processes in exchange for speed to investigational new drug application submissions. Process development is then re-initiated in light of the positive clinical signal enabling late-stage development and commercialization of the product. Process optimization can potentially alter critical quality attributes (CQA's), and consequently elicit functional changes of the proteins. To mitigate this risk, a thorough understanding of process-to-product impact is necessary.

Experimental approach: The definitive screening design methodology was used to screen a large number of factors and several factors were identified to have an impact on the CQA's. Leveraging these factors along with other cell culture process manipulations, several CQA's can be modulated toward the desired levels. In addition, the impact of CQA's on antibody functions was assessed using a surrogate *in vitro* assay.

Conducting the functional assay was time and resource intensive. To address this issue, a predictive model was developed based on historical cell culture process development data. This model was validated against results obtained from actual experiments designed to assess the impacts of CQA's on the functional assay. The predictions based on the CQA's had good alignment with the functional assay results.

Results and discussion: The relationships among factors in cell culture process, CQA's and functional response were established. The newly developed cell culture process not only yielded more titer, but also achieved desirable CQA's and functional response. These findings enhanced our process-to-product understanding which can be applied to future programs.

Bibliography, Acknowledgements: None

Disclosure of Interest: None declared





PO002

FAST AND STREAMLINED TECHNOLOGY TRANSFER TO CONTRACT MANUFACTURING ORGANIZATION FOR EARLY-STAGE CLONAL ANTIBODY PRODUCTION

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Background and novelty: Contract manufacturing organizations (CMOs) have become increasingly important to the biopharmaceutical industry. The advantages of leveraging CMOs include increasing manufacturing capabilities and experience, cutting costs and reducing time-to-market, etc. To realize the full potential of outsourcing manufacturing, it is essential to develop a strategy to ensure a successful and streamlined technology transfer between the sponsor company and CMO.

Experimental approach: The interdisciplinary Fast And Streamlined Technology Transfer (FASTT) team, consisting of members from both Seattle Genetics and CMO, was formed to streamline and optimize the efficiency of technology transfer across CMC projects to increase throughput, compress timelines, and provide benefit to both companies without increasing risk to product quality and patients. The FASTT strategy focuses on: 1) implementing new technology transfer workflow which eliminates redundancies in developments; 2) streamlining practices to ensure consistent process and technology transfer among CMC projects; 3) addressing recurring concerns; 4) continual technology transfer process improvement and communication between sites.

Results and discussion: Overall, the FASTT strategy significantly reduces technology transfer time by approximately 6 months.

Bibliography, Acknowledgements: None.

Disclosure of Interest: None declared





PO003

DIELECTRIC IMPEDANCE SPECTROSCOPY FOR NON-DESTRUCTIVE QUALITY ASSESSMENT OF 3D CELLULAR CONSTRUCTS

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Background and novelty: In any three dimensional (3D) biofabrication process, assessing critical biological quality attributes of 3D constructs such as viable cell number, cell distribution and metabolic activity is critical to determining the suitability and success of the process. One major limitation in current state-of-the-art is the lack of appropriate methods to monitor these quality attributes in situ in a non-destructive label-free manner. In this study, the feasibility of using dielectric impedance spectroscopy to address this gap was investigated. Dielectric spectroscopy has been successfully used to monitor cell concentration as a critical process parameter in a variety of bioprocesses. However, this is the first study where it has been used to test cell-encapsulated hydrogel 3D constructs.

Experimental approach: First, we tested the fundamental hypothesis that the dielectric signatures (permittivity signals) of 3D alginate constructs with different encapsulated-MG63 (human osteosarcoma) cell concentrations (1, 2.5, 4, and 6.5 million cells/mL) are distinct. Furthermore, the β -dispersion parameters for MG63-encapsulated in alginate (6.5 million cells/mL) were characterized.

Results and discussion: There was a statistically significant difference between the relative permittivity of constructs with the four different encapsulated-MG63 concentrations ($p < 0.05$). Within the tested range, the relationship between cell concentration and relative permittivity was noted to be linear ($R^2 = 0.986$). These results demonstrate that dielectric impedance spectroscopy can be used to monitor critical quality attributes of cell-encapsulated 3D constructs. Owing to the measurement efficiency and non-destructive mode of testing, this method has tremendous potential as an in-process quality control tool for 3D biofabrication processes and the long-term monitoring of cell-encapsulated 3D constructs.

Bibliography, Acknowledgements: -

Disclosure of Interest: None declared





PO004

OPTIMIZED PEI-BASED TRANSFECTION REAGENTS FOR PRODUCTION OF CLINICAL GRADE VIRAL VECTORS

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Background and novelty: Gene- and cell therapy-based medicines are experiencing resurgence due to the introduction of “next generation” transfer viral vectors, which have demonstrated improved safety and efficacy. Adeno Associated Virus (AAV) and Lentivirus are very commonly used in therapeutics and often produced using PEI-mediated transient transfection in HEK-293 or HEK-293T cells. The critical raw materials needed for cGMP vector production must be sourced from approved suppliers and should have gone through a rigorous testing program to reduce the risk of introducing adventitious agents into the production process. Polyplus-transfection now provides PEIpro® and PEIpro®-HQ, the unique PEI-based transfection reagents suitable for use in process development and in cGMP biomanufacturing, respectively.

Experimental approach: Suspension and adherent HEK-293 and HEK-293T were transfected with different transfection reagents.

Results and discussion: PEIpro® and its high-quality counterpart PEIpro®-HQ are particularly well suited for therapeutic virus production. Both reagents are PEI (Polyethylenimine) based and are free of components of animal-origin. They have been selected for their high transfection efficiency using low DNA amount. PEIpro® and PEIpro®-HQ undergo stringent quality controls for use in process development. In addition, impurity profile, residual organic solvent and heavy metal content are also tested in PEIpro®-HQ to assess the purity of the reagent. Moreover, each lot of PEIpro®-HQ is provided with an extensive documentation, making PEIpro®-HQ perfectly suitable for use as a qualified raw material for the production of clinical batches of viruses in GMP processes.

Bibliography, Acknowledgements: Polyplus-transfection would like to thank the whole chemistry and biology R&D teams of Polyplus-transfection.

Disclosure of Interest: None declared





PO005

EFFECTS OF REAL-TIME BIOPROCESS CONTROL USING RAMAN SPECTROSCOPY

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Background and novelty: The quality of therapeutic CHO cell culture proteins is a result of the entire production process. Producing therapeutic proteins with consistent characteristics is a challenging task which requires a high level of process understanding and maintaining process conditions within an optimal batch trajectory. *In situ* Raman spectroscopy enables simultaneous monitoring of multiple product quality relevant parameter and allows for in-process corrections. Precise navigation and smart adaptation of critical upstream process conditions based on Raman spectroscopy will reduce the complexity of producing high quality products.

Experimental approach: Process development, pilot, and manufacturing scale CHO cell culture batches are utilized to demonstrate the feasibility of monitoring and effect of controlling multiple critical process parameter in real-time using Raman spectroscopy.

Results and discussion: Case studies in the presentation will illustrate the impact of tight Raman based bioprocess control to the cells, the cell culture environment, the yield, and finally to the quality of therapeutic proteins [1]. Results of the case studies will provide valuable insights into simple but very effective control strategies and explicate the successful transfer of analytical Raman methods from process development to GMP manufacturing.

Bibliography, Acknowledgements: 1. Berry, Brandon et al. (2016) Quick Generation of Raman Spectroscopy Based In-Process Glucose Control to Influence Biopharmaceutical Protein Product Quality During Mammalian Cell Culture. *Biotechnology Progress* 32, no. 1: 224–34.

Disclosure of Interest: None declared





PO006

A MACHINE LEARNING APPROACH FOR NON-INVASIVE CELL DENSITY DETERMINATION IN ADHERENT CELL CULTURES

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Background and novelty: Cell counting is a cornerstone of cell culture, underpinning evaluation of experimental outcomes, comparison of results across laboratories, and ultimately establishment and quality control of cell culture processes. In adherent cultures, direct measurements of cell densities have traditionally required cell detachment and therefore precluded continuous monitoring. We propose the use of a trainable machine vision algorithm for the non-invasive determination of cell density in adherent cultures based on phase contrast microscopy (PCM) images.

Experimental approach: All machine vision algorithms were developed using MATLAB and JAVA. Experimental validation was carried using Chinese hamster ovary cells (CHO), mouse embryonic stem cells (mESC), neonatal fibroblast cells (HDFn), monkey kidney cells (CV-1), mesenchymal stem cells (MSC) and osteosarcoma cells (MG-63). The output of the algorithm was compared with well-established cell enumeration methods (manual and automated).

Results and discussion: We present QUANTAST, a method to accurately and non-invasively determine cell density in adherent cultures. Cell line-specific regression models were trained based on global texture analysis of PCM images. QUANTAST was found to be 2.7-fold more accurate than manual counting methods and 4.3-fold more accurate than automated cell counters. Beyond the monitoring of 2D cultures, application of QUANTAST to microcarrier analysis is also demonstrated. Additionally, we demonstrated non-invasive measurements of metabolic rates when coupling QUANTAST with on-line oxygen sensors[1] and at-line medium analysis. QUANTAST is accurate, non-invasive, versatile and accessible to any laboratory with basic microscopy capabilities, paving the way for the establishment of robust process analytical technologies for the monitoring of adherent cell culture processes.

Bibliography, Acknowledgements: [1]Super, A., Jaccard, N., *et al.* (2016), *Biotechnol. J.*, doi:10.1002/biot.201500479

Disclosure of Interest: None declared





PO007

INVESTIGATION OF THE APPLICABILITY OF ATR-IR FOR THE ANALYSIS OF MONOCLONAL ANTIBODY AGGREGATION IN BIOPROCESSES

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Background and novelty: Antibody aggregation can occur in different stages of a biopharmaceutical production process and can be a severe problem already in the upstream process. The determination of aggregates in cell culture samples or supernatants proves to be difficult, though. Size exclusion chromatography and microscopic methods are used to identify aggregates, but they cannot be used as online measurement methods in production processes. However, spectroscopic methods such as two dimensional fluorescence spectroscopy (2D-FS) and attenuated total reflection infrared spectroscopy (ATR-IR) can in principle be used for this purpose.

Experimental approach: In this study, we investigated if ATR-IR can be used to analyze aggregates of mAbs. For this purpose, different types of mAb aggregates, induced by methods such as high salt concentrations, heat or freeze thawing, and supernatants from mAb producing processes were analyzed by ATR-IR and 2D-FS as a reference method, which already proved to be applicable for the analysis of mAb aggregates. The obtained ATR-IR spectra were analyzed by common deconvolution methods and multivariate methods, respectively.

Results and discussion: Changes in the Amid I band in ATR-IR spectra indicate normally the increase of beta-sheets of aggregated proteins compared to their monomer. Since antibodies mainly consist of beta-sheets, no change could be observed in this band by common deconvolution analysis. However, when using principal component analysis to analyze the ATR-IR spectra, the different samples could be differentiated appropriate to their aggregation status. Also correlation of the spectra to other data obtained by SEC or microscopic methods was possible when using partial least square regression. The results suggest that ATR-IR might be a well suited method for the establishment of soft sensors which provide real-time information on the aggregation status of a mAb-producing bioprocess.

Bibliography, Acknowledgements: -

Disclosure of Interest: None declared





PO008

DEVELOPMENT OF MEASUREMENT, MONITORING, MODELLING AND CONTROL STRATEGIES IN PRODUCTION PROCESSES WITH CHO CELL CULTURES

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Background and novelty: A better process understanding and control of biopharmaceutical production processes is important for the safety and efficacy of drugs. Therefore, critical process parameters which affect critical quality attributes need to be defined, analysed, and controlled. However, real-time monitoring of important process parameters, like cell or glucose concentration, is often hampered by the lack of direct measurement systems. In order to gain real-time information, different online obtained signals can be combined, enabling the implementation of soft sensors. These can be used for process control, for example automated feeding strategies.

Experimental approach: Fluorescence spectroscopy features high sensitivity and robustness and can be implemented as online measurement system in the production process, which is an important prerequisite for the development of soft sensors. The intrinsic fluorescence signal of the cell culture is a mixture of fluorescence signals that originate from different components such as aromatic amino acids, ATP, NAD(P)H, FAD and vitamins, that change during the production processes. We have investigated the applicability of 2D fluorescence spectroscopy for the online monitoring of CHO cell cultures.

Results and discussion: The excitation/emission spectra of different batch and fed-batch fermentation processes were recorded for this purpose. Multivariate data analysis was used to investigate the multi-factorial relationships of the measured data. In a first step, principal component analysis was used to organize the data, extract the relevant information and to identify outliers. In a second step, partial least square regression was applied to correlate the 2D fluorescence spectra to offline measured process parameters like viable cell concentration, glucose concentration or product titer to generate reliable soft sensors for online monitoring of important production parameters.

Bibliography, Acknowledgements: -

Disclosure of Interest: None declared





PO009

HIGH-THROUGHPUT SAMPLE PROCESSING AND ANALYTICS OF SAMPLES DERIVED FROM HIGH-THROUGHPUT DOWN SCALE BIOREACTOR SYSTEMS

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Background and novelty: High level process understanding or multivariate statistical approaches require an elevated data density and a high number of experiments. This evokes the need for high-throughput cultivation systems. Many robotic bioreactor systems have been developed in the last years, of which some are commercially available (e.g. ambr®, Sartorius Stedim). With up to 48 small-scale disposable bioreactors these fully controlled systems enable parallelization and automation of cultivations. However, operators have to cope with the large amount of samples generated. This entails the need for fast high-throughput sample processing and analytics.

Experimental approach: For metabolite and titer concentration as well as product quality measurements process samples are collected by the ambr cultivation system. Cells need to be removed by centrifugation before samples can be distributed to different racks and tubes required by analytical devices (Cedex Bio HT Analyzer, Roche Diagnostics). Moreover, samples are prepared for amino acid analysis and micro-purification. We tested a pipetting robot (Fluent®780, Tecan) with an integrated centrifuge to accelerate this workflow.

Results and discussion: To generate a high-throughput sample processing method we established a Tecan pipetting robot to automate centrifugation and sample distribution steps for analytical devices. Compared to manual sample handling, the processing time for 48 samples was reduced from 60 to approximately 30 minutes and the operator only has to spend about 5 minutes hands-on. Hence, we used a Tecan Fluent to link ambr bioreactors to a Cedex Bio HT bioprocess analyzer and established a fast and automated workflow for accurate and efficient sample processing.

Bibliography, Acknowledgements: n/a

Disclosure of Interest: None declared





PO010

IMPROVEMENT OF CHO SPECIFIC PRODUCTIVITY USING TYROSINE AND CYSTEINE DERIVATIVES

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Background and novelty: Industrial fed-batch cultivation is used for the production of therapeutic proteins. Amino acids are key elements for the cellular metabolism but also for the quality of the recombinant protein since their availability was linked to sequence variants or specific modifications like trisulfide bonds. To avoid such modifications, the chemical modification of amino acids is an interesting alternative to modulate their overall solubility, stability or chemical reactivity.

Experimental approach: In this study, we analyzed the effect of using S-sulfocysteine sodium salt and phosphotyrosine di-sodium salt in neutral pH feeds on CHO cell growth and specific productivity in small scale and bioreactor experiments. The mechanisms of extra- or intracellular amino acid metabolization were investigated as well as the interaction with other media components. Additionally, gene expression arrays and western blot analyses were used to decipher the mechanisms of increased productivity. Finally, LC-MS based methods were used to study the impact of the chemical modification on several IgG critical quality attributes.

Results and discussion: The use of S-sulfocysteine and phosphotyrosine in a single feed at neutral pH allowed a process simplification and an increased specific productivity. In depth characterization of the monoclonal antibody indicated no change in the glycosylation, or charge variant pattern whereas peptide mapping experiments were not able to detect any integration of the modified amino acids in the sequence of the monoclonal antibody. Finally, the mechanism of increased productivity pointed out to an anti-oxidative cellular response. Altogether, this work provides tools to simplify and improve fed-batch process performance through the use of a single feed containing highly soluble and stable cysteine and tyrosine derivatives.

Bibliography, Acknowledgements: -

Disclosure of Interest: None declared





PO011

MIXING AND TEMPERATURE CHARACTERIZATION OF SINGLE-USE MIXER SYSTEMS

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Background and novelty: Single-use mixing systems are widely used in biomanufacturing. However, process transfer from stainless-steel to single-use vessels is challenged by insufficient knowledge about physical performance. Here we characterized 50, 100, 200, and 500 L single-use mixers based on two key parameters: heat transfer and mixing capacity. We evaluated heating-cooling properties as well as liquid-liquid and solid-liquid mixing times for various volumes and impeller speeds.

Experimental approach: Temperature characterization was performed at temperature intervals of: 5°C-20°C; 20°C-37°C; 37°C-20°C; and 20°C-5°C. Impeller direction and speed were constant. Heating/cooling times were established by calculating the time to reach 95% of the temperature step change (t_{95}). Liquid-liquid mixing was investigated by adding acid to the liquid surface while recording the pH for variable impeller speed, working volume, and viscosity. Probes were placed at several bag locations to investigate mixing homogeneity. Mixing times were established by calculating the time to reach 95% of a pH step change (t_{m95}). Solid-liquid mixing was evaluated for salt (PBS) and powdered medium (HyClone™ HyCell™ CHO). For both solids, mixing time was assessed at two different volumes and impeller speeds by calculating t_{m95} of the conductivity step change.

Results and discussion: Heating was achieved within 2 h for all mixer sizes tested. Cooling time was 2 h for mixer sizes up to 200 L and 3 to 4 h for the 500 L mixer. Homogenous mixing of liquids was achieved within 45 s for all mixer sizes and viscosities tested. For solid-liquid mixing, PBS was mixed within 2 min and the medium powder was dissolved within 4 min. These results should aid implementation of single-use mixers in new facilities and help in process optimization and scale-up.

Bibliography, Acknowledgements: Characterization of Xcellerex™ XDM and XDUO single-use mixers 29237251 AA 12/2016 gelifesciences.com/xcellerex

Disclosure of Interest: None declared





PO012

DEVELOPMENT OF A CHO CELL CULTURE PLATFORM FOR MONOCLONAL ANTIBODY PRODUCTION: FROM CLONE GENERATION TO PILOT PLANT SCALE-UP

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Background and novelty: Chinese Hamster Ovary (CHO) cells have become the industry standard for mAb production. There is a desire to decrease the duration of process development, from clone generation to production at pilot scale and tech transfer to a GMP facility. A generic platform approach can help simplify process development, reducing its length and cost. The National Research Council Canada (NRC) has developed its own 'CHO^{BRI}' cell platform for mAb production using a cumate gene switch to produce proteins in transient and stable systems, generating hundreds of milligrams to grams of material for early evaluation.

Experimental approach: Stable CHO cell pools producing high levels of the protein of interest are generated within two weeks post-transfection. Using a ClonepixTM device and semi-solid medium plating, 400 colonies are screened for productivity. The top 96 clones are selected following a primary fed-batch suspension culture and a few iterations. The most promising 48 clones are then evaluated with a generic fed-batch approach at small-scale using commercially available culture medium and feed. Final clone selection is performed in bench-scale bioreactors (1 L). Subsequently, custom fed-batch development is available to maximize antibody productivity. Analysis of product quality attributes is typically conducted to evaluate the impact of process improvement on product quality. Process robustness is then assessed at 3-10 L scale; 20-200 L pilot plant scale productions are used to generate material for DSP development and testing for research purposes.

Results and discussion: The NRC has produced several stable CHO clones expressing mAbs, from transfection of the desired gene and clone selection to fed-batch optimization and scale-up of the bioreactor process to a pilot plant scale, ready for tech transfer to CMOs. This poster presentation showcases recent studies performed at the NRC.

Bibliography, Acknowledgements: None

Disclosure of Interest: None declared





PO013

ON THE ROAD TOWARDS THE PRODUCTION OF NANOPARTICLES FOR UTILIZATION IN DEFINED MEDIA

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Background and novelty: Nowadays, the encapsulation of molecules into nanoparticles is a promising opportunity to increase the bioavailability and stability of labile or poorly soluble ingredients for cells. Beyond medical applications, they can also be applied for biotechnological approaches. In this study, the particles are used to tune customized cell culture media by providing encapsulated supplements. However, the production and cleaning procedure of particles has to be adapted to novel particles, as the composition of defined media differs from body fluids known from medical applications of particles.

Experimental approach: A series of new polymeric particles was produced and investigated regarding their utilization in chemically defined media. For this purpose, polymers based on commercial and self-made polyesters and polymethacrylate were used to encapsulate different ingredients, such as the hardly soluble cysteine or labile components such as insulin. Different encapsulation methods were applied based on nanoprecipitation, emulsion, and microfluidic approaches. We further compared different stabilizers and cleaning procedures concerning their influence on particle stability, efficiency, encapsulation, and particle size. Finally, the cellular uptake of nanocarriers was analyzed using flow cytometry.

Results and discussion: Particles of different sizes (100 to 300 nm) and compositions were formed, showing that the encapsulated ingredient influences the optimal procedures for particle production and cleaning. The solubility of the ingredient was found to be the most important parameter for particle production, whereas particle size and stability revealed an influence on the cleaning procedure. Regarding the cellular uptake, the efficiency can be tuned by the surface charge of the particles and the media composition.

Bibliography, Acknowledgements: We thank the German Federal Ministry of Education & Research (BMBF # 031A518B Vectura) for funding.

Disclosure of Interest: None declared





PO014

INOCULUM PERFORMANCE UNDER DIFFERENT CULTURE CONDITIONS: ONE CLONE, MULTIPLE BEHAVIOR.

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Background and novelty: In manufacturing processes of monoclonal antibody, the Upstream Process normally consists of a series of inoculum generation steps and a production step. The inoculum generation steps are required to generate sufficient cells to inoculate the Production Bioreactor, which is where product is made. Production Bioreactor step is considered to be very crucial as product is made in this step. A typical requirement for the inoculum generation stage is to expand the cells in each step while keeping them in their exponential growth phase. The cells spend a significant portion of process time in this stage from vial to shake flasks and seed bioreactors. During these passages, the cells encounter some environmental changes like pH, Osmolality, conc. of nutrients and waste metabolites. These small changes can have a profound impact on the cells when experienced over multiple passages. The effect of this cumulative impact may not be apparent during the seed stage but can be evident in the Production step.

Experimental approach: In this work we present the data from experiments with cells maintained under different inoculum culture conditions and evaluating the behaviour in terms of their growth performance in Production Bioreactor. The conditions include parameters like varying pH, Osmolality, Temperature etc. Based on the initial screening experiments, the interaction studies for the chosen conditions were performed using a DOE model.

Results and discussion: The results of our experiments indicate that cells when maintained under different inoculum culture conditions can behave significantly different when used in the production process. We also identified that these conditions impact the overall cell line stability of a clone. Thus manipulation of culture conditions suitably in the early inoculum propagation steps enables improving performance and stability of a cell line, thereby increasing process robustness.

Bibliography, Acknowledgements: Downstream Team, Biocon Ltd.

Disclosure of Interest: None declared





PO015

ADVANTAGES AND CHALLENGES OF A CONTINUOUS UPSTREAM PROCESS

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Background and novelty: In the recent years, there is a renewed interest in continuous processing for the manufacture of Biopharmaceuticals primarily driven by cost advantages (reduced capital cost, higher product formation) and availability of better cell retention devices. The continuous process helps in overcoming some of the disadvantages of a fed batch for e.g.: Prevention of accumulation of toxic metabolites, Ability to achieve and maintain higher cell densities for longer batch durations etc.

Experimental approach: A perfusion process was developed in the lab for multiple products using 1/2L bioreactors, evaluating different retention devices such as ATF, Hollow fibre technology and spin filter system. The factors responsible for better cell growth and higher product yield were determined and optimized for consistent process performance. These processes were also compared with their respective Fed batch processes.

Results and discussion: In this poster, the optimization of continuous process for mAbs is discussed with a focus on how the continuous process is different and offers certain advantageous over fed-batch processes. We provide few case studies comparing fed-batch and continuous modes of process for the same set of mAbs. We show that in continuous processing, production efficiency increases multi-fold by increase in cell conc., cell specific productivity and extension of batch duration. However, these processes are operationally complex to run. On the other hand, Fed-batch processes are relatively simpler and easy-to-run. In this poster, with the help of these case studies, we attempt to assess the advantages, limitations and challenges of both types of processes.

Bibliography, Acknowledgements:

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Disclosure of Interest: None declared





PO018

ANALYSIS OF PRODUCT QUALITY ATTRIBUTES BY MIR SPECTROSCOPY

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Background and novelty: Cell culture processes require excellent control of process parameters and cell metabolism because these factors substantially influence product quality. The product quality of monoclonal antibodies, for instance, is strongly affected by changes in pH, temperature and substrate concentrations (1). However, the analysis of substrates, metabolites and product quality attributes in cell culture broth is costly, time consuming and requires the use of numerous analytical methods, many of which cannot be carried out at-line. For this reason, real-time process control is difficult. Newly developed technology utilising mid infrared (MIR) spectroscopy may help to simplify this process by providing a sensitive method for analysing numerous analytes in aqueous samples within a few minutes.

Experimental approach: In order to test this approach, samples from 12 fed-batch cultivation runs differing in process pH values and feeds were analysed, on the one hand, by classical methods using gel electrophoresis and liquid chromatography and, on the other hand, using MIR spectroscopy with principal component analysis (PCA).

Results and discussion: The profiles obtained via MIR spectroscopy contain precise molecule-specific information about glycosylation patterns and aggregate formation. This allowed for a clear correlation with attributes measured by classical methods and could therefore also be used to classify acceptable and unacceptable product quality. MIR technology also provides additional information on protein structure. Overall, the application of MIR spectroscopy for the analysis of product quality provides valuable information quickly and without elaborate sample preparation.

Bibliography, Acknowledgements: (1) Hossler, P.; Khattak, S.F.; Li, Z.J. (2009): Optimal and consistent protein glycosylation in mammalian cell culture. *Glycobiology* 19 (9): 936-949

Disclosure of Interest: None declared





PO019

INFLUENCE OF MEDIA SUPPLEMENTS ON NANOPARTICLE PERFORMANCE

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Background and novelty: In the field of biotechnological process engineering, the choice of the ideal cell culture media represents an important factor for the efficiency and quality of biotechnological products. Up to now, manufacturers created special media for growth and transfection based on decades of experience with common non-viral vectors like poly(ethylene imine). In the expanding field of polymeric vectors and particles a rethinking of traditional media supplements and their influence on different types of polymeric systems has to be considered to enhance efficiency.¹ In particular, the effect of parameters of the vector and the interaction between particle and additives can have a great influence on the performance and should therefore be investigated.

Experimental approach: In this study the influence of common supplements on cationic and anionic charged polymethacrylate copolymer and *poly(lactic-co-glycolic acid)* particles was investigated. Therefore, chosen inorganic and organic supplements have been varied in their combination and concentration based on a chemically defined media to examine particle aggregation, surface adsorption and uptake efficiency. In addition to basic investigations on viscosity and ionic strength, confocal laser scanning microscopy, dynamic light scattering and flow cytometry have been used for a better understanding on adjustable parameters.

Results and discussion: All in all, organic supplements showed a great influence by the promotion or prevention of particle aggregation and, thereby, uptake depending on the nature of the supplements, whereas the inorganic compounds showed no impact. The results of the zeta-potential and size measurements in combination with uptake experiments confirm a good uptake by the reduction of particle aggregation.

Bibliography, Acknowledgements: We like to thank the German Federal Ministry of Education & Research (BMBF # 031A518B Vectura).

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Disclosure of Interest: None declared





PO020

HIGHTHROUGHPUT SCREENING AND MULTIVARIATE ANALYSIS IDENTIFY CRITICAL COMPONENTS DURING CHO MEDIA AND FEED DEVELOPMENT

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Background and novelty: A challenging aspect of culturing recombinant cell lines is quickly identifying and optimizing critical components to achieve a desired endpoint such as optimized glycosylation profiles. Biopharmaceutical industry pressure to shorten the duration of media and feed development required the generation of a High Throughput System (HTS) that allowed a global evaluation of cell culture parameters during screening to target subsequent optimization.

Experimental approach: Model cell lines are screened with a minimum of 78 media or feed mixtures derived from our diverse library. Growth conditions and metabolites along with product titer and quality are evaluated for each condition. Multivariate analysis (MVA) of the media or feed formulations and cell culture endpoints allows for the correlation of individual components to desired cell culture targets. Targeted optimization of these components can be performed using subsequent Design of Experiment (DOE) assays.

Results and discussion: Due to the diversity of components in the media panel, cell culture outputs (e.g. peak viable cell density, cumulative titer, lactate production, glycosylation, etc.) vary significantly in the HTS media screen. The HTS feed screen yields equally divergent results. Using the various key outputs as responses during MVA of the formulations, the critical components affecting these parameters are elucidated. Each component can then be optimized using DOE for assay construction. These assays determine which components and best concentration of each are the ideal combination for each individual cell line. This methodology is also applied when designing a platform media system. This targeted approach to determine new formulations not only decreases the amount of time required, but also allows incorporation of key outputs at the start of development.

Bibliography, Acknowledgements: We would like to thank the Analytical R&D team for their continued support.

Disclosure of Interest: None declared





PO021

BIOPROCESS ENGINEERING STRATEGIES FOR ENHANCED GAG-VLPs PRODUCTION IN STABLE INSECT CELLS

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Background and novelty: Insect cells have been widely used for recombinant protein production, mostly using the lytic baculovirus vector system. Stable expression in such hosts has been increasingly explored to circumvent baculovirus-related issues, but the protein titers achieved are still seemingly low. Bioprocess optimization schemes are therefore critical to increase productivities. In this work, we assessed the impact of i) adaptive laboratory evolution to hypothermic culture conditions and ii) supplementation with productivity enhancers on Gag-Virus like particles (VLPs) production in stable insect cells.

Experimental approach: Stable Sf9 and High Five cells tagged with eGFP-fused Gag protein were adapted to hypothermic culture conditions (22°C) by sequential sub-culturing over 2-3 months. In addition, cell cultures were supplemented with chemicals known to promote expression of recombinant proteins in mammalian cells systems, sodium butyrate (NaBu) and dimethyl sulfoxide (DMSO). Gag-VLPs titers were estimated by ELISA and product stability demonstrated by electron microscopy.

Results and discussion: The production of Gag-VLPs was enhanced under hypothermic culture conditions (up to 30-fold). Noteworthy, the adaptation process seems to be critical for maximizing titers as cells cultured directly at 22°C (without adaptation) induced lower titers. Supplementing NaBu and DMSO to cell cultures proved to be beneficial for protein expression (up to 7-fold improvement). The synergistic effect of these strategies on Gag-VLPs production is currently being evaluated. This work demonstrates the potential of the bioprocess engineering strategies herein proposed for improving production of Gag-VLPs in stable insect cell lines.

Bibliography, Acknowledgements: Support from EU FP7/2007-2013/ under REA grant agreement n° [602640] and Fundação para a Ciência e Tecnologia (PhD fellowship SFRH/BD/90564/2012).

Disclosure of Interest: None declared





PO022

BIOPROCESS INTENSIFICATION AND OPTIMIZATION USING MACROSCOPIC PREDICTIVE MODELS OF CELL CULTURE PROCESSES

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Background and novelty: Optimal feeding during biopharmaceutical fed-batch production is dependent on cell metabolism which can be characterized by mathematical models. However, throughout the bioreactor production, the cells adapt to the changing extracellular conditions resulting in different metabolic phases. This study provides insights into the predictive capacities of systematic and simple cell modeling approaches of metabolism, growth and production of monoclonal antibodies (mAb) during CHO fed-batch culture which remains correct upon scale up.

Experimental approach: Different experimental conditions in 2 L CHO fed-batch cultures were analyzed. Amino acids, glucose, lactate, ammonium and mAb were quantified. We applied the metabolic steady state concept and used a segmented linear model to predict cell metabolism. The external metabolite rates are expressed as a linear function of the specific growth rate with various breakpoints associated to metabolic shift [1]. In a next step a fully predictive cell growth model was established by incorporating growth kinetics for the identified phases.

Results and discussion: Using the cell metabolism model structure and parameter values from a 2 L training data set, it was possible to predict metabolic rates of new fed-batch cultures in both 2 L and 2000 L scales. The final mAb titer can also be predicted even if the cells are starved in some essential metabolites. The cell growth model combined to a linear piecewise regression model of cell metabolism also allows us to get an accurate *in silico* prediction of the impact of untested feeding strategies on cell culture performance. For both models an entire and complex metabolic network model is not needed which makes them easily accessible.

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Disclosure of Interest: None declared





PO023

DIFFERENTIAL ANALYSIS OF IGG PRODUCT QUALITY BY INTACT MASS ANALYSIS FOR FED-BATCH-CULTIVATED CHO CELLS UNDER GLUCOSE LIMITATION

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Background and novelty: Chinese hamster ovary (CHO) cell culture has been widely used for production of monoclonal antibodies in the pharmaceutical industry. Previous studies have shown that the cell specific productivity in CHO cells can be increased by glucose limitation (Wingens et al., 2015). Introducing a productivity enhancing effect it is possible that this also affects the quality of the product such as glycosylation or other posttranslational modifications.

In this work, we are focusing on the impact of glucose limitation and increased productivity on the product quality of a monoclonal antibody produced in a fed-batch cultivation of CHO cells.

Experimental approach: CHO cells were cultivated both under limiting (0.2 – 0.5 g/L glucose) and non-limiting glucose conditions (1.5 – 3.0 g/L) in fed-batch mode in a multiple vessel parallel single-use system. Both cultivations were performed in a chemically-defined, animal-component free CHO growth medium (Xell AG) and fed with similar volumes of CHO feed medium (Xell AG).

Viable cell density and viability were determined using an automated cell counting system. The product titer was determined via Protein A HPLC, the analysis of product quality was performed by intact mass analysis using HPLC-MS.

Results and discussion: The CHO cell culture cultivated under glucose limitation reached a 54% higher viable cell density than the reference culture. The product titer was even increased by 98%.

The product quality showed significant changes under glucose limitation. As expected, the glycation level decreased from 3% to 1% compared to the reference culture. The truncation level of C-terminal lysine at the heavy chain of the mAb increased from 79% to 88%. The glycosylation was also significantly influenced by the glucose limitation: The non-fucosylated variants increased from 3% to 6%, the degree of galactosylation increased from 31% to 39%.

Bibliography, Acknowledgements: -

Disclosure of Interest: None declared





PO024

BROAD ANALYTICAL PLATFORM DEVELOPMENT FOR ADVANCED PROCESS MONITORING AND CONTROL IMPLEMENTATION

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Background and novelty: Today, monoclonal antibodies (mAbs) are produced based on a rigid quality by testing approach. Tight process control prevents to react on process variations and subsequently to achieve a consistent high quality output. However, by applying in-depth process knowledge and advanced process control (APC) techniques the operation becomes highly flexible. Implementation of such a Quality by Design (QbD) concept requires a broad analytical platform, achieving reasonable at- and off-line data of good quality for robust process model development. Though, due to the amount of data needed, the analytical part will become inherently the bottleneck.

Experimental approach: In this project an industrial relevant mAb was produced by Chinese hamster ovary (CHO) cells in fed batch mode in shake-flask (300 mL) and continuous stirred tank reactor (CSTR) cultivations (15 L & 100 L). Certain design of experiments (DoE) concepts were applied for the experimental runs.

Results and discussion: Different at-line analytical methods were developed and established for fast, efficient and flawless determination of certain attributes and parameters. For instance, charge distributions and aggregation behaviour of mAb's was proven to be measurable directly out of the supernatant without preprocessing. A sedimentation velocity analysis leads to assumptions about cell size and can indicate viability and cell aggregation. Supplementary, establishment of a cheap and simple method for determination of the host cell protein (HCP) content allows to assess an ideal harvest time. Parts of these data were already sufficiently incorporated into the development of a hybrid shaker model. In future, the established methods will be important for advanced process monitoring and control including model predictive control (MPC) for CSTR.

Bibliography, Acknowledgements: .

Disclosure of Interest: None declared





PO025

OPTIMISATION OF TRANSIENT EXPRESSION PLATFORM TO INCREASE TITRE AND THROUGHPUT

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Background and novelty: Development of antibody therapeutics, from early stage research through to preclinical and clinical development, requires ever increasing amounts reagents. In order to meet the challenge of furnishing a diverse and full pipeline we utilise several different transient platforms. Through continuous optimisation, streamlining and automation of component parts of our panel of platforms (utilising both HEK293 and CHO host cells with a variety of transfection methods) we now have the capability to produce microgram to gram quantities of panels of purified antibodies and antibody fragments in as little as 4 weeks from receipt of plasmid DNA.

Experimental approach: utilising several different transient platforms CHO/HEK optimised component parts of our panel of platforms

Results and discussion: Now have the capability to produce microgram to gram quantities of panels of purified antibodies to produce microgram to gram quantities of panels of purified antibodies and antibody fragments in as little as 4 weeks from receipt of plasmid DNA.

Bibliography, Acknowledgements: Protein Sciences Team, UCB

Disclosure of Interest: None declared





PO026

NEW SOLUTIONS FOR VIRUS RISK MITIGATION IN CELL CULTURE MEDIA

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Background and novelty: The contamination of bioreactors with infective agents like bacteria, mycoplasma and viruses is a risk and a widely debated topic currently within the biopharmaceutical industry. A variety of methods exist to effectively prevent contamination with bacteria or mycoplasma however the risk from viruses, in particular small non-enveloped viruses, which have caused multiple contaminations recently, is a greater challenge.

Experimental approach: Virus clearance technologies, effective for small non-enveloped viruses, are important during upstream processing and we will demonstrate that size exclusion-based filtration (20 nm nominal pore size) provides a robust, rapid and cost effective strategy for upstream protection of cell culture media and additives.

Results and discussion: This presentation will focus on new developments within virus retentive filtration in upstream operation. The characteristics of the new membrane including virus retention performance of > 4 log reduction for small non-enveloped viruses will be presented for different cell culture media. In addition, we will also present data proving that 1000 LMH/4h filtration time at 2 bar is a realistic filter throughput with commercial media, that filtered media has no impact on cell culture performance and cell culture media composition and strategies to achieve optimal performance.

Bibliography, Acknowledgements: Birte Kleindienst - Junior Product Manager Virus Clearance, Purification Technologies Division, Sartorius Stedim Biotech

Birte Kleindienst studied at the University of Applied Sciences in Hamburg and holds a Master Degree with honour in Pharmaceutical Biotechnology. She joined Sartorius within the Purification Technologies division in 2015 and took over the product responsibility for virus clearance beginning of this year.

Disclosure of Interest: None declared





PO027

OBSERVATIONS OF CELL CULTURE PERFORMANCE AND PRODUCT QUALITY UPON SUBCLONING OF CHO PRODUCTION CELL LINES

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Background and novelty: There has been much discussion recently regarding the importance of single-cell clonality at the time of production cell line isolation, in the context of recombinant protein manufacturing (1). While new methods have been implemented to methodically verify single-cell clonality, many current production cell lines were developed before such techniques were established. We are curious to understand if heterogeneity in phenotype and product quality exists in daughter cell lines derived from subcloning of existing CHO production cell lines that were produced with varying methods.

Experimental approach: We subcloned different CHO production lines. Some were originally cloned with technology that verifies single-cell per well cloning via imaging. Another was produced with limited-dilution cloning and relied on a mathematical model to predict the probability of a single cell per well at the onset of cloning. We observed the cell growth and product quality characteristics of subclones derived from various parental lines.

Results and discussion: There were similar levels of non-trivial variation in cell culture performance and product quality in all three sets of subclones.

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Disclosure of Interest: None declared





PO028

HYDRODYNAMICS OPTIMIZATION IN HUMAN CELL PERFUSION CULTURE

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Background and novelty: High cell density perfusion operation is a way to produce biopharmaceuticals at high yield. Cell separation based on hollow fiber filter alternating tangential flow filtration (ATF) and tangential flow filtration (TFF) has been reported to potentially generate very high densities [1][2]. To achieve high density of healthy cells, the culture conditions should not be mechanically detrimental to the cells. Although CHO cells are resistant to mechanical damage, human HEK293 cells, interesting for the production of biologics, are more sensitive. We present here a study to obtain the optimal hydrodynamics conditions for reduced mechanical damage compatible with HEK293 cells achieving for the first time very high cell densities.

Experimental approach: We review the different mechanisms responsible for mechanical damage in bioreactor with ATF or TFF, compare these latter and optimize the settings of the bioreactor and separation device for HEK293 cell perfusion culture in stirred tank scale-down bioreactors.

Results and discussion: We identify the shear rate potentially damaging HEK293 cells and relate it to the mechanical forces of the system. The effects of the impeller configuration and of the re-circulation flow rate in the separation device are studied theoretically and experimentally, and optimized for perfusion cultures of HEK293 cells producing EPO or producing C5. Using this optimized perfusion system, we obtain cell densities stabilized at 80×10^6 cells/mL in stirred tank scale-down bioreactors, showing high production of the product of interest.

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Acknowledgements: This work has been carried out at the Wallenberg Centre for Protein Research with co-funding of the Knut and Alice Wallenberg Foundation and AstraZeneca-MedImmune.

Disclosure of Interest: None declared





PO029

CONTROLLING TERMINAL SIALYLATION OF MAB THROUGH CULTURE CONDITION

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Background and novelty: Monoclonal antibodies or mAbs are one of the bestselling biologics on the market and one of the most effective. Their ability to treat a wide range of diseases specifically and with minimal side-effects has transformed them into one of the most important treatments for combating cancers and autoimmune diseases. One of the main areas that pharmaceutical companies are concerned with, in regards to mAbs, is the production of homogenous glycans, as mammalian systems do not always produce these. Glycans are oligosaccharide chains attached to proteins that assist with various functions of the protein. The glycans can affect mAb half-life, efficacy and safety based on the composition of sugars within the chain. Bioprocessing is one route that can be used for this controlling the composition.

Experimental approach: Changing growth conditions effects the cell's metabolism and enzyme activity which consequently effects the composition of glycan chain. The aim of this project, is increasing the sialic acid content on the glycan chain. Sialic acid, the terminal sugar added to glycan, and confers a longer half-life and bioavailability a drug. pH shifts have been shown to be an effective method for controlling sialylation. Three cell lines, producing one product, are being run on an ambr24 system. Each cell line is to be run at 7.1, 6.8 and a shift from 7.1 to 6.8 pH within the run.

Results and discussion: Earlier preliminary bioreactor runs have returned promising results for the effectiveness of a pH shift on sialylation. The ambr run will determine the effectiveness of a shift across multiple cell lines.

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Disclosure of Interest: None declared





PO030

INTEGRATED MEDIA BLENDING INCREASES EFFICIENCY OF CLONE SELECTION

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Background and novelty: In cell line development, many evaluation steps are performed to reduce number of candidates until selection of the manufacturing clone. They are often done in the same chemically-defined media, leading to selection of the best adapted clone to the screening medium and not the best one per se. Media optimization is usually performed at larger scale only once the final clone is selected. By combining clone selection and media blending, a better performing clone may already be selected at an early stage and reduce further optimization steps.

Experimental approach: Media components were classified into 11 different groups, and subsequent blending (160 conditions) performed with three different mAb producing CHO clones. Two-week fed-batch cultures were carried out in 96 deep-well plates. Viable cell density, viability and product titer were monitored. The groups exhibiting the strongest effects on productivity were identified using multivariate data analysis.

Results and discussion: The media blending produced a great growth and productivity diversity for each clone. The viable cell-densities varied between 6 to 21 mio cells/mL, and the product titer at harvest between 1,6 and 6,3 g/L. For most groups, the observed effects were clone-specific, highlighting the relevance of media blending during clone selection. For every clone there was a condition that allowed to obtain better titers than the proprietary media, showing a clear advantage of blending early in selection.

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Disclosure of Interest: None declared





PO031

HOW CELL CULTURE AUTOMATION BENEFITS UPSTREAM PROCESS DEVELOPMENT

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Background and novelty: We developed an automated, multiwell plate (MWP) based screening system for suspension cell culture which is now routinely used in cell culture process development. It is characterized by a fully automated workflow with integrated analytical instrumentation and uses shaken 6-24 well plates as bioreactors which can be run in batch and fed-batch mode with a capacity of up to 768 reactors in parallel. A wide ranging analytical portfolio is available to monitor cell culture processes and also cooperation with internal HT analytic groups to characterize product quality.

Experimental approach: This poster presents an overview about the benefits of using cell culture automation in late stage process development based on 2 examples of current applications. For this purpose we show the experimental results of the development work of two late state projects using the in-house developed automated cell culture system.

Results and discussion: For one project the final product concentration could be increased by factor 2.5 by a media screening and changing to the in-house media platform. The second example shows the capability of the automated cell culture system by reducing trisulfides significantly in just one experiment. These two examples show the potential of cell culture automation as a routine tool in process development.

Bibliography, Acknowledgements: The authors would like to thank:

All Roche Penzberg portfolio project teams

The Roche Penzberg pilot plant and GMP facility team

Management Pharma Biotech Development and Bio-TED SC

Disclosure of Interest: None declared





PO032

IMPACT OF PROCESS AND CHO CELL ENGINEERING ON ANTIBODY YIELD AND QUALITY

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Background and novelty: To meet the needs of the growing antibody market, it is crucial to develop effective fed-batch cell culture processes that can ensure high product yield and quality. In this study, we have assessed the relative impact of both process and cellular engineering strategies on the production yield and quality of an antibody.

Experimental approach: We performed a comparative metabolic analysis of a CHO cells line and a derivative clone expressing the PYC2. Both cell lines were grown in the presence of 3-^[13C] lactate. The isotopic enrichment of several intracellular species were measured by mass spectrometry. Both cell lines were cultivated in fed-batch cultures, performed using different concentrated feed solutions and addition of manganese and galactose, and we have compared the resulting glycan distribution obtained via HILIC analysis.

Results and discussion: The PYC2 cells led to a reduction in waste accumulation that is associated with a significant increase in product titer in batch culture. Metabolic analysis revealed that, in parental cells, glycolysis and lactate production were relatively maintained over the time, while TCA was significantly decreased. In contrast, PYC2 cells displayed a reducing glycolysis activity, a shift to lactate consumption and a concomitant increased in TCA fluxes. Despite exhibiting greatly different metabolism and antibody productivities, only minor variations in galactosylation were observed between the PYC2 and the parental cell lines. The maximum product titer is however reached earlier with PYC2-expressing cells, allowing to harvest the cultures earlier and circumvent the adverse effects of increased culture duration on antibody glycosylation. Our study also shows that galactose and manganese supplementation can mitigate the negative impact of culture longevity on antibody glycosylation.

Bibliography, Acknowledgements: Toussaint, C., Henry, O., and Durocher, Y. (2015).

Disclosure of Interest: None declared





PO033

NEW MODELS FOR PREDICTING MICROCARRIER JUST-SUSPENDED STATE IN BIOREACTORS

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Background and novelty: Today, rather close attention is paid to mesenchymal stem cells as a tool for regenerative medicine. Due to their need to adhere on a solid surface to grow and proliferate, 3D systems involving microcarriers suspended in a stirred tank bioreactor are studied. To reach a large scale production required to meet the high cell dose demand, some challenges have to be overcome, including the understanding and the control of hydromechanical stress generated in the vessel. This work is more particularly focused on the determination of the critical impeller agitation rate N_{js} , ensuring complete beads suspension. It is indeed generally assumed that this value is a good compromise between sufficient nutrients concentration homogenization, mass transfer and hydromechanical stress encountered by the cells. However, no robust correlation predicting N_{js} can be found in literature.

Experimental approach: To fill this lack, a design of experiments was carried out, dealing with 5 impeller designs, 3 D/T ratios defining the impeller diameter on the vessel diameter, 3 C/T ratios defining the impeller clearance off the base on the vessel diameter, 5 particles volume fractions and 2 different microcarrier types. N_{js} was thus measured for 90 operating conditions, using the 1-2s Zwietering criteria.

Results and discussion: A dimensional analysis was assessed to evaluate the impact of each parameter and to establish an empirical correlation. Simultaneously, an innovative strategy based on Computational Fluid Dynamics simulations was conducted in order to predict N_{js} and a new calculation method was proposed. The N_{js} values obtained numerically were then validated with the experimental data.

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Disclosure of Interest: None declared





PO034

BIOSIMILARS DEVELOPMENT CASE STUDY: HOW TO MATCH BOTH GLYCOPROFILE AND CHARGE PROFILE?

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Background and novelty: Development of biosimilars has drawn the attention of many big and small actors of the pharmaceutical industry. Glycosylation and charge profiles are two key quality attributes that must be similar in the developed monoclonal antibody (mab) and the original molecule. In this study, we present two cases of biosimilar mabs development in Chinese Hamster Ovary (CHO) cells where matching both glyco- and charge profiles raised questions about their relationship in two different configurations.

Experimental approach: The expressing clones were created with the same parental CHO cell line. In order to match the originator glycoprofile, the galactosylation level had to be decreased in the first case and increased in the second case. The second case was initially thought to be the easiest to solve since efficient and easy-to-use feeding strategies allowing higher galactosylation levels have been developed and commercialized. However, enhancing glycosylation similarity happened to reduce charge profile similarity. In contrast, in the first case, the culture parameters required to match the glycosylation profile also allowed to reach the right charge profile.

Cell culture parameters (pH, temperature) were screened in 3L single-use [Mobius] bioreactors. Feeding strategies for galactosylation adjustment were screened in 50-mL spintubes and confirmed at the 3L scale. The glycoprofiles were assessed by hydrophilic interaction liquid chromatography (HILIC) and the charge profiles were determined by capillary isoelectric focusing (cIEF).

Results and discussion: The impact of pH and Ex-Cell Glycosylation Adjust (GAL+) on glycosylation and charge profiles will be shown. The relationship between pH, glycosylation profile and charge profile in this CHO cell line will be discussed.

Bibliography, Acknowledgements: Acknowledgments: Mammalian UpStream and Analytical Development Teams – Merck Biodevelopment – Martillac (France)

Disclosure of Interest: None declared





PO035

CASCADING EFFECTS IN BIOPROCESSING: THE IMPACT OF CELL CULTURE ENVIRONMENT ON MAMMALIAN CELL BEHAVIOUR AND HOST CELL PROTEIN SPECIES

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Background and novelty: One of the reasons for the rejection of new drugs during clinical trials is the presence of host cell proteins (HCPs) in the drug. HCPs are immunogenic contaminants that can compromise patient safety. Proteases and chaperones compromise the integrity of product. Current downstream purification platforms are challenged by HCP-mAb co-elution. A Quality by Design strategy to reduce HCPs is by tracing their source back to upstream culture and eliminating it. Previous study showed that upstream culture parameters, e.g. harvest time and culture temperature, significantly affect HCP profiles. However, little is known about how host cells coordinate their molecular machinery under different culture environment that results in different HCP profiles.

Experimental approach: Studies were performed to link the bioreactor temperature of CHO cell culture producing IgG₄ with cell health and cell cycle distribution (NucleoCounter), mAb yield (BLitz), HCP level (HCP ELISA kits) and HCP species (LC-MS/MS).

Results and discussion: Cells were more robust under mild hypothermia: over 90% of cells were maintained in a healthy state until the decline phase, and the onset of apoptosis was less evident compared to the results for physiological temperature. IgG₄ titre and HCP level at harvest were comparable between the two cases. However, mild hypothermia reduced the HCP variety by 36%, including 50% and 30% lower proteases and chaperones, respectively. The differences in HCP variety at harvest resulted in a significantly different HCP profile post-Protein A purification between the two cases. Half of the critically immunogenic HCPs species as determined by the CHOPPI tool were different between the two cases. This study shows that cell culture conditions significantly affect the HCP profile at harvest and that of purified samples.

Bibliography, Acknowledgements: We like to thank Dr Bernadette Byrne for the access to Life Science lab.

Disclosure of Interest: None declared





PO036

DEVELOPMENT OF A NOVEL POLOXAMER 188 SHEAR PROTECTANT

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Background and novelty:

Poloxamers 188 is a surface-active nonionic polymer used in cell culture media as shear protectant. It was demonstrated to increase the robustness of mammalian cells to shear from sparging [1,2] as well as agitation [3]. Performance variation between lots has been reported [4,5]. It seems that current lot-to-lot variation would require users to investigate each lot in relevant scale-down models [6]. Therefore, we developed a novel Poloxamer 188 with reliably high protective effect [7,8].

Experimental approach:

Several cell-based tests were performed to assess the protective effect of Poloxamer 188 (or lack thereof) on mammalian cells, as well as various analytical techniques to understand the underlying chemical properties of the different lots [7,8].

Results and discussion:

We have investigated ~150 Poloxamer 188 lots from several suppliers as well as lots provided to us by biopharma customers facing issues with Poloxamer. A classification of lots by their protective effect allowed to derive specifications positively correlated with shear protection. These were used to develop a cell culture optimized Poloxamer 188 which reliably protects mammalian cells from shear stress in suspension culture.

The novel Poloxamer performs reproducibly superior than lots across different suppliers, lots, quality grades and – in one case – two manufacturing sites investigated.

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Disclosure of Interest: None declared





PO037

EXPERIENCES WITH A PARALLELIZED AND AUTOMATED SMALL SCALE FERMENTATION SYSTEM REGARDING SCALABILITY OF RESULTS IN PROCESS DEVELOPMENT

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Background and novelty: DoE experiments have become a standard tool during upstream process development. Screening experiments with full factorial designs are mostly set up in high throughput robotic systems which are highly automated but limited e.g. in pH control. As pH is seen as one of the most critical parameters for cell growth and product quality the best results from such screening experiments are confirmed in parallelized small scale fermentation systems with reduced DoE designs.

Experimental approach: In Pharma Technical Development the use of a parallelized small scale fermentation system for cultivation of mammalian cell lines was established in order to increase the throughput of fermentations under controlled conditions. The parallelized and highly automated 250 mL fermentation system fills the gap between the robotic screening system and the 2 L scale which is mainly used for process design studies.

Results and discussion: Results from fermentations in the 250 mL system will be compared with cultivations in 2 L and 1000 L scale for different projects. Despite cell growth and metabolic data also product quality data will be shown. Initial differences between data from the 250 mL and 2 L scale were eliminated by implementing a consistent pH measurement device and method as well as using the information generated by offgas analysis.

Bibliography, Acknowledgements: The author would like to thank Tanja Leitner, Stephan Schroot and Roman Greppmair for their contribution during the establishment of the mentioned small scale fermentation system at Roche Pharma Technical Development.

Disclosure of Interest: None declared





PO038

A NOVEL PLATFORM FOR HIGH THROUGHPUT CELL LINE SCREENING & DEVELOPMENT

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Background and novelty: Cell line development has become faster and more clone and product analytics data are being generated during this process. Subsequently, the timely availability of all the relevant data needed to decide which cell line to pursue has become a bottleneck in the cell line development workflow. In addition, extremely heterogeneous data types need to be thoroughly compared in order to select the best producer cell line. Therefore, new integrated workflow support and data analysis methods are needed to ensure sound decision making.

Experimental approach: We have developed a new end-to-end platform for bioprocess development, which includes a cell line development workflow system supporting seeding, selection, passaging, analyzing, cryo-conservation and processing in bioreactors including micro-bioreactors, such as ambr®. This platform enables partially or fully automated cell line selection and assessment processes, thereby increasing process efficiency and quality. The system tracks the full history of all clones - from initial transfection all the way to their evaluation in bioreactor runs - and combines this information with molecule, product quality, and clone analytics data. It directly integrates with all instruments, such as pipetting robots, bioreactors, and bioanalyzers. It can be applied to antibodies (IgGs, novel formats) and other therapeutic proteins (e.g., fusion proteins).

Results and discussion: Here, we present concrete use cases to demonstrate how the platform streamlines the generation and assessment of mammalian production cell lines, shortening typical cell line development campaigns and enabling decision-making across campaigns.

Bibliography, Acknowledgements: Allison Kurz, Genedata AG, Basel, Switzerland, email: bioprocess@genedata.com

Disclosure of Interest: None declared





PO039

INCREASE THROUGHPUT IN DESIGN AND PRODUCTION OF NON-ANTIBODY TOOL PROTEINS AND CELL LINES

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Background and novelty: The availability and quality of the tools being used for drug targets, other protein reagents, or cell lines represent important prerequisites for successful drug discovery and development projects. The tools are typically produced by cell and protein sciences or technology groups and are supplied to small-molecule and large-molecule research and screening as well as to structural biology and PK-PD groups. Tool proteins and cell lines are usually applied in many types of bioassays and compound characterization, in high throughput screening, and in crystallization studies.

Experimental approach: We have developed workflow support platforms which enable the high-throughput design, engineering, validation, and production of these important tool proteins and cell lines. The systems include comprehensive tracking and annotation of proteins and cell lines, their domain variants (e.g., truncated or mutated), corresponding expression constructs, expressed and purified materials, related analytics and quality control data, together with sample inventory and warehousing information, as well as transfer and QC documentation (e.g., auto-generated Certificate-of-Analysis).

Results and discussion: We will present how our platforms enable high-throughput or highly parallelized workflows and a systematic evaluation of protein production and cell line development.

Bibliography, Acknowledgements: Allison Kurz, Genedata AG, Basel, Switzerland, biologics@genedata.com

Disclosure of Interest: None declared





PO040

CASE STUDY OF HIGH CELL DENSITY CELL CULTURE AND CLARIFICATION POST-FLOCCULATION WITH TOTAL SINGLE USE SOLUTIONS AT A 1800L SCALE

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Background and novelty: Advances in mammalian expression systems have resulted in increased monoclonal antibody titers and cell density cultures with corresponding elevations in cellular debris and cellular impurities. This evolution combined with the reduction of the bioreactor sizes down to 1000L or 2000L, in such cases, drastically reduce the performances of classical unit operations such as centrifugation or conventional depth filtration

Although the effectiveness of flocculation for clarification has been frequently demonstrated, adoption at a production scale of 2000 L is not yet common.

Experimental approach: In this study from bench to 2000 L single-use bioreactor scale, we could implement and witness the benefit of combining cell harvest pretreatment with a polycationic flocculating agent (polydiallyldimethylammonium chloride or pDADMAC), followed by specific depth filters, able to handle very large particle sizes (> 20 µm).

Results and discussion: This robust process has not only allowed for reaching higher filtration capacity and yield with enhanced impurity removal, it has also exhibited its linear scalability and great flexibility with limited foot print and investment. We also demonstrated process simplification with pre-treatment achieved directly in the Bioreactor with bottom-mounted impeller, thus avoiding the need for an additional mixing step between the bioreactor and depth filter. The area requirement of the subsequent depth filtration is reduced compared to classical depth filters.

Bibliography, Acknowledgements: Acknowledge:

Nicolas Fouques, Cyril Boucher, Laura Kirakossian Merck Healthcare Aubonne

Disclosure of Interest: None declared





PO041

APPLICATION OF DIELECTRIC SPECTROSCOPY TO DETERMINE BIOMASS AND METABOLIC STATUS IN A CELL CULTURE PROCESS

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Background and novelty: Dielectric spectroscopy based online sensor technology provides a novel, non-invasive means to achieve continuous monitoring of cell culture processes. Exploiting this technology offers significant opportunities that enable risk managed pharmaceutical development, manufacture and quality assurance. The technology fits well within the Process Analytical Technology (PAT) framework.

Experimental approach: In this study, a CHO K1 cell line was initially grown in batch configuration. In addition to standard pH & DO probes, the bioreactors were equipped with a biomass sensor. Data from the sensors and offline data on cell concentration, viability, productivity and concentration of metabolites was recorded. Further studies were performed using a fed-batch process developed for the cell line. Multiple regression analysis was used to establish the optimal frequency for biomass measurement. Principal component analysis was used to establish a correlation between parameters estimated from the characteristic beta dispersion curve for the culture and offline measurements. Verification of established correlations was performed through experiments in which extremes of the proven acceptable ranges were induced to allow further characterisation of the process design space with the online sensor.

Results and discussion: A continuous online measurement for culture biomass was established. The data produced from the sensor correlates well with the results achieved using the currently employed cell counting methodology (Vi-cell trypan blue exclusion). Data obtained from multiple frequency scanning was used to make inferences on cell metabolic state and overall cell health. Measured changes in the cell membrane capacitance during the culture were evaluated in tandem with offline data like the cell culture phase, cell size & nutrient limitation etc. Further work was outlined to validate these correlations within the design space.

Bibliography, Acknowledgements: N/A

Disclosure of Interest: None declared





PO042

VALIDATION OF A GMP COMPLIANT PROCESS TO PRODUCE RECOMBINANT ADENO-ASSOCIATED VIRUS VECTORS IN HEK 293 SUSPENSION CELLS

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Background and novelty: Adeno-associated virus (AAV) is one of the most popular vectors for gene therapy applications. The evaluation of vectors in pre-clinical studies and potential applications in the clinic requires scalable, high-yielding and compliant vector production processes. Even though much progress has been made in the last years, AAV production remains a major challenge for translating basic research into clinic. Here we describe the GMP-compliant production of rAAV by transient transfection of HEK 293 suspension cells grown in serum-free medium and the subsequent purification of vectors by immunoaffinity chromatography (IAC).

Experimental approach: Transfections were carried out via polyethyleneimine (PEI) based transfection of a 2-plasmid system. As a proof of concept rAAV2/9 expressing a GFP reporter gene were produced at a scale of one liter in orbital shaken bioreactors. To evaluate identity and purity of rAAV samples we performed SDS-PAGE or western blot analyses. Electron microscopy was applied to assess the ratio of full particles versus empty particles. To validate our process, we did a comparison with rAAV batches produced with adherent HEK 293 and purified on iodixanol gradient.

Results and discussion: The production of rAAV particles using suspension cells and IAC did yield viral genome titers of up to 10^{13} VG/L. These titers were equal or higher than titers achieved by production with adherent cells. Furthermore we observed an increased in vitro infectivity of vectors produced with the novel process. By using IAC we could achieve highly pure vector preparations and a recovery of $\geq 70\%$. As a final validation, we are currently evaluating transduction efficiency and transgene expression in the striatum of adult mice.

The presented process offers a novel method to produce rAAV for pre-clinical and clinical trials, scalable, highly pure and compliant to GMP.

Bibliography, Acknowledgements: Work funded by Swiss Translational Medicine

Disclosure of Interest: None declared





PO043

INVESTIGATION OF A DOWNSTREAM PROCESSING FOR THE APPLICATION OF ONCOLYTIC MEASLES VIRUSES IN THE GENE THERAPY

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Background and novelty: Strategies for fighting cancer nowadays still rely on chemotherapies, mostly implying unwanted and severe secondary effects. In this case, oncolytic viruses, such like the Edmonston strains of measles virus (MV) can be promising and less toxic alternatives.

Although alterations in the receptor specificity towards tumor cells, studies showed that a dose of 10^{11} TCID₅₀ (50% tissue culture infectious dose) active MV particles were needed for a full remission in an advanced stage multiple myeloma. Additionally, regulations pose high demands on the purity of therapeutical products.

Experimental approach: Having established a production process to generate 10^{11} TCID₅₀ ml⁻¹ in a stirred tank reactor, we are now faced with the development of an efficient downstream process. As the measles virus is enveloped and shows a high sensitivity against physicochemical parameter like high temperature, low pH and conductivity as well as shear forces, the challenge is to generate active virus in a high yielding purification process.

Results and discussion: At large scale, membrane filtration represents a beneficial trade-off. Membrane-based filtration like depth filtration can process large volumes of fermentation broth providing first purification and resulting in the recovery of active virus particles.

However, in terms of selectivity, adsorptive materials are a potentially efficient method for final virus purification. Instead of just filtration by size, adsorptive materials can separate based on the electric interaction between charged components of a liquid phase including viruses and ionic groups.

With the development of this tailor-made production process of an oncolytic measles virus, we are able to preserve the required virus titers.

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Disclosure of Interest: None declared





PO044

INVESTIGATING THE WARBURG EFFECT: HOW HIGH EXTRACELLULAR LACTATE AFFECTS INDUCED PLURIPOTENT STEM CELL METABOLISM AND PLURIPOTENCY

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Background and novelty: Induced pluripotent stem (iPS) cells hold the potential to dramatically improve cell-based therapies. Yet, in order for stem cell therapies to become clinically feasible, stem cells of sufficient quantity and quality must be generated. Rapidly proliferating cells, including iPS cells, rely on glycolysis and lactate fermentation to generate energy, even in the presence of sufficient oxygen; this process is referred to as the Warburg effect. Lactate has been shown to cause hypoxic gene expression in both tumor and noncancerous cells grown in normoxic conditions. Yet, there remains an incomplete understanding of the role of lactate in stem cell metabolism and pluripotency in glucose containing media.

Experimental approach: This study specifically examined the impact of various extracellular lactate levels on the metabolic activity and pluripotency in human K3 iPS cells, a liver-derived cell line. K3 iPS cells were grown in Essential 8 Flex, either control or supplemented with elevated lactate. Cell numbers and extracellular metabolite concentrations were quantified throughout the cultures to determine extracellular metabolite fluxes.

Results and discussion: The high extracellular lactate resulted in altered cell metabolism, including decreased lactate production, while the glucose consumption rate remained unchanged. These results support the hypothesis that there is redistribution of glucose-derived carbon in metabolism under high extracellular lactate, with a larger portion of this carbon entering the tricarboxylic acid (TCA) cycle. Additionally, these results illustrate that the glycolytic flux is unaffected by lactate for iPS cells. The implications of these findings towards understanding iPS cell metabolism and designing cell culture conditions to limit lactate accumulation will be discussed.

Bibliography, Acknowledgements: Thermo Fisher for providing custom Essential 8 media for these studies.

Disclosure of Interest: None declared





PO045

MONITORING THE PRODUCTION OF AAV VECTORS IN INSECT CELLS BY FLUORESCENCE SPECTROSCOPY

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Background and novelty: Adeno-associated viruses (AAV) are among the most promising viral vectors for gene therapy. AAV can be produced in the insect cell-baculovirus expression vector system (IC-BEVS), combining scalability with high cell density. Yet, solutions for real-time monitoring of AAV production are still lacking. Here we apply Fluorescence Spectroscopy (FS) to baculovirus-infected insect cell cultures, correlating the spectra to critical process parameters.

Experimental approach: Sf9 cells were co-infected with two baculovirus at low or high multiplicities of infection (MOI), and the culture was followed by FS. Sampling occurred at different time points to measure cell density, viability, AAV titre (packed genomes, total and infectious particles) and key metabolites concentration in culture media. For these parameters, independent calibration models were built based on FS data and chemometric methods.

Results and discussion: Cells infected at low MOI reached higher cell density before baculovirus induced cell-growth arrest, producing more total AAV particles. Besides, harvesting on day 5 rather than 4 almost doubled total AAV particles recovered. We have also found differences in the metabolite uptake/secretion rates of cultures producing different AAV serotypes. Further work will be conducted to understand if the observed differences are consistent and represent a metabolic shift induced by the different serotypes.

Preliminary results for Sf9 producing another virus-like particle have shown that FS can be used to predict cell density and viability with a normalized RMSE of 14.9% and 16.5% (respectively) in leave-one-out cross validation. We expect this approach to allow non-invasive online monitoring of IC-BEVS-based AAV production, including the ability to find in real-time the best harvesting time.

Bibliography, Acknowledgements: Funding from *Fundação para a Ciência e a Tecnologia*, project EXPL/BBBBIO/1129/2013, grants SFRH/BPD/72523/2010 and PD/BD/105873/2014.

Disclosure of Interest: None declared





PO046

TAILORING N-GLYCOSYLATION BY RATIONAL CELL CULTURE MEDIUM DESIGN

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Background and novelty: For more than 20 years, the industry has mainly invested in productivity enhancements. Driven by the biosimilar development, the focus of cell-culture process development shifted and the modulation of quality attributes has gained substantial interest¹. This study aims to establish a toolbox of novel media supplements to modulate quality and a new Parallel Experimental Design Method by which many modulators can be tested in one experiment.

Experimental approach: CHO cells expressing recombinant mAbs were cultivated in fed-batch mode. From 96-deepwell experiments viable cell density, viability, product titer and N-glycans (2AB-UPLC) were monitored. Multivariate analysis identified the best performing glycosylation modulators. Intracellular nucleotide sugars (CE) and gene expression (NGS seq) were measured in shake tubes. Metabolite profiling in 3.5-L bioreactors was used to build a multivariate model linking metabolites with the glycan fingerprint.

Results and discussion: The 96-DWP screening produced a large glycan diversity^{2,3}. Subsequent DoE in shake tubes confirmed the initial results and greatly improved the glycan biosimilarity. Further enhancements enabled to generate extreme glycosylation variants, which induced significant responses in biological activity assays. Moreover, metabolites correlating with time-dependent glycan data were pinpointed and the glycan distribution of an external data set predicted. Our data highlight the great potential of cell culture medium optimization to modulate product quality.

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Disclosure of Interest: None declared





PO047

DEVELOPMENT OF DOE BASED FED-BATCH STRATEGIES FOR HIGH-PRODUCING CHO CELL CULTURES

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Background and novelty: Fed-batch culture is commonly employed to maximize cell and product concentrations in upstream bioprocesses. Typical standard platform processes rely on fixed-volume bolus feeding of concentrated feed supplements at regular intervals. However, such static approaches might result in over- or underfeeding. To mimic more closely the dynamics of a fed-batch culture, we developed a dynamic feeding strategy responsive to the actual nutrient needs of a mAb-producing recombinant CHO cell line.

Experimental approach: A dynamic feeding strategy was elaborated for a high-producing CHO cell line using a three-step Design of Experiment (DoE) approach. First, eight different chemically defined feed supplements (HyClone™ Cell Boost, GE Healthcare), were initially spiked to the basal medium in different combinations. Feeds that best matched the clone-specific nutrient requirements and supported the highest cell and antibody concentrations during batch cultivation were selected. Second, the feed ratio of selected feeds was further optimized during fed-batch cultivation by continuous feeding of different feed combinations at regular intervals. Third, the best-performing combination during constant (static) feed additions was compared to six different (dynamic) feed protocols that were based on different target substrates.

Results and discussion: In step 1, we identified essential feeds and eliminated non-beneficial feeds. Further, the results provided a good starting point for subsequent fed-batch cultivation to further optimize the feed ratio of the beneficial feeds. In step 2, we fine-tuned then fixed the respective feed ratio. In step 3, we investigated different dynamic feeding strategies and showed the applied protocol's importance. Ultimately, using the developed DoE based protocol we rapidly established a three-step, fed-batch strategy that increased antibody concentrations almost 6-fold from 1.1 to 5.9 g/L.

Bibliography, Acknowledgements: GE Healthcare for funding

Disclosure of Interest: None declared





PO048

TRANSCRIPTOME ANALYSIS IN HIGH-PRODUCING CHO CELL CULTURES: STRATEGIES TO DESIGN HIGH-PERFORMING CELL CULTURE MEDIA

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Background and novelty: Concentrated feed media are commonly added to bioprocesses to increase cell growth and productivity. We compared a standard batch culture in a chemically defined basal medium (ActiPro™) with a batch culture grown in the same basal medium supplemented with a single shot of concentrated feed media (Cell Boost™). This single-shot strategy triggered 80% higher peak cell and 3× higher antibody concentrations. The qP remained constant at 70 pg/cell/d in feed-spiked batch cultures but continuously declined to 10 pg/cell/d in controls. Therefore, we investigated transcription-level changes in cellular physiology using microarray experiments and analyzed differential gene expression.

Experimental approach: CHO cells were grown in a bioreactor under controlled conditions to compare two different batch modes with or without supplementation with a single shot of concentrated feed on day 0. Daily mRNA expression was analyzed on microarrays to determine transcriptomic differences between cultures.

Results and discussion: In both cultures, mRNA expression levels continuously increased throughout cultivation. However, adding a single-feed spike boosted the cellular physiology to a substantially more active proliferative state as indicated by identified gene ontology terms related significantly to cell cycle and primary metabolism, cellular division, and nucleobase formation or regulation. More importantly, feed spiking significantly enriched sets of genes relating to transcription, DNA replication and repair, cell growth and proliferation, and inhibition of apoptosis according to gene set enrichment analysis. Several cellular targets were identified; most were not previously reported in relation to recombinant protein production. These targets might be important regulators for maintaining cellular physiology in a productive state and could potentially be exploited for bioprocessing.

Bibliography, Acknowledgements: GE Healthcare for funding

Disclosure of Interest: None declared





PO049

COUPLING SE-UPLC WITH LC-MS/MS FOR UNDERSTANDING AGGREGATE AND SHOULDERS ELUTING WITH MONOCLONAL ANTIBODIES PRODUCED IN CHO CELLS

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Background and novelty: Size exclusion chromatography (SEC) is the predominant method used in biopharmaceutical industry to measure the aggregation, degradation and impurities of mAbs produced in Chinese hamster ovary (CHO) cells. Biosimilar market is becoming increasingly important to provide affordable drugs to the patients. The aggregate level of the biosimilars is one of the critical quality attributes which has to be fulfilled. Downstream process affects the size profiles of the mAbs, in some cases yielding ambiguous species that need to be well characterized. SEC alone is not sufficient to define these ambiguous species, especially when they are in the form of shoulders eluting with the major monomer peak. This is where MS becomes a useful tool for the characterization of unknown species, which can be critical in terms of toxicity, immunogenicity or efficacy.

Experimental approach: In this study, a biosimilar mAb developed in CHO was used as a case study. The size exclusion analysis showed a shoulder peak eluting with the monomer. Both the shoulder and the monomer was fractionated and analyzed in mass spectrometry. Three different strategies were used to understand the shoulder. Intact mass, reduced mass and peptide mapping analysis were run to figure out the differences between shoulder and the monomer.

Results and discussion: SE-UPLC was coupled with LC-MS/MS to figure out the aggregate reasons and shoulder problems which can lead the directions for the downstream process development of CHO. Combining the peptide mapping, reduced and intact mass analyses; we were able to identify all post-translationally modified mAb forms and other degradation and aggregation forms.

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Disclosure of Interest: None declared





PO050

HOW TO EFFICIENTLY SCALE UP CLINICAL MANUFACTURING OF THE ONCOLYTIC VIRUS VSV-GP AND MOVE QUICKLY FROM BENCH TO BEDSIDE

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Background and novelty: Oncolytic virotherapy is an emerging, increasingly recognized discipline in cancer treatment. It uses replication-competent oncolytic viruses with the ability to specifically destroy cancer cells via immune and non-immune-mediated mechanisms. The Vesicular Stomatitis Virus (VSV) is one member of this new class of innovative anti-cancer therapeutics utilizing the defective interferon-mediated anti-viral response of cancer cells. To generate VSV-GP, VSV was modified by replacing the wild-type glycoprotein with the lymphocytic choriomeningitis virus (LCMV) glycoprotein to conceal VSV-GP from the human immune system and abrogate neurotoxicity associated with VSV infection.

Experimental approach: In order to ensure potency, purity and safety of VSV-GP intended for use in oncolytic virotherapy, we designed an efficient and scalable clinical manufacturing process based on animal component-free mammalian cell culture. It enables high infectious virus titers required to enable local, intratumoral administration at limited dose volumes, as well as highly purified and stabilized single-particle virus formulations compulsory to ensure safety during systemic administration.

Results and discussion: We will discuss how the lessons learned from implementing manufacturing platforms for antibody and vaccine production help to overcome complexities related to oncolytic virus propagation in adherent cells, as well as downstream virus purification and formulation. We will further illuminate how virus stability directly affects the efficiency of scaling up manufacturing and why early time investments in manufacturing science may increase future clinical success.

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Disclosure of Interest: None declared





PO052

A PAT APPLICATION FOR THE MONITORING OF VIABLE CELL DENSITY AND AUTOMATING FEEDING STRATEGIES IN MAMMALIAN CELL CULTURES FOR IMPROVED PERFORMANCE

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Background and novelty: Present pharmaceutical industry practice uses fed-batch bioprocesses with daily bolus or continuous feeds for production of recombinant protein in mammalian cell cultures. These feeding strategies can result in over feeding cell cultures which leads to higher osmolality, metabolite accumulation, and suboptimal cell culture performance. Advances in process analytical technologies (PAT) allow online monitoring, continuous data acquisition and feedback control which has aided in obtaining improved and more reliable cell culture performance.

Experimental approach: Biocapacitance probes have gain traction as a marker for measuring online biomass. These probes can be used to control feeding strategies and avoid accumulation of metabolites, potentially increasing cell culture performance. To develop a dynamic feeding strategy, we implemented this technology into our control system at bench and pilot scale.

Results and discussion: Equations were determined using different permittivities at multiple scanning frequencies in order to predict viable cell density (VCD). Then, different automated feeding strategies were tested in mammalian cell cultures using online VCD. It was found that an automated feeding strategy could be a valid alternative to the traditional bolus and continuous fed-batch bioprocesses. The application of these biocapacitance probes could benefit in automating bioprocesses with better cell culture performance.

Bibliography, Acknowledgements: N/A

Disclosure of Interest: None declared





PO053

IMPROVEMENT OF A PLATFORM MEDIA AND FEED SCREEN FOR MASTER CELL BANK CANDIDATES

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Background and novelty: In a competitive market, timelines must be shortened to be able to produce protein for clinical trials as quickly as possible. A number of steps in the process can be made as generic (platform) as possible in order to achieve these time savings. As a CMO, Catalent's proprietary GPEX[®] cell line development technology eliminates one of the most time-consuming tasks in the process: cell line stability. With transduction efficiency close to 100% the need for any selection or amplification is eliminated. Because the gene insertion targets different genomic locations, the cell lines are inherently stable. The elimination of antibiotic selection and stability studies during cell line development significantly shortens the timeline for a master cell bank candidate.

Experimental approach: In Upstream Process Development, we utilize a platform media and feed screen that works for the expression of both antibodies and recombinant proteins. In this screen we narrow 20 clones to a single master cell bank candidate with a single media and feed strategy. While this approach helps to shorten the timeline, these conditions are usually not fully optimized. Therefore new media and feeds are evaluated to try and improve the existing platform process. In this study the upstream process development of two different antibody-producing cell lines will be presented.

Results and discussion: Media and feeds from Irvine Scientific were included in the initial screen of both cell lines alongside our existing Platform screen. Overall, the Irvine feeds and media consistently outperformed the other media and feeds in the screen. As a result our Platform process has been modified to include Irvine products.

Bibliography, Acknowledgements: Bryan D Monroe; Primus Consulting, LLC
Diane Wyatt; Irvine Scientific

Disclosure of Interest: None declared





PO054

PERFUSION MEDIA DEVELOPMENT USING CELL SETTLING IN AUTOMATED CELL CULTURE SYSTEM

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Background and novelty: An increased number of biopharmaceutical companies are interested in implementing perfusion cell culture processes, maintaining high cell densities ($>50 \times 10^6$ vc/mL) with minimal perfusion rates (<2 vvd). In order to obtain the required medium depth for intensified perfusion without the generation of by-products, medium component concentrations must be balanced correctly. Currently, the efficiency of perfusion media development is limited by the lack of a commercially available cell separation device that can operate with working volumes under 1L.

Experimental approach: In order to increase the throughput in small scale bioreactors, we have implemented an automated simulation of perfusion process that uses cell settling as a means of cell retention. This method allows the exchange of medium in each individual vessel with perfusion rates ranging from 0.5 to 1vvd. Using automation, the total perfusion rate can be divided in several smaller media exchanges performed at equal time interval. This represents a significant improvement over other small scale models that exchange media only once per day.

Results and discussion: In this study we will show the metabolic profile of critical metabolites before and after cell sedimentation as well as the comparison of growth, production and protein quality to benchtop scale bioreactors. By applying the optimized process we have developed for [ambr®15] microbioreactors in simulation of perfusion mode, we were able to achieve $>90\%$ cell retention efficiency and maintained steady states at a selected target cell densities with different cell lines. We will show as well the application of this method to evaluate perfusion medium formulations, as well as supplements developed to modulate protein quality profile. Lastly, we will show the application of this method to identify the $CSPR_{min}$ for a given formulation, and how we were able to distinguish medium depth for growth and for production independently.

Bibliography, Acknowledgements: N/A

Disclosure of Interest: None declared





PO055

CHARACTERIZATION AND OPTIMIZATION OF DIFFERENT APPROACHES FOR VLP PRODUCTION IN INSECT CELLS

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Background and novelty: Insect cells have been one of the platforms used for the production of Virus-Like Particles (VLPs). The characterization of the infection and production process of such multicomplex structures has been often performed by means of one-variable-at-a-time experiments. In this study, advanced statistical techniques together with a combination of novel analytical procedures were used to model and optimize different insect cell-derived expression methodologies.

Experimental approach: A comparison between High five and Sf9 cells for the production of fluorescently tagged HIV-1 VLPs was performed with the baculovirus expression vector system (BEVS), transient transfection and stable expression. The VLP production process was evaluated by means of flow cytometry and time lapse confocal microscopy whereas HIV-1 VLPs were characterized according to Electron Microscopy techniques, Nanoparticle Tracking Analysis, double cushion ultracentrifugation and Fluorescence-based techniques. Also, desirability functions and RSM-based DoE methodologies were used to define best operation conditions for the different expression systems[1].

Results and discussion: The study of the parameters influencing the transient transfection process revealed the important role of PEI and DNA concentrations for efficient PEI:DNA complex formation. Regarding BEVS, the use of DoE techniques emphasized the time of harvest as the key factor influencing product quality. Confocal imaging showed the formation of complex megastructures inside the cytoplasm of infected cells. Also, non-assembled monomers were found to be an important source of contamination of HIV-1 VLPs produced with this system.

Bibliography, Acknowledgements: [1] Myers, R., et al., Response Surface Methodology: Process and Product Optimization Using Designed Experiments. 2016.

Disclosure of Interest: None declared





PO056

DEVELOPMENT OF AN IN-LINE NANOFILTRATION STEP FOR VIRUS REMOVAL IN A CONTINUOUS CELL CULTURE PROCESS

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Background and novelty: Viral risk for bioproducts has been a major safety concern over the last decades. For this reason, manufacturers of bioproducts are requested to assure viral safety for a large span of virus type by health authorities. The viral reduction techniques spanning non-enveloped viruses and amenable to large-scale manufacturing scale are multiple such as chromatography, nanofiltration, high temperature short time or ultra-violet C irradiation. The choice of the viral reduction technique is driven by the origin of the viral risk, the bioproduction process and the bioproduct. In this context, Shire developed an innovative approach on a viral removal step of von Willebrand recombinant factor process given the complexity of the molecule, the viral risk mainly associated to raw material in cell culture, and the continuous mode of culture of bioreactors.

Experimental approach: In-line nanofiltration of media was developed at small-scale, implemented and validated to feed >2'000 L bioreactors working on continuous mode. At small-scale, Minute Mice virus model was chosen to assess the clearance capabilities. Process parameters such as transmembrane pressure, flow rate, process interruptions and media formulation were assessed. At large-scale, process parameters were developed to include such a step into a continuous mode of cell culture.

Results and discussion: Under manufacturing conditions, nanofiltration is processed continuously for about 2 months, with a maximum of 20'000 L/m², allowing an additional viral clearance of a factor 6 without impact on cell culture performances. This breakthrough process modification is bringing facts that the implementation of viral reduction step is amenable to complex processes and products.

Bibliography, Acknowledgements: -

Disclosure of Interest: None declared





PO057

TRANSFER OF GMP-COMPLIANT MANUFACTURING OF NATIVE AND GENETICALLY MODIFIED HUMAN MESENCHYMAL STEM CELLS TO A MULTIPLATE BIOREACTOR TECHNOLOGY

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Background and novelty: Mesenchymal stem cells (MSC) are a promising candidate for cell-based therapies. Allogeneic setting allows for rapidly available and cost effective “off the shelf” products but necessitates a manufacturing strategy able to provide a large quantity of cells under GMP conditions. Traditional 2D expansion technologies require large footprints and are too labor- and cost intensive for commercialized MSC manufacturing. Thus, large scale expansion technologies are needed for the manufacturing of clinical grade MSC.

In this study, we used MSC from bone marrow of healthy volunteers and transferred the manual GMP manufacturing expansion process (CellSTACK®) to the multiplate bioreactor (Xpansion®).

Experimental approach: Cultivation parameters such as inoculation density, medium exchanges or run time were kept constant in the bioreactor and the control. Both cultures were monitored (glucose, lactate, pH) and cultures in the bioreactor were controlled (pH, DO). Native and genetically modified MSC of three bone marrow donors were expanded in different bioreactor scales (10, 50 and 100 plates).

Results and discussion: The comparative study, including 11 runs at three different bioreactor scales, showed that the MSC yield in the multiplate bioreactor was equivalent or even significantly higher than in control. A maximum cell yield of 9.96×10^8 native MSC and 5.39×10^8 genetically modified MSC could be harvested from a cultivation area of $30,600 \text{ cm}^2$ (50 plates). MSC characteristics, such as CD73+/CD90+/CD105+/CD34-/CD45- phenotype, adipocyte and osteoblast differentiation, CFU-F formation, migration potential and absence of tumorigenicity, were maintained and comparable to the control in all runs.

Hence, the multiplate bioreactor represents an attractive platform for large scale, GMP-compliant expansion of native as well as genetically modified MSC-based cell therapeutic drug products.

Bibliography, Acknowledgements: -

Disclosure of Interest: None declared





PO058

MICROCARRIER-BASED CULTIVATION OF HUMAN MESENCHYMAL STEM CELLS IN THREE DIFFERENT SUSPENSION BIOREACTOR SYSTEMS

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Background and novelty: Microcarrier-based cultivation in bioreactors represents an attractive platform for large scale, GMP-compliant expansion of allogeneic MSC-based cell therapeutics.

We compared the performance of the previously established protocol for microcarrier-based expansion of native MSC in three single-use suspension bioreactors (2L UniVessel®, 3L Mobius® and 0.5L Vertical-Wheel™), chosen based on their principal suitability for the GMP-compliant use.

Experimental approach: One and the same MSC population was expanded three times in every bioreactor. Type of microcarriers (Solohill®) as well as cultivation parameters were kept constant in all three bioreactors. t°, pH and DO were regulated in the stirred tanks in contrast to the wheel bioreactor. pH, metabolite concentration and cell number were determined daily in all systems.

Results and discussion: In each of three comparative runs, the MSC density in the wheel bioreactor was by trend the highest, followed by the 3L (Mobius®) and the 2L (UniVessel®) systems. The expansion factor of the MSC population after 6 days cultivation was 11.8-16.3 in the wheel bioreactor, 6.0 – 12.5 in the 3L system and 5.7 – 9.6 in the 2L system. The MSC phenotype CD34-/CD45-/CD73+/CD90+/CD105+ could be detected in all runs irrespective of the bioreactor type. Additionally, the CFU-F formation and adipocyte and osteoblast differentiation were analyzed.

The tested protocol for the MSC cultivation showed the optimal performance in the 3L stirred tank (Mobius®) and in the wheel bioreactor. However, the effect of scale up on the performance in the wheel bioreactor remains to be studied. The applied cultivation strategy appeared less suitable for the 2L bioreactor (UniVessel®), suggesting that further protocol adaptation is required to reach optimal MSC cultivation parameters in this system.

Bibliography, Acknowledgements: -

Disclosure of Interest: None declared





PO059

DIAMINE OXIDASE N-GLYCOSYLATION SITE ASN110 IS HIGHLY CONSERVED IN EVOLUTION AND ESSENTIAL FOR SECRETION

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Background and novelty: Human diamine oxidase (hDAO) is the key enzyme in the extracellular catabolism of histamine. Increased histamine concentrations contribute to the symptomatology in patients with mastocytosis, mast cell activation syndrome (MCAD), urticaria and life-threatening anaphylaxis. Treatment with histamine receptor antagonists has significant limitations.

Three of the four N-glycosylation sites of hDAO, Asn110, Asn538 and Asn745, are highly conserved from Homo sapiens down to zebrafish. For the first time we investigated the function of the different N-glycosylation sites using mutation analysis.

Experimental approach: Recombinant hDAO (rhDAO) was produced in CHO and HEK cell lines. Native DAO was purified from human amniotic fluid, human Caco-2 cells and a commercial porcine kidney protein preparation. We further obtained CHO derived recombinant human vascular adhesion protein 1 (rhVAP-1), a related copper amine oxidase. Glycan profiles were determined via mass spectrometry. All glycosylation sites were mutated by substituting Asn for Gln and expressed in CHO and HEK cell lines.

Results and discussion: Glycosylation site Asn110 was consistently occupied by high-mannose glycans, which is rarely seen in mammalian proteins. The other sites showed the expected complex-type glycosylation patterns. Asn137 in hVAP-1, the Asn110 corresponding glycosylation site, was also exclusively populated by high-mannose glycans.

Asn110Gln mutation caused complete inhibition of rhDAO secretion at normal mRNA levels. rhDAO-wt was detected in the ER and Golgi, whereas the Asn110Gln mutant was exclusively visible in the ER. Mutations of the other N-glycosylation sites allowed expression of rhDAO at reduced levels. These findings imply a critical role of Asn110 during protein folding and secretion. It is likely that the high-mannose glycan stabilizes the local protein structure and is inaccessible to further trimming and processing reactions.

Bibliography, Acknowledgements: -

Disclosure of Interest: None declared





PO060

IMPROVEMENTS ON A SCALABLE BIOPROCESS FOR THE 2KL PRODUCTION OF AN ANTIBODY WITH THE SINGLE-USE TECHNOLOGY

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Background and novelty: The production of antibodies by Chinese Hamster Ovary (CHO) cells is one of the current largest biopharmaceutical activities. One of the main challenges faced at time of development is to get a high titer with a Good Manufacturing Practices (GMP) scalable bioprocess and without any changes on the quality attributes of the molecule.

In this project, the aim was to optimize a process which was successfully transferred from 3L [Mobius] single-use development bioreactors to 2kL [Mobius] single-use GMP production. The main points to be improved were the titer and the control of the pCO₂ value with the constraint to keep a 2kL GMP scalable process without any significant change on the quality and without changing the production medium.

Experimental approach: The work was performed by screening different feeding and supplementation strategies using spin tubes (60 conditions) with the aim to increase titer and productivity. In parallel, the parameters of the 3L single-use bioreactors were optimized to reduce the pCO₂ value. Finally, the best conditions from both containers were combined in 3L bioreactors to get the final process to be transferred in the 2kL bioreactor.

Results and discussion: The final results on these fed-batch cultures indicated that the optimizations in bioreactor alone allowed in increasing the titer by 25% without any change in the medium and feeds strategy. The combination of the best conditions from spin tubes with the new parameters in bioreactors led to a 3X titer with a cheaper feeding strategy and without modifying the quality (including glycans and bioassay activity).

This development strategy allowed a significant improvement of the existing production process and highlighted the potential of the single-use technology to develop GMP scalable processes in a quick and efficient way.

Bibliography, Acknowledgements: Acknowledgments: Mammalian UpStream and Analytical Development Teams – Merck Biodevelopment – Martillac (France)

Disclosure of Interest: None declared





PO061

**AN 'INDUSTRY FIRST' 500L BIOREACTOR CHO TRANSIENT CULTURE:
DEVELOPMENT OF LARGE SCALE TRANSIENT EXPRESSION CAPABILITIES**

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Background and novelty: MedImmune has developed a proprietary high yielding, scalable and easy to use CHO cell based transient expression system. The system is used routinely and very successfully for early stage material supply for projects within the R&D organisation at both AstraZeneca and MedImmune.

Experimental approach: To further build on the success achieved to date with the system there is a continued desire to optimise the process even further including; increasing expression levels, increasing scalability and improving product quality.

As part of the continued development of the transient expression system, work has been performed to develop a process for use in a 500L Single Use Bioreactor (SUB). Here we present data on the scalability of the CHO transient process and the successful scale up to 500L, which, to our knowledge, is an industry first. Comparable product quality profiles have been observed in material generated from stirred SUB and rocking bioreactor cultures at different scales, suggesting that this transient process can be used to rapidly generate 100's of grams of recombinant protein and potentially accelerate drug development timelines. However, differences were observed between SUB and rocking bioreactor cultures; with stirred SUB cultures of all scales tending to show increased cell growth profiles and lower specific productivity (Qp) values. Investigative work into the mechanisms of the differing culture performances across scales has shown that pCO₂/pH, agitation rate and DO levels at the time of transfection as well as modulation of transfection complex parameters have an influence on transfection efficiency and subsequent culture performance.

Results and discussion: Data will be presented showing the above observations as well as successful mitigation strategies which is now yielding in excess of 1g/L of IgG at SUB scales.

Bibliography, Acknowledgements: Cell Culture and Fermentation Sciences, BPD, MedImmune

Disclosure of Interest: None declared





PO062

COMBINING METABOLIC AND PROCESS ENGINEERING STRATEGIES TO IMPROVE RECOMBINANT GLYCOPROTEIN PRODUCTION AND QUALITY

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Background and novelty: Increasing recombinant therapeutic protein titers and ensuring consistent protein quality remains a challenge in cell culture bioprocess development. While PYC2-overexpression has proven effective at improving glucose utilization efficiency in mammalian cell cultures, its impact on protein glycosylation has not been assessed and strategies to further exploit the altered metabolism of PYC2 cells have not been fully investigated.

Experimental approach: We have assessed the effect of PYC2-overexpression in HEK293 cells on the quality of interferon α 2b (IFN α 2b). The product was characterized by mass spectrometry analysis and with a surface plasmon resonance assay using specific lectins. We have then explored the use of glutamine substitution strategies to further reduce waste metabolite accumulation and improve glycoprotein production and quality.

Results and discussion: In batch cultures, PYC2-expressing cells were shown to sustain a significantly higher percentage of intact glycosylated IFN α 2b in comparison with parental cells, which was correlated with a prolonged viability and reduced accumulation of waste metabolites. Replacing glutamine by pyruvate led to further improvements in cell growth and product yield. As batch cultures highlighted the ability of PYC2-overexpressing cells to grow and sustain high viability in absence of glutamine, we performed fed-batch cultures by daily feeding a glutamine-free concentrated nutrient solution. This resulted in a 2-fold improvement in IFN α 2b titer and also increased product sialylation in comparison to a similar fed-batch protocol with glutamine supplementation. These results demonstrate that the favorable culture conditions promoted by PYC2-overexpression can have a beneficial impact on protein quality and may confer improved performances in glutamine-free cultures. This offers an attractive approach to mass-produce high quality therapeutic glycoproteins.

Bibliography, Acknowledgements: .

Disclosure of Interest: None declared





PO063

ESTABLISHMENT OF AN AUTOMATIZATION SYSTEM FOR A CONTINUOUS INTEGRATED BIOPHARMACEUTICAL MANUFACTURING PROCESS

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Background and novelty: Through the PAT initiative the utilization of spectroscopic techniques, multivariate data analysis (MVDA) and advanced control algorithms gained increasing interest in the biopharmaceutical industry in the recent years. Despite several successful implementation cases, there is still a huge gap towards an efficient centralized data mining combined with online use of MVDA and the integration of process knowledge into a supervisory control frame.

Experimental approach: To master these challenges, an IT platform for a fully continuous integrated biopharmaceutical manufacturing process was developed. The potential of online Raman spectroscopy was tested in both, downstream and upstream. Moreover, diverse spiking strategies and advanced modeling algorithms were investigated to improve the predictive power. To develop and tune the control algorithms dedicated runs were performed.

Results and discussion: The developed IT platform enables the efficient collection and centralized storing of all process data. In addition, it can interact with the control systems of process units to close control loops. Advanced MVDA and mechanistic models as well as control and optimization tools, can be integrated. In particular, the possibility to decently predict central process variables including glucose, viable cell density and product titer, all amino acids and even quality attributes (aggregates and glycans), outlines the key role of online Raman spectroscopy in supervisory control. Such an advanced control system enables to handle process perturbations and to optimize diverse objectives such as productivity, efficiency and product quality design. The IT platform with the innovative control system provides a greatly important basis to intensify the main advantages of continuous manufacturing and follows the trend of industry 4.0.

Bibliography, Acknowledgements: The authors acknowledge the support from the teams of Kaiser Optical Systems and Siemens SIPAT.

Disclosure of Interest: None declared





PO064

COMPREHENSIVE ANALYSIS OF THE IMPACT OF TRACE ELEMENTS IN MEDIA ON CLONE DEPENDENT PROCESS PERFORMANCE AND PRODUCT QUALITY

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Background and novelty: State-of-the-art biopharmaceutical processes are accounting concomitantly for process performance and product quality. Even though high yielding, robust processes are the cornerstones of any process development, product quality parameters such as structural integrity, charge variances and post-translational modifications are progressively becoming the focus of the developmental work. In conjunction with host cell line selection and process performance parameters, media components are crucial for the continued progress in rational modulation of product quality attributes affecting biological activity, immunogenicity, half-life or stability. Among media components, trace elements (TE) are of particular interest as they play a pivotal role in various cell metabolism pathways [1].

Experimental approach: In a comprehensive I-optimal DOE approach, the effect of six TE in various concentration levels and combinations in serum-free media was studied for four different CHO-K1 cells lines in an ambr® 15 setup. A scrutiny of the process performance parameters such as cellular growth, productivity, amino acids and vitamins consumptions rates for each of the conditions was performed. The process performance evaluation was accompanied by a product quality analysis. Furthermore, metabolic flux analyses were performed based on the nitrogen balance.

Results and discussion: Based on extensive analytical data, the obtained response surface model provides a clear insight into the impact of particular TE and their combinations on process performance and product quality. The high model quality enables discriminations between clone dependent and clone independent effects. Analyzing specific rates in combination with metabolic flux analysis improves the understanding of metabolic restructuring of the cell lines under distinct TE levels and combinations.

Bibliography, Acknowledgements: Bruhlmann D, et al. 2015. Biotechnol Prog 31(3):615-29.

Disclosure of Interest: None declared





PO065

DEVELOPMENT OF AN ANALYTICAL APPROACH FOR ON-LINE MONITORING AND CONTROL OF MONOCLONAL ANTIBODIES QUALITY

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Background and novelty: Due to regulatory concerns and economic impact, ensuring product quality and consistency is now one of the main challenge faced by the biopharmaceutical industry. For monoclonal antibodies (mAb), glycosylation is one of the most important quality attributes as it impacts on mAb structure integrity, and ultimately on both clinical efficacy and safety. Many factors affect mAb glycosylation and its inherent heterogeneity, including the host cell, the culture medium, the mode of operation and the operating conditions. In this context, the capacity to monitor and control on-line the antibody glycosylation, from early- to late-stage process development, would be of salient interest to reduce the time and cost to market.

Experimental approach: In order to address this unmet need, we have designed an improved SPR biosensor assay to measure the kinetics of interaction between a mAb and the extracellular domain of the FcγRIIIa receptor bound at the biosensor surface.

Results and discussion: Of salient interest, we also demonstrated that various binding kinetic signatures, especially different dissociation kinetics could be correlated with distinct mAb glycosylation patterns and with therapeutic efficacies, as deduced from mass spectrometry and a surrogate ADCC assay, respectively. In parallel, we have also harnessed a SPR biosensor directly to a bioreactor, which permitted the at-line determination of the concentration of antibodies by hybridoma cells during a bioreactor culture. We now plan on combining both approaches to determine on-line the glycosylation profile of the produced mAbs. Our ultimate goal is to design a unique and highly innovative bioprocess control tool that can be readily applied in an industrial bio-manufacturing setting.

Bibliography, Acknowledgements: The authors would like to thank Gilles Saint-Laurent for his help with molecular biology.

Disclosure of Interest: None declared





PO066

DEVELOPMENT AND ASSESSMENT OF A ROBOTIC HIGHTHROUGHPUT PLATFORM FOR ANTIBODY PURIFICATION MINIPURIFICATION

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Background and novelty: Reducing timelines, reducing costs: These key factors are strategic in bio-pharmaceutical industries to accelerate process development and drug delivery to patients. Enhancing throughput of bioprocess development has become increasingly important for the screening and optimization of cell culture processes. This challenge requires high throughput tools. In a previous study (1) we showed that ambr® 15, a robotically driven, mini-bioreactor system developed by TAP-Sartorius, could be advantageous to accelerate process development. The use of ambr® 15 system allows to test a large numbers of experimental conditions in a single experiment. Therefore the large amount of production samples to be characterized for PQA increases as well: the bottleneck has moved from the generation of samples at the production bioreactor step to in-process analysis.

For product quality attribute analysis at lab scale, protein purification is generally carried out on >10mL columns which is not compatible with the size of ambr® 15 bioreactors and are relatively low throughput methods. The development of robots for small scale purification purposes is a great opportunity for us to tackle this bottleneck. by enabling high throughput samples purification at smaller scale (200µL).

Experimental approach: In this study we first assessed the robustness of our minipurification approach by comparing the reproducibility between the Robocolumns to our classical purification procedure (yield, pH, and eluate volume). In addition we have also compared the PQA profiles (Charge variant and size exclusion) of the eluates.

Results and discussion: We demonstrated that the small scale purification is robust and comparable to our classical purification procedure in terms of purification and PQA outputs. This study showed Predictor Robocolumn shows similar performances to the AktaXpress system.

Bibliography, Acknowledgements: (1) Delouvroy *et al* BMC Proceedings 2015 9 (Suppl 9) :P78

Disclosure of Interest: None declared





PO067

VIABLE CELL DENSITY MONITORING IN BIOREACTOR WITH LENSLESS IMAGING

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Background and novelty: The traditional measurement for bioreactor cell count and viability rely on using the Trypan Blue exclusion method once a day. While automatic cell counters have reduced the statistical manual error, sampling the bioreactor remains a contamination risk and is prohibiting process control as the sampled volume becomes significant. Lensless Imaging Technology is a new method for accurately determining cell concentration and viability without staining. This technique directly acquires the light diffraction properties of each individual cells through their holograms images without any objective, lens or focus settings. Living and dead cells have significant holographic patterns that can be distinguished and precisely counted.

Experimental approach: We compare cell counts and viability between the reference method and our Lensless Imaging device, the Cytonote counter. Measures are performed once a day on samples from 12 bioreactors, from the inoculation to the end of the culture. We also assessed the repeatability of our method.

In a second part, a Lensless Imaging prototype is setup as a measurement chamber directly connected to a perfusion bioreactor, for continuously receiving the bioreactor broth, and therefore reproducing an in situ measure.

Results and discussion: With a concentration range up to 40×10^6 cells /ml and viability range at 75-100%, we obtained a correlation factor of 0.98 between the two compared methods. The large field of view allows the analyze of several thousand cells within a single image, keeping the statistical variability of the measure as low as 3%.

Our measurement chamber prototype has demonstrated its capability for continuous Viable Cell Density and viability monitoring. We are now working at designing a steam sterilizable probe, and we envision Lensless Imaging to become the future method of choice for on-line monitoring of suspension cells cultures.

Bibliography, Acknowledgements: Acknowledgment to Cedric Allier from CEA Leti, Grenoble, France.

Disclosure of Interest: None declared





PO068

TIME-DEPENDENT PRODUCT HETEROGENEITY IN MAMMALIAN CELL FERMENTATION PROCESSES

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Background and novelty: A consistent product quality is a major goal in the production of bio-therapeutics, especially recombinant glycoproteins. Whereas the polypeptide chain is unlikely to change during a production process, posttranslational modifications and protein folding are sensitive to fluctuations in process conditions.

We focus on protein glycosylation as one important indication for product quality. During a batch process conditions change continuously. At the beginning, the supply situation for the cell is excellent, but secreted material remains a long time in the culture fluid. Later during cultivation metabolite concentrations increase, whereas the exposition time of the protein to the culture fluid is much shorter. Altogether this results in product heterogeneity in a batch culture.

Experimental approach: Four different cell lines producing four different recombinant glycoproteins were investigated in this study. With a growth phase dependent sampling we analyzed products from different growth phases. Product was isolated from supernatant, glycans were released, permethylated and analyzed by MALDI-ToF mass spectrometry. The investigated proteins were Antibody, Antithrombin III from CHO clones and α_1 -Antitrypsin, C1-Inhibitor from human clones, respectively.

Results and discussion: We found that glycan antennarity is quite stable. Except for IgG the degree of core fucosylation was also very constant. The antibody process showed less core fucosylation in the latest process phase, which is favorable for a higher ADCC performance. Three products showed antennary fucosylation which seemed not to change very much in different phases. The degree of antennary galactosylation decreased noticeably for three products from the first phase to the last phase. An incomplete galactosylation will result in truncated glycans and, inevitably, in undersialylated antennas. Consequently, sialylation was found to be the highest in the early phases.

Bibliography, Acknowledgements: ...

Disclosure of Interest: None declared





PO069

ACOUSTIC WAVE SEPARATION – A SCALABLE DISRUPTIVE TECHNOLOGY FOR CONTINUOUS CLARIFICATION OF FED BATCH CELL CULTURE PRIOR TO CAPTURE CHROMATOGRAPHY

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Background and novelty: With advances in fed batch cell culture leading to higher cell densities and higher product titers there is a drive to improve the efficiency and speed of the cell harvest and clarification stage to generate Harvested Cell Culture Fluid (HCCF) for subsequent downstream processing. With the evolution of continuous processes there is a preference for a continuous feed of HCCF available for direct load to the continuous chromatography stage. Conventional clarification is typically operated in batch mode requiring storage of feed or HCCF during the process.

Experimental approach: Acoustic Wave Separation (AWS) technology involves the use of low frequency acoustic forces to generate a 3 dimensional standing wave across a flow channel. Cell culture from a fed batch bioreactor enters the flow channel and as the cells pass through the 3D standing wave they are trapped by the acoustic forces. The trapped cells migrate to the nodes and clump till such time as their buoyancy decreases and they settle out of the suspension by gravity. This yields a partially clarified HCCF which can be polished using a small area depth filter.

Results and discussion: We report the continuous clarification of fed batch culture of a CHO-S based cell line expressing a humanised IgG1 MAb without compromising product quality. We demonstrate the ability to clarify cell culture at cell densities of 30 - 100 million cells/mL, in a continuous manner at flow rates of up to 3.6 L/h. The technology is scalable and we have demonstrated clarification flow rates of 50 L/h suitable for use with up to 2000L bioreactors. The partially clarified HCCF is polished in a continuous mode using depth filtration but typically requires 3-5x less depth filter area than used for a traditional depth filtration process. This offers substantial economic benefits in a process

Bibliography, Acknowledgements: Thanks to the FDS teams for supporting this work

Disclosure of Interest: None declared





PO070

UNDERSTANDING THE EFFECT OF HIGH GAS ENTRANCE VELOCITY ON CHINESE HAMSTER OVARY (CHO) CELL CULTURE PERFORMANCE AND ITS IMPLICATIONS ON BIOREACTOR SCALE-UP

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Background and novelty: There are three main potential sources for cell shear damage in stirred tank bioreactors. One is the potential high energy dissipation in the immediate impeller zones; another is small gas bubble burst, and the third is high gas entrance velocity emitting from the sparger. The first two have been thoroughly addressed for the scale-up of CHO cell culture with commonly successfully practiced agitation ranges and the use of shear protectants to guard against bubble burst. However, GEV remains a potential scale-up problem across scales and different sparger designs.

Experimental approach: GEV as high as 200 m/s due to high gas flow rates and relatively small sparger hole diameters was hypothesized to cause significantly lower cell culture performance at large scale when compared to a satellite small scale with GEV of < 1 m/s. A small scale study with GEV as high as 265 m/s supported this hypothesis. Based on anecdotal evidence, a critical GEV of > 60 m/s for CHO cells is proposed, whereas previously 30 m/s had been reported for NS0 cells by Zhu et al.

Results and discussion: Analysis of the GEV effect on the same cell line using an apoptosis assay by flow cytometry suggests that cell death may be caused by apoptosis rather than by the presumed necrosis. Cell cycle analysis of the same culture also pointed to a higher apoptotic population under high GEV. Implementation of new spargers at large scale with a higher number of larger diameter holes designed to impart lower GEV did improve the cell culture performance and help close the scale-up gap. Such new sparger design was even more critical when hole plugging was observed during large scale cultivation hence exacerbating the GEV impact. Furthermore, development of a scale-down model based on mimicry of the large scale GEV profile as a function of time was proven to be beneficial for reproducing large scale results.

Bibliography, Acknowledgements:

Disclosure of Interest: None declared





PO071

EFFICIENT PROTEIN PRODUCTION BY TRANSIENT GENE EXPRESSION USING INSECT CELLS

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Background and novelty: Transient gene expression allows rapid production of various recombinant proteins for early-stage preclinical and clinical developments of biologics. Insect cells have been recognized as an excellent platform for the production of functional recombinant proteins [1, 2]. In the present study, the production of an antibody Fab fragment by transient gene expression in lepidopteran insect cells was examined.

Experimental approach: The DNA fragments encoding the heavy chain (Hc) and light chain (Lc) genes of an Fab fragment were respectively cloned into the plasmid vector pHAneo, which contained the *Bombyx mori* actin promoter downstream of the *B. mori* nucleopolyhedrovirus (BmNPV) IE-1 transactivator and the BmNPV HR3 enhancer for high-level expression [3]. *Trichoplusia ni* BTI-TN-5B1-4 (High Five) cells were co-transfected with the resultant plasmid vectors using linear polyethyleneimine. When the transfection efficiency was evaluated, a plasmid vector encoding the enhanced green fluorescent protein (EGFP) gene was also co-transfected. Transfected cells were incubated with a serum-free medium in a static or shake-flask culture.

Results and discussion: Transfection and culture conditions were successfully optimized by flow cytometry of EGFP expression in transfected cells and the yield of the secreted Fab fragment measured by enzyme-linked immunosorbent assay (ELISA). Under optimal conditions, the yield of more than 100 mg/L of Fab fragment was achieved in 5 days in a shake-flask culture. Transient gene expression using insect cells may offer a promising approach to high-throughput production of candidate proteins for the development of biologics.

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Disclosure of Interest: None declared





PO072

IMPLEMENTATION OF DIFFERENT CULTURE STRATEGIES FOR INCREASING CELL DENSITY IN HEK293 CULTIVATIONS IN BIOREACTOR

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Background and novelty: The main drawbacks when designing cell culture fed-batch strategies to achieve high viable cell density (VCD) are the accumulation of by-products and the increase of osmolarity. Even further complications arises when a growth inhibitory recombinant product is expressed by the cultured cells, since upon reached a critical product concentration the cell growth is inhibited. In those cases perfusion must be the preferred culture strategy in order to achieve high productivities.

Experimental approach: Two sets of experiments were performed in 2-liter bioreactor using two HEK293 cell lines that produce an intracellular GFP (HEK293-GFP) and an Interferon Gamma (HEK293-IFN- γ) respectively. Four different culture strategies were implemented: Batch, Fortified-Batch, Fed-Batch and Perfusion. In addition, two different culture parameters were studied: the nutrient concentration in feed media and pH culture.

Results and discussion: In fed-batches performed with HEK293-IFN- γ , higher VCD ($10.9 \cdot 10^6$ cells/mL) was obtained when the nutrient feed concentration was reduced, demonstrating that high osmolarity affects significantly the cell growth. However, fed-batch cultures performed worse than fortified-batch in terms of VCD ($12.5 \cdot 10^6$ cells/mL) since the product expressed inhibits the cell growth. Therefore, a perfusion system allows overcoming this limitation. In contrast, when non-growth inhibitory product is expressed (HEK293-GFP), a fed-batch without pH control to trigger concomitant glucose/lactate consumption is performed to overcome osmolarity limitation. This approach suppressed lactate accumulation and avoided alkali buffer addition, reducing the broth osmolarity and yielding to higher VCD ($25 \cdot 10^6$ cells/mL).

Bibliography, Acknowledgements: Liste-Calleja, Leticia, et al. "Lactate and glucose concomitant consumption as a self-regulated pH detoxification mechanism in HEK293 cell cultures" *Applied microbiology and biotechnology* 99.23(2015):9951-9960.

Disclosure of Interest: None declared





PO073

PROCESS INTENSIFICATION FOR THE PRODUCTION OF ANTIMICROBIAL PEPTIDES WITH STABLY TRANSFORMED DROSOPHILA MELANOGASTER S2 CELLS

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Background and novelty: Antimicrobial peptides (AMPs) such as representatives of the Gloverin family are valuable resources for pharmaceutical industry and can serve as leads for the development of novel antibiotics. In order to access this potential an effective recombinant expression process is mandatory, which includes the selection of a suitable expression host as well as process optimization during scale up.

Experimental approach: Here we describe the production of a Gloverin-family AMP derived from the greater wax moth *Galleria mellonella* using a stably transformed *Drosophila melanogaster* S2 cell line. Based on the polyclonal population that is usually obtained after transfection of separate expression and selection plasmids, we isolated highly productive single cell clones by limiting dilution and achieved a 100% increase in productivity. Further optimization on the cellular level included a statistical planned screening to determine optimal conditions for induction of the employed Metallothionein promoter. The online measurement of the cell suspensions dielectric properties and turbidity enabled an efficient process control and monitoring of the cells physiological status.

Results and discussion: Based on this information, 25 mg/L of the AMP was expressed at the 1-L bioreactor scale ensuring efficient timing for induction and harvest. The functional Gloverin was recovered by affinity chromatography and the products antimicrobial properties against two different model strains of *Escherichia coli* were tested, indicating the successful isolation of an active peptide. Current focus of this work is the adaptation from batch to perfusion culture in order to take advantage of the robustness of S2 cells and to enhance the final protein yield.

Bibliography, Acknowledgements: We acknowledge the Hessen State Ministry of Higher Education, Research and the Arts for the financial support within the LOEWE-Program.

Disclosure of Interest: None declared





PO074

APPROACH TO OUTSOURCE THE MANUFACTURING OF A CELL CULTURE MEDIUM

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Background and novelty: The Dry Powder Media (DPM) is the most common format used in the cell culture industry. It is determinant on process productivity. However, the particularity of process-cell line combination often demands the customization of the medium. A solution could be to develop an in-house formulation and outsource its manufacturing. Frequently, the bioproducers are far-off from DPM manufacturing processes. Thus, they do not possess enough technical arguments to select a manufacturer or to design a rational and effective scheme for the development of a high-quality DPM starting from its liquid homemade formulation. Here we propose some premises to face the selection of a proper manufacturer and a methodology aimed at the assessment of the DPM manufacturing and its scaling-up, minimizing the risks of failure

Experimental approach: Three manufacturing technologies at different scales were compared through the production of two homemade media formulation. The DPM was evaluated based on four performance parameters (PP), previously defined according to the features required for the industrial bioprocess. The flow characteristics, particle size distribution, Carr's index and other quality requirements completed the analysis to assess the manufacturing technologies

Results and discussion: Potential sources of poor performance or failure were identified through its impact on the PP and the DPM quality. Based on that, a group of technical aspects was outlined as starting point to establish a methodology for turning the homemade liquid formulation developed in the lab into industrial DPM. Those premises must allow achieving the target performance of the DPM in a relatively short-time and in a cost-effective approach from the standpoint of a bioproducer, being also the first tool for selecting a proper manufacturer. A general methodology in three stages is proposed to assess the manufacturing development and its scaling-up

Bibliography, Acknowledgements: Joshi/J Pharm Sc. Tech Vol.3(7),2011

Disclosure of Interest: None declared





PO075

MEASURING AND MANIPULATING THE HYDRODYNAMIC ENVIRONMENT OF AN iPSC-DERIVED CARDIOMYOCYTE DIFFERENTIATION PROCESS

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Background and novelty: Cardiomyocytes, derived from induced pluripotent stem cells (iPSCs), have the potential to be used for cardiac repair. Differentiation consistency and yield have been shown to improve when based upon recapitulation and emulation of cardiogenesis *in vivo*. Correia(2014) developed a stirred bioreactor protocol to mimic the physiological environment within the developing heart. This utilised a hypoxic and mechanical environment through manipulation of the agitation profile. Hypoxia, combined with intermittent agitation, resulted in 1000-fold improvement in cardiac differentiation yield in comparison to the continuous normoxic protocol¹.

Experimental approach: This work investigated the hydrodynamic environment to identify and quantify the flow characteristics leading to the increase in differentiation yield in the aforementioned study. Laser Doppler Anemometry was used to identify characteristic flow frequencies under different agitation modes. A proof of concept biological study using iPSCs was undertaken, manipulating the hydrodynamic environment by adjustment of one time component.

Results and discussion: The use of intermittent agitation resulted in the presence of low frequency patterns. It can be inferred that the presence of low frequencies is responsible for the observed improvement in cardiogenic differentiation and that this can be further optimised through manipulation of three significant time components identified in this study. The rotation time, i.e. the impeller stirrer speed, the dwell time during which agitation is stopped, and the interval time between in-motion and stationary phases.

An improvement in cardiomyocyte yield was obtained through manipulation of the low frequency patterns by adjustment of the time component, dwell time. This work demonstrated that through tuning of hydrodynamic cues, optimisation of iPSC cardiac differentiation is possible.

Bibliography, Acknowledgements: Correia *et al. Stem Cell Rev.*(2014)

Disclosure of Interest: None declared





PO076

STRATEGIES FOR MICROCARRIER-BASED STEM CELL PRODUCTION: NEW HARVEST ENZYMES AND DEFINED MEDIA FOR hMSC

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Background and novelty: Many clinical trials using human mesenchymal stromal/stem cells (hMSC) in applications for regenerative medicine underline the therapeutic potential of these cells. However, for enabling extensive clinical applications, a large-scale expansion processes is yet to be found. Advantageous is the use of suspended microcarriers in a dynamic system (e.g. stirred tank reactor) where important parameters are the choice of microcarriers for cell expansion, a defined growth medium, the scale-up and the enzymatic harvest procedure. Additionally, an understanding of the flow characteristics and mixing time is important for homogeneity in the microcarrier-based process.

Experimental approach: Evaluating the growth and harvest of bone-marrow derived hMSCs and the immortalized cell line hMSC-TERT, various microcarriers were tested. A chemically defined medium [1] was used for cell expansion. For the detachment of the cells, new recombinantly produced proteases, originally found in insects, were investigated.

Results and discussion: The chemically defined medium was used for expansion under dynamic culture conditions and we confirmed that cells showed surface marker profile and differentiation capacity as stated by the ISCT. Missing the serum components, the chemically defined medium led to slower cell attachment and growth. The seed train from small spinner flask to bioreactor scale was carried out via bead-to-bead transfer. The usage of a peptidase from non-animal origin was suitable for detachment of cells. The mixing characteristics in a single-use STR with multiple phases (liquid, microcarriers, gas bubbles) were investigated at different power inputs suitable for stem cell expansion [2]. We demonstrated that dynamic bioreactor systems offer a good opportunity to generate large amounts of high-quality hMSCs under controlled and monitored process conditions.

Bibliography, Acknowledgements:

[1] DOI: 10.1155/2016/5246584

[2] DOI: 10.1016/j.procbio.2016.05.010

Disclosure of Interest: None declared





PO077

EXTENDED GENE EXPRESSION AT BIOREACTOR SCALE

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Background and novelty: One of the intrinsic limits of TGE is the plasmid loss over time which leads to a production diminishment. In order to overcome it, the so-called Extended Gene Expression (EGE) was developed. This protocol consists in prolonging the production phase through medium exchange and retransfection in shake flask. This work focuses on the proof of concept that this protocol is doable at bioreactor scale, maintaining production and product quality. The recombinant protein expressed in this approach was Gag-GFP, which forms VLPs upon expression in a host cell line.

Experimental approach: HEK293 cultures were transfected using PEI and Gag-GFP plasmid. Retransfections were done at 48 and 96 hpt. A 3-liter bioreactor was used to test EGE at large scale. Acoustic filter was used for perfusion in the bioreactor at a rate of 0.5 rvd. Flow cytometry and fluorescence in the supernatant were used for analyzing expression and production of the VLPs. NTA and TEM were used for product quality assessment.

Results and discussion: Cell density in the bioreactor was higher than in the shake flask and viabilities were held over 60%. VLP production was comparable in both systems: 23 µg of Gag-GFP protein/mL of working volume. This means that the bioreactor was able to produce a total amount of 30 milligrams of Gag-GFP protein compared to 0.44 milligrams obtained at the shake flask scale. TEM observation confirmed the presence of VLPs. NTA was used to analyze the total amount of particles in the purified supernatant as well as the fraction of fluorescent particles, corresponding to VLPs. Both TEM and nanosight confirmed product quality. In conclusion, this work demonstrates that Extended Gene Expression is perfectly scalable to bioreactor.

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Disclosure of Interest: None declared





PO078

A NOVEL APPROACH OF HIGH THROUGHPUT CELL LINE SCREENING SPECIFIC FOR PERFUSION PROCESSES

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Background and novelty: The present biomanufacturing landscape drives the need for process intensification. Merck has perfusion legacy with some commercial products that were developed in the 90s. Since then, with the advances in media design and the monoclonal antibody driven portfolio, development efforts have been focusing on fed-batch. If clear strategies are proposed to select cell lines for fed-batch, no specific screening methodology dedicated to continuous processes has been described. This presentation will discuss a novel approach of cell line screening for perfusion processes.

Experimental approach: To support clone screening for perfusion there is need for high throughput solutions. Existing small scale systems were used to mimick perfusion conditions. Ideas on how to use these systems to evaluate clone performance for perfusion processes will be presented.

Results and discussion: First a methodology for the screening of 470 clones in fed-batch mode was established. From this initial screening, 12 clones were selected and tested in a series of small scale models ranging from microliters to several liters. This large screening was performed to asses relative clone performance in each model by comparing the ranking obtained in the different scale down systems based on a number of process criteria and/or quality attributes [1]. Currently a novel clone screening approach designed specifically for perfusion is being developed. It will be using the same 12 model clones and small scale systems adapted for continuous cell culture parameters. This work should help to define clone selection criteria specific for perfusion process development.

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Disclosure of Interest: None declared





PO079

MODULATING ANTIBODY GALACTOSYLATION THROUGH CELL CULTURE MEDIUM FOR IMPROVED FUNCTION AND PRODUCT QUALITY

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Background and novelty: The production of therapeutic antibodies requires high titers and excellent product quality to ensure efficient manufacturing and potent drug efficacy. The *N*-glycan profile, especially galactosylation, is a critical quality aspect that can alter antibody binding and function. A major factor in shaping the galactosylation profile of production antibodies is cell culture condition, such as culture medium. Herein, we demonstrate the ability to modulate antibody galactosylation through key media components. In addition, we improved upon an assay capable of measuring antibody induction of complement-dependent cytotoxicity. Together, these tools allow for the optimization of cell medium, a better understanding of the relationship between antibody structure and function, and excellent protein quality.

Experimental approach: Various factors affiliated with galactosylation were assessed in their ability to increase or decrease galactosylation levels of a therapeutic antibody produced in CHO fed-batch cultures. Cell growth and titers were monitored throughout the culture process while the glycan profiles were evaluated after the harvest. An *in vitro* complement-dependent cytotoxicity assay was utilized to evaluate the effect of galactosylation on antibody function.

Results and discussion: By varying media components, we were able to increase or decrease the level of galactosylation. The media composition was further adjusted to obtain a cell medium that resulted in a desired galactosylation profile without compromising VCD, percent viability, and titer. Evaluation by the CDC assay showed that changes in galactosylation significantly altered cell cytotoxicity and EC₅₀ values. The ability to modify galactosylation with media supplements, measure glycan profiles, and evaluate antibody effector function allows for cohesive control of antibody product quality that can be further utilized to improve antibody drug efficacy

Bibliography, Acknowledgements: .

Disclosure of Interest: None declared





PO080

MULTIMODAL SPECTROSCOPIC BIOPROCESS MONITORING FOR IN-LINE DETECTION OF CHO CELL VIABILITY

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Background and novelty: The use of spectroscopic sensors for bioprocess monitoring is a powerful tool within the process analytical technology (PAT) initiative of the FDA [1]. In-line measurements are particularly important during cost-intensive manufacturing of biopharmaceuticals in order to facilitate early process fault detection, minimize the risk of contamination and observe real time product release. Spectroscopic sensors enable simultaneous in-line bioprocess monitoring of various critical process parameters including biological and chemical variables during the cultivation process. The monitoring potential can be increased by multimodal combination of spectroscopic techniques.

Experimental approach: In this study, mammalian cell cultivation (CHO-K1) in a bioreactor was chosen as a benchmark process for bioprocess monitoring by 2D-fluorescence and UV/Vis spectroscopy. The use of a new 2D-fluorescence sensor with three relevant excitation wavelengths enabled the detection of compounds involved in cellular growth and metabolic activity. In addition, absorption and scattering in the UV/Vis spectra provided information about the morphology, total and viable cell count.

Results and discussion: Spectral data were analyzed by multivariate data analysis including principal component analysis (PCA) and partial least square (PLS) regression. Results of the PCA demonstrate the comparison of different cultivations and present the number of principal component which are necessary to describe the process variance. PLS regression generates quantitative predictions of process variables representing the cell count and viability. Benefits and limitations of both techniques are discussed and possible applications as well as the advantage of combination of different sensors are presented in this work.

Bibliography, Acknowledgements: [1] FDA. Guidance for industry PAT—a framework for innovative pharmaceutical development, manufacturing, and quality assurance. Rockville: FDA; 2004. p. 16.

Disclosure of Interest: None declared





PO081

DEVELOPMENT OF A SCALABLE PROCESS FOR MANUFACTURING CGMP GRADE RECOMBINANT HUMAN LAMININ 521 BASED ON THE CAP-GO EXPRESSION SYSTEM.

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Background and novelty: Laminins are the major components of Basement Membrane (BM), which plays a central role in cell-matrix interaction and is the foundation for cells to grow on. Laminins are heterotrimeric glycoproteins composed of three covalently linked chains that are termed α , β and γ , each of which consists of 5 (α 1-5), 4 (β 1-4) and 3 (γ 1-3) genetic variants. LAM521 is composed of an α 5, β 2 and γ 1 chain and has a molecular weight of 760 kD. It is known to be capable of supporting *in vitro* survival and growth of pluripotent cells through interaction with α 6 β 1 integrin. Previous studies have shown a great success of using LAM521 in cultivation of hESCs or iPSCs for cell-based therapeutic applications. Although the research grade rhLAM521 is commercial available, a scalable process to produce LAM521 in GMP quality has been missing so far. We used the CAP-Go technology, which is highly suitable for expression of complex and difficult-to-produce proteins, to develop a new, GMP-compliant, scalable production process for LAM521.

Experimental approach: Based on human CAP-Go suspension cells a single cell clone highly expressing LAM521 was generated. A fed-batch process in stirred tank bioreactor was developed and optimized. A clarification scheme and the downstream process using two chromatographic steps and viral inactivation steps were developed as well.

Results and discussion: We were able to develop a cell line producing rhLAM521 at high titers. After up- and downstream process development, LAM521 with purities of >98% could be produced. As the process is entirely animal component free and viral clearance steps are included in the process, the purified protein presents a safe product for cell therapy applications. Moreover, all process steps are scalable and only GMP compliant materials are used. Thus the process was easily transferable to GMP facilities for commercial production.

Bibliography, Acknowledgements: /

Disclosure of Interest: None declared





PO082

DEVELOPMENT OF A HIGH-THROUGHPUT PLATFORM TO SUPPORT CELL CULTURE MEDIA AND FEED SCREENING

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Background and novelty: Industry practice for mammalian cell culture media and feed development typically employs a high throughput screening (HTS) platform along with large sets of experiments. Modern HTS systems often include robotic liquid handlers to replace labor intensive steps. To align with advancements in the field, a semi-automated HTS platform was developed to facilitate in-house media and feed development for early stage biologics projects.

Experimental approach: Selecting appropriate instruments and integrating them into a seamless system are the keys to a HTS platform. The developed HTS platform includes 24 deep well plates (DWPs) as the culture vessels, Advance Microscale BioReactor (AMBR15) for formulation preparations in an aseptic environment, a cell imager for viability and cell growth analysis, a liquid handler for assay sample preparations, and a high throughput metabolite analyzer. The representative cell growth in DWPs to shake flask and the compatibility of its layout to AMBR15 makes 24 DWPs the best option to the platform despite of the limited throughput. In addition, the liquid handler function of AMBR15 was chosen for formulation preparations because of its user friendly Design of Experiments (DoEs) interface. A Macro program was written to enable the easy import of DoEs design from major statistics software into AMBR15.

Results and discussion: Performance qualification of each component were first performed prior to implementing the HTS platform. A case study of applying this semi-automatic HTS platform to support a complex feed screening using definitive screening design is presented. This experiment, containing more than 60 feed formulations in duplicates, was handled by one operator and delivered a 40% improvement in productivity within 4 week period. This screening platform not only improves process throughput, operational precision, and traceability, but it also reduces the labor for the formulation preparation.

Bibliography, Acknowledgements: N/A

Disclosure of Interest: None declared





PO083

“DE NOVO” HIGH DENSITY PERFUSION MEDIUM: INCREASED PRODUCTIVITY AND REDUCED PERFUSION RATES

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Background and novelty: A perfusion medium requires high concentrations of specific nutrients while balancing other components to support intensified perfusion processes (cell densities $>50 \times 10^6$ vc/mL and cell specific perfusion rates (CSPR) <40 pL/cell*d). By using a combination of design of experiment (DOE), multivariate analysis (MVA), and spend media analysis, we developed a perfusion medium that can sustain extended steady states and maximizes volumetric productivity for multiple cell lines and proteins. We also validated that the medium is stable at room temperature and 4°C for over a year after hydration.

Experimental approach: A “de novo” perfusion formulation was designed from a DOE mixture and MVA using our proprietary media library in high density batch culture. Additional optimization was performed by a combination of factorial designs and MVA to achieve increased volumetric productivity and lower CSPRs while maintaining cell density and product quality. Spent media from perfusion bioreactors was analyzed in order to understand nutrient consumption. This information was used to rebalance the concentration of specific amino acids, vitamins and fatty acids to support targeted CSPRs. The final formulation was tested for extended steady states in bioreactors for production and product quality stability.

Results and discussion: The perfusion medium was evaluated in bioreactors using alternating tangential flow (ATF) cell retention devices with multiple CHO cell lines and proteins. Steady states were maintained at cell concentrations over 50×10^6 vc/mL with perfusion rates of 1 to 3vvd. The optimization of the medium supported the reduction of the minimum CSPR from initially over 80pL/cell*d to under 40pL/cell*d while maintaining or increasing specific productivity. Lastly, we demonstrated this “de novo” perfusion medium can sustain steady states over 20 days with stable growth rates, viability, volumetric productivity and product quality.

Bibliography, Acknowledgements: N/A

Disclosure of Interest: None declared





PO084

CONTINUOUS SUSPENSION CELL CULTURE MONITORING IN BIOREACTORS USING QUANTITATIVE PHASE IMAGING.

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Background and novelty: Monitoring of cell counts and viability are performed daily using Trypan-Blue cell exclusion as a method of choice. Quantitative phase imaging (QPI) is a new quantitative imaging technique that allows cell count and viability monitoring in a continuous, label-free set-up, making results available in real-time for the whole run. Compared to classical microscopy, QPI offers the ability to refocus images post-acquisition and the collection of quantitative phase information (optical density, not captured by the human eye), covering shape and density of an object. QPI helps the operator to track total cell density and cell viability at any time.

Experimental approach: OVIZIO's iLine-F has been benchmarked with an off-line method making use of sampling and automated Trypan-Blue staining. Vi-Cell XR (Beckman Coulter) was used as reference. A bioreactor equipped with a BioConnect (OVIZIO's continuous, closed-loop, sampling device) plugged into an iLine-F was inoculated with CHO cells in CD-CHO medium (Life Technologies) at a final volume of 2L. Several culture modes were applied. Cultures were sampled daily for Vi-Cell XR cell count. Cell counts, viability measurement and a set of 59 parameters per cell were generated twice per hour by the iLine-F. Cell starvation and death were also captured.

Results and discussion: A correlation factor R^2 of 0.993 was obtained for the viable cell density (0.987 for viability) demonstrating that results achieved with the label-free QPI method are in line with current methods. The iLine-F shows the benefit of having the full trend which can be more relevant than a single point, once a day. The availability of full data at single cell level, for a whole experiment, allows the use of the iLine-F for PAT approach. The great amount of data produced can be used to perform various statistical analysis on the cell population to define and control critical parameters of the cell culture process.

Bibliography, Acknowledgements: NA

Disclosure of Interest: None declared





PO085

**METTLER TOLEDO PCO₂ PROBE
EVALUATION/IMPLEMENTATION FOR A PERFUSION
BASED CELL CULTURE SYSTEM**

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Background and novelty: Dissolved carbon dioxide (pCO₂) is a key process parameter for this perfusion cell culture and it is critical to keep it well controlled. Prior to using a pCO₂ probe, pCO₂ was monitored offline using BGA pCO₂, and controlled indirectly by adjusting the pH set-point in the small scale-down model.

Experimental approach: In this study, pCO₂ probes from Mettler Toledo were evaluated in the small scale bioreactor and compared with offline BGA readings.

Results and discussion: Some of the pCO₂ probe readings drifted over time from BGA reading, but with recalibration of pCO₂ probes every 8 days, the pCO₂ probe reading tracked well with the BGA reading (within 3 mmHg). Adjustment based on the offline measurement deemed to be necessary. The second and third batch verification runs show equivalent accuracy and adjustment frequency as the first batch evaluation runs. Tuned pCO₂ probe control demonstrated that pCO₂ can be controlled much tighter at the set point than indirect pH probe control controlled pCO₂. The probes showed no sign of performance declining after >300 days of in process use. The Mettler Toledo pCO₂ probe has been recommended to be used in commercial perfusion production.

Bibliography, Acknowledgements: Bibliography: Dr jiyi zhang is a process/development manager for cell culture team at Sanofi. She is specialized at process development for animal cell/yeast/bacteria platform.

Acknowledgements: The authors would like to thank Department of Technology Genzyme Geel for the support and close collaboration. Additionally, special thanks to Manufacturing, QA Departments, Genzyme Geel, and MSAT network for its support.

Disclosure of Interest: None declared





PO086

ONLINE CAPACITANCE MEASUREMENT FOR BIOMASS MONITORING OVER CULTIVATION SCALES AND PLATFORMS

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Background and novelty: Online capacitance measurement has become a well-established PAT tool in biopharmaceutical applications using traditional multi-use (MU) bioreactor and fermentation equipment. Simultaneously, as this PAT tool emerges in MU processes, there is a clear trend to single-use (SU) cultivation equipment in process development up to production. Consequently, prototypes of SU capacitance sensors for viable biomass detection have been developed and presented to the community throughout the past years. In 2015, SU capacitance measurement has become commercially available with significant uptake in market place.

Upstream capacity adaptation and bioprocess transfer is a challenging procedure in process development. During the scaling process, different types of bioreactors are used. Today, SU cultivation equipment is available with good scalability from small process development to production scale. In this scale-up context, online capacitance measurement for viable biomass monitoring can be a key parameter to monitor consistent cell growth and support the verification of process scaling.

Experimental approach: CHO cultivation experiments were carried out in SU and MU bioreactors using different scales from 5 L to 2000 L working volume. Two different CHO fed-batch processes have been evaluated involving online capacitance measurements (Sartorius BioPAT® ViaMass).

Results and discussion: The comparison of the results of different scales show scalability of the two model process. This was demonstrated by offline sample analysis (e.g. VCD and titer trends) as well as by the online capacitance signals. Here, the online viable biomass measurement in terms of capacitance detection showed to be an attractive tool to support scaling and process characterization. The experiments and results will be presented and comparability between scales/systems as well as specific differences will be discussed.

Bibliography, Acknowledgements: Sartorius PAT and Upstream teams

Disclosure of Interest: None declared





PO087

IMPROVING MAMMALIAN CELL CULTURE PROCESS DEVELOPMENT BY MODEL-BASED DESIGN OF EXPERIMENT

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Background and novelty: Increased process understanding have evolved from the Process Analytical Tool initiative (PAT) and the Quality by Design (QbD) methodology. In contrast to one-factor-at-a-time methods, statistical Design of Experiment (DoE) methods are widely used to develop biopharmaceutical processes. Even if high-throughput systems can handle these numbers of experiments in parallel, the heuristic restriction of boundaries and the high number of factors results in stepwise iterations with multiple runs. Therefore, the combination of model-based simulations with DoE methods for the development of sophisticated cell culture processes is a novel tool for process development. This method is used to reduce the number of experiments during DoE and the time needed for the development of more knowledge-based cell culture processes.

Experimental approach: The concept of model-based DoE (mDoE) was applied to the optimization of the initial L-glutamine and D-glucose concentrations of a CHO cell culture process. At first, a mathematical model was fitted to average data of an IL-8 Antibody producing cell line. Secondly, experiments were planned by mDoE with a reduced set of experiments. This optimization method was compared to a fully experimental DoE with the same constraints.

Results and discussion: This case study describes and evaluates the concept of model-based DoE, which enhances the generation of deeper understanding during process design. Furthermore, cultivation strategies for mammalian cell lines can be compared and evaluated before experiments have to be performed in laboratory scale. This results in a significant reduction in the number of experiments required during process development and process establishment. The strategy is intended for the use in multi-single-use-devices to speed up process development.

Bibliography, Acknowledgements: We kindly acknowledge the Cell Culture Technology group (Bielefeld University) for providing the cell line used in this work.

Disclosure of Interest: None declared





PO088

USING RADIO-FREQUENCY IMPEDANCE TO CONTROL CONTINUOUS HIGH DENSITY PERFUSION CULTURE WITH THE ALTERNATING TANGENTIAL FLOW SYSTEM.

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Background and novelty: Many cGMP cell culture processes are now based on a continuous high cell density perfusion bioreactor system. Tight control of the perfusion or concentrate addition rate allows the bioreactor to be operated under the optimum conditions for maximum recombinant protein production.

Experimental approach: An automatic perfusion rate control system based on the Alternating Tangential Flow or ATF system (Repligen, USA) was combined with an on-line live biomass monitor probe using Radio Frequency Impedance (Aber Instruments, UK). The system operated in a completely closed loop i.e. no samples need to be taken to obtain process information. For a continuous increase in perfusion, a live biomass probe is interfaced with the harvest pump, such that the perfusion rate is increased as a linear function of the cell density determined by the biomass probe, based on a desired cell specific perfusion rate (CSPR).

Results and discussion: In this paper we show three examples of combining the ATF and live biomass probes. In the first example the bioreactor perfusion was controlled at a constant CSPR by the biomass probe to automatically increase the feed rate as cell density increased. The probe accurately estimated the viable cell density throughout the run with cell densities up to 110 million viable cells /mL. In the second example the probe was shown to immediately spot a sudden increase in live cell density caused by too much media being pumped out in error. For the final study, the capacitance was scanned within a wide range of frequency values (100–19,490 kHz). For the measured spectroscopic data, partial least squares regression (PLS), Cole–Cole, and linear modeling were applied and compared in order to predict VCD. The Cole–Cole model and the PLS model provided reliable prediction over the entire cultivation.

Bibliography, Acknowledgements: L. Parta and D. Lazai, Gedeon Richter; C. Agarabi, FDA

Disclosure of Interest: None declared





PO089

OVERCOMING CHALLENGES IN SCALING DOWN A PERFUSION CELL CULTURE MANUFACTURING PROCESS

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Background and novelty: Prior to transferring a commercial process for the manufacture of a recombinant Factor VIII protein, scale down models at bench (5L wv) and pilot (150 L wv) scales were developed.

Experimental approach: The process itself was poorly characterised, and had limited scale down information associated with it. Numerous challenges were encountered during the development of the models, including a lack of fundamental mixing information, an unusual gassing strategy, and use of manual counting to assess cell growth throughout the process. Equipment differences exacerbated the issues, as the development lab adapted from a previous high cell density process to this low cell density culture.

Results and discussion: These challenges required innovative problem-solving approaches but were overcome, with both cell culture performance and product quality comparable to the originator commercial process.

Bibliography, Acknowledgements: Many thanks to the cooperation received from our contract manufacturer.

Disclosure of Interest: None declared





PO090

A TECHNOLOGY ROADMAPPING PROCESS TO TRANSFORM THE BIOPHARMACEUTICAL MANUFACTURING INDUSTRY

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Background and novelty: The complexity of the current industry structure has held back innovation, with many biomanufacturers trying to develop new technology in isolation, whilst supply partners often guess common industry requirements. The BioPhorum Operations Group's (BPOG's) technology roadmapping collaboration has brought together 26 of the biopharmaceutical industry's top companies, along with leading academics, to establish an industry technology strategy that is already starting to align the industry's innovation efforts.

Experimental approach: A strong steering committee was established with a shared vision of the future of biomanufacturing. The drivers and metrics from this shared vision triggered the mobilization of over 90 industry experts onto 6 teams focused on the key enabling technologies of in-line monitoring & real-time release, process technology, automation, modular & mobile, knowledge management and supplier partnerships. Each team has now established detailed technology roadmaps that combine to provide a roadmap for the next 10 years.

Results and discussion: In this presentation, we will talk about highlights from the roadmap as well as the problems, challenges and solutions, and how the work of the 6 teams integrates. The first publication of the roadmap will be refreshed and updated regularly, and we are looking for input and comment now and after publication in May.

Bibliography, Acknowledgements: The Technology Roadmap is the work of the Technology Roadmapping collaboration group within the Biophorum Operations Group and is made up of the major manufacturers, leading academics, supply partner R&D Heads, regulators and worldwide regional hubs.

Disclosure of Interest: None declared





PO091

LATEST DEVELOPMENTS IN SCALABLE, HIGH-TITER TRANSIENT PROTEIN EXPRESSION IN THE EXPICHO EXPRESSION SYSTEM

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Background and novelty: The ExpiCHO™ transient expression system offers a turnkey solution for generating high-titer recombinant proteins for therapeutic drug development, reagent generation, as well as an alternative platform for proteins that express poorly in 293-based systems. In the time since its introduction, the ExpiCHO system has become a prominent part of the drug development workflow for many biopharmaceutical laboratories.

Experimental approach: In the present study we provide protocols for optimal protein expression in the ExpiCHO system for volumes ranging from 1mL in 96 well plates up to greater than 1L in vented shake flasks. Additionally, we provide data on the implementation of the ExpiCHO system on the ambr®15 micro bioreactor system and recommendations for automated protein expression protocols. Lastly, we present data on scale up of the ExpiCHO system to 10L expression volumes using the WAVE Bioreactor™ 20/50 system.

Results and discussion: Together, these protocols allow for rapid, high titer transient CHO protein expression from mL to L volumes to meet a broad range of experimental demands.

Bibliography, Acknowledgements: We would like to acknowledge Jian Liu, Wanhua Yan, Kyle Williston and Henry Chiou for their contributions to the poster.

Disclosure of Interest: None declared





PO092

UNDERSTANDING OF DECREASED SIALYLATION OF FC-FUSION PROTEIN IN HYPEROSMOTIC RECOMBINANT CHINESE HAMSTER OVARY CELL CULTURE: N-GLYCOSYLATION GENE EXPRESSION AND N-LINKED GLYCAN ANTENNARY PROFILE

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Background and novelty: For the large-scale production of therapeutic glycoproteins, fed-batch culture has been widely used of its operational simplicity and high titer. However, repeated feeding of medium concentrates and/or addition of a base to maintain optimal pH lead to increase in osmolality. The hyperosmolality affects glycosylation in a protein-specific manner, however, the mechanism behind such osmolality-dependent variations in glycosylation in rCHO cells remains unclear.

Experimental approach: To better understand the effect of hyperosmolality on the glycosylation of a protein produced from rCHO cells, we investigated 52 *N*-glycosylation-related gene expression and *N*-glycan structure in Fc-fusion protein-producing rCHO cells exposed to hyperosmotic conditions. Furthermore, to validate the effect of hyperosmolality on protein glycosylation, we performed hyperosmotic culture supplemented with betaine, an osmoprotectant, and then analyzed the *N*-glycan structure and mRNA levels of *N*-glycan antennary genes.

Results and discussion: After three days of hyperosmotic culture, nine genes (*ugp*, *slc35a3*, *slc35d2*, *gcs1*, *manea*, *mgat2*, *mgat5b*, *b4galt3*, and *b4galt4*) were differentially expressed over 1.5-fold of the control, and all these genes were down-regulated. *N*-glycan analysis showed that the proportion of highly sialylated (di-, tri-, tetra-) and tetra-antennary glycans was significantly decreased upon hyperosmotic culture. Addition of betaine to the hyperosmotic culture significantly increased the proportion of highly sialylated and tetra-antennary glycans, while it increased the expression of the *N*-glycan antennary genes (*mgat2* and *mgat4b*). Taken together, the results obtained in this study provide a better understanding of the detrimental effects of hyperosmolality on *N*-glycosylation, especially sialylation, in rCHO cells.

Bibliography, Acknowledgements: -

Disclosure of Interest: None declared





PO093

DISRUPTIVE COST-EFFECTIVE ANTIBODY MANUFACTURING PLATFORM BASED ON CUTTING-EDGE PURIFICATION PROCESS

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Background and novelty: The demand for monoclonal antibodies is growing exponentially, pushing for new production capacities. Manufacturing of such biologics is usually done in large factories, using high-volume bioreactors, requiring high CAPEX. This high CAPEX is a barrier preventing small- or medium-size players to enter the market, and limits the availability of therapeutic proteins to high-income countries only.

Experimental approach: Univercells is proposing a disruptive biomanufacturing process able to reduce the CAPEX by 90%, while offering competitive COGS and productivity up to 500 kg mAbs/year therefore enabling the manufacturing of affordable biologics in emerging markets. We are focusing on processes implementing single-use cost-effective bioreactors operated in fed-batch or perfusion modes, with in-line clarification & capture processes operated in simulated continuous mode. We have introduced an innovative clarification technique in the form of host cell impurities precipitation operated directly in the production bioreactor. This significantly reduces the HCP/HCD burden on downstream processing and allows to use smaller chromatography columns/membranes in batch or continuous mode. It also favors implementation of monoclonal antibody capture steps different from protein A affinity chromatography.

Results and discussion: This innovative solution will allow to reduce significantly the cost of manufacturing of antibodies (targeted at less than 75 \$/gram), and offers an excellent opportunity for emerging countries to enable local manufacturing of affordable therapeutic proteins. The case study of the process development of an anti-MERS antibody (from the NIH) will be presented, along with assessment of manufacturing options, and presentation of results such as cell culture dynamics, productivity, and key process quality indicators. An evaluation of the cost model of the complete production facility will be demonstrated.

Bibliography, Acknowledgements: N/A

Disclosure of Interest: None declared





PO094

SCIENTIFIC STRATEGY FOR RESIN SELECTION AND SINGLE-USE FILM ARCHITECTURAL DESIGN FOR CELL CULTURE SYSTEMS

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Background and novelty: Bioprocessing widely implements single-use technologies including multiple polymer layers with stabilizing additives that might negatively impact cell culture performance or protein quality. To overcome this challenge, a first principles approach was taken to design a film by selecting materials compliant with pharmaceutical standards (e.g., EP 3.1.3). To reduce the potential for leachates, polymer resins with anti-block properties were selected for external layers of the film, eliminating the need for small molecule additives (e.g., stearates). Film matrix porosity also influences small molecule migration through contact layers. Therefore, incorporating polymer resins that formed a tighter molecular matrix limited the potential for leachable molecules and facilitated a film architecture designed for biocompatible single-use film.

Experimental approach: Polymer resins and additive combinations were evaluated based on chemical extraction profiles, biocompatibility data, and cell culture screening with sensitive CHO cell lines. The architectural placements of polymers and additives were balanced based on the film processing requirements, mechanical stresses during cell culture applications, and other design requirements for film performance (e.g., gas barrier, puncture resistance). The optimization of the film architecture for physical durability and minimized porosity was evaluated using mechanical stress testing (e.g., flexural fatigue), gas transmission rates, and time-dependent extraction studies.

Results and discussion: With an optimized selection of materials and structural design, a film with a reduced chemical profile of extractable molecules was produced. For example, one antioxidant known to negatively impact cell growth was below the limit of detection (2 ppb). This film supported > 90% cell viability and > 90% population doubling with a mAb-producing CHO cell line.

Bibliography, Acknowledgements: Daniel Nelson, GE Healthcare

Disclosure of Interest: None declared





PO095

SCALE-UP OF HIGH AREA FILTERS FOR MICROFILTRATION OF CELL CULTURE MEDIA

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Background and novelty: Sterilizing grade membrane filters are often packaged in pleated formats. A wide variety of pleat configurations are possible including high density pleat geometries that allow for increased productivity per device, smaller filter footprint, and improved filtration economics. Depending on the particular pleat configuration, up to 100% more membrane area can be fitted into a high area cartridge filter compared to a conventional pleat pattern. A high pleat density arrangement can, however, present unique challenges when scaling up from discs to cartridges. In this study, the factors that impact membrane filter scalability for filtration of cell culture media were investigated.

Experimental approach: Several variants of sterilizing grade filters were assessed for throughput capacity, for each of several types of cell culture media challenge streams that were formulated to represent a wide range of particle size distributions. Pleated cartridge performance was compared to flat discs and to a new small scale pleated scaling tool.

Results and discussion: It was found that for most streams, high area filters exhibited near linear throughput capacity scalability with both flat discs and pleated scaling tools. However, for some streams containing a population of very large particles (relative to the size of the membrane pores), particle access to the membrane surface can be inhibited, resulting in diminished scalability to flat discs. The new small scale pleated scaling tool, however, closely mimics the filtration behavior of the full scale high area filter for all the streams tested. This scaling tool can be used to confirm expected scaled-up performance. In addition, a model was developed that can be used to assess which applications can most benefit from high area pleated devices and which applications may be best served using conventional pleat configurations.

Bibliography, Acknowledgements: N/A

Disclosure of Interest: None declared





PO096

ANALYSES OF PRODUCT QUALITY OF COMPLEX POLYMERIC IGM PRODUCED BY CHO CELLS

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Background and novelty: Immunoglobulin M (IgM) antibodies are secreted by B cells as the first defense against invading pathogens during primary immune response. Some IgM antibodies already gained the orphan drug status, which shows their unique capability in therapy of rare diseases. Potential fields for applications are discovered with increasing knowledge about these molecules. It seems that the most active forms are pentameric and hexameric IgMs. Unfortunately, recombinant production of IgMs is rather difficult as secretion and correct polymer formation results in low expression yields and mixtures of polymers.

Experimental approach: We established stable producing CHO DG44 cell lines to analyze cellular and extracellular factors that influence quantity and quality of the produced recombinant polymeric IgM in future studies [1]. One quality parameter is polymer distribution, which can be measured directly in cell culture supernatant using densitometric analyses [2]. Additionally, we developed a very efficient single-step-affinity purification strategy to analyze pure IgMs. For more precise measurements of the IgM isoform distribution we separated the purified polymers by SEC HPLC.

Results and discussion: Process development on IgM purification using the POROS Capture Select human IgM affinity matrix enabled the recovery of highly pure fractions. Through optimization, by combining mild pH and high salt concentrations, the relatively low elution yields were increased by a factor of 5. Our model IgMs were produced in a ratio of approximately 4:1 pentameric to dimeric IgM, measured concordantly with both analytical methods. Applying densitometry and SEC-HPLC we will investigate how culture conditions influence polymer formation in future.

Bibliography, Acknowledgements: [1] Chromikova et al. 2015. Cytotechnology 67:343-356

[2] Vorauer-Uhl et al. 2010 J Immunol Methods 359:21-27

Disclosure of Interest: None declared





PO097

DEVELOPMENT OF A HIGH THROUGHPUT SCALE DOWN MODEL FOR A HIGH CELL DENSITY PER.C6@-BASED ADENOVIRUS PERFUSION PRODUCTION PROCESS

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Background and novelty: Currently, no small scale (<2L) cell culture system is commercially available for high cell density perfusion cultivations to use in high throughput screening studies. To increase throughput for process characterization at Janssen, a shaker flask-based scale down model was developed. Though, the control possibilities of shaker flask cultures are technically very limited and different compared to a bioreactor controlled process. In addition, the sensitivity of the shaker flask should enable to detect and characterize the effects of process parameters on critical quality attributes of the vaccine produced at large scale.

Experimental approach: Experiments were conducted to determine the best possible cultivation conditions for the shaker flask to mimic as closely as possible the 10L bioreactor high cell density perfusion process used for adenovirus production such as for Ebola and HIV vaccines. Next to the standard cell culture outputs, the PreSens shake flask reader was implemented to obtain pH and DO profiles. Subsequently, the shaker flask scale down model was qualified by comparing the critical quality attributes outputs to those of the 10L bioreactor process.

Results and discussion: Iterative experiments were performed to evaluate the influence of cultivation parameters in shaker flasks. A daily medium exchange was implemented to mimic the perfusion mode of the large scale process. Using the cultivation conditions for which the process performance parameters (as cell growth, pH and DO) and most importantly the virus titer and kinetics showed the best fit, shaker flask infections were conducted which confirmed equivalence of critical quality attributes between the large and reduced scale processes. By implementing the shaker flask model we have a tool available to screen at a higher throughput a subset of process parameters and reduce timelines of a process characterization project.

Bibliography, Acknowledgements:

Disclosure of Interest: None declared





PO098

CHO SINGLE CELL SUBCLONING AND STABILITY OVER PROLONGED PASSAGING IN THE PRESENCE AND ABSENCE OF SELECTION PRESSURE

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Background and novelty: Cell stability is important in biopharmaceutical processes, particularly in the case of perfusion processes that can be operated for weeks or months. The growing interest of industry in perfusion has also led to more interest in tools to assess and control cell stability. We have previously performed single cell subcloning of a recombinant cell clone expressing a glycoprotein and in the present work a comparison between the original clone and the best-performing subclone was done in terms of product formation, mRNA levels, gene copy number and DNA methylation.

Experimental approach: CHO cells were cultivated in chemically defined animal component-free media (SFM4CHO, HyClone; TC-LECC, Xell AG) and were passaged in spintubes every 3-4 days for up to 60 days, in the presence and absence of selection pressure. Product formation was monitored by measuring biological activity and product concentration. Analyses of product transcript levels and gene copy number by qPCR and methylation by genomic sequencing of bisulfite converted DNA were performed.

Results and discussion: The presence of the selection pressure did not impact the viability of the cells over passaging but led to a decrease specific growth rate and productivity compared to the cultures without antibiotics. The prolonged use of selection pressure did not avoid productivity loss over time. The comparison between the subclone (high producer) and the original clone (low producer) evidenced subcloning as a useful strategy. The mRNA levels suggested that the productivity loss might be related to reduced transcription activity. No effects in gene copy number or promotor methylation were detected. Further studies at the molecular level are recommended to better understand cellular stability, as well as the mechanisms underlying the higher productivity shown by the subclone.

Bibliography, Acknowledgements: Financial support from CNPq, FAPERJ, Capes, Hemobrás and BNDES is acknowledged.

Disclosure of Interest: None declared





PO099

EVALUATION OF SIGNAL PEPTIDES FOR ENHANCED PRODUCTION LEVELS IN CHO DG44 CELLS

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Background and novelty: The high-titer production of biopharmaceuticals using “Chinese hamster ovary” (CHO) cells is an important pillar of the pharmaceutical industry. A potential bottleneck for profitable production is the successful secretion of the synthesized proteins through the endoplasmatic reticulum (ER) and the Golgi apparatus into the culture medium. The transport into the ER is directed by the signal peptide (SP), a protein sequence at the N-terminus of the product. Since natural SP sequences and efficiencies vary between proteins and species, the utilization of different SPs can lead to enhanced protein expression in already existing production systems.

Experimental approach: At first we compared the influence of four different natural SPs on the secreted amount of an IgG4 model antibody using a CHO DG44 host cell line. Employing different pool generation strategies, the best producing pools obtained with each SP and the standard SP were analyzed regarding their fed-batch performance. After identification of SP(9) as a promising candidate, in a second approach, the influence of this SP on the secretion of four additional antibody products was investigated. For this purpose, large and mini pools were generated expressing the respective product with the standard signal peptide as well as with SP(9) and fed-batch experiments were performed for final evaluations.

Results and discussion: For the first approach, the results revealed a 2.4-fold increase in average final fed-batch antibody titer of SP(9) when compared to the standard SP approach (standard SP = 0.44 g/L; SP(9) = 1.50 g/L). The other SPs performed worse than the standard SP.

In the second approach, an improved performance was observed for all products when comparing SP(9) and the standard SP with an increase in average final fed-batch titers ranging from 27 to 353 %. Taken together, the results contribute to the optimization of Sartorius Stedim Cellca cell line development process.

Bibliography, Acknowledgements:

Disclosure of Interest: None declared





PO100

INVESTIGATING THE CHANGE IN A CHARGE VARIANT PROFILE OF A MONOCLONAL ANTIBODY FROM A COMMERCIAL MAMMALIAN CELL CULTURE PROCESS

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Background and novelty: An elevated level of a charge variant was observed in the drug substance of several commercial manufacturing batches of a monoclonal antibody. Analysis of upstream and downstream intermediates of the manufacturing process demonstrated that the increased charge variant level was already seen in the harvested cell culture fluid of the cell culture part of the process. An extensive root cause investigation was initiated to elucidate the upstream factor(s) causing the shift in the charge variant profile. This presentation gives an overview of the root cause investigation including the experimental small-scale studies performed to prove root cause.

Experimental approach: Trouble shooting was initiated with a comprehensive data mining exercise covering the manufacturing process, operations and raw materials. A shortlist of most likely root causes was compiled and included changes in production bioreactor pH and seeding cell density, upstream raw material lot-to-lot variability and changes to an iron chelating medium component. Impacts of these potential root causes were studied in a qualified 3-L bioreactor scale-down model representative of the large-scale manufacturing cell culture process. Peptide mapping was also performed to characterize the various charge variant peak fractions analytically.

Results and discussion: The investigation revealed a large impact of basal medium lot-to-lot variability and much smaller effects of production bioreactor pH and seeding cell density on the charge variant shift. Peptide mapping suggested that two different enzymatic modifications led to changes in the charged species. In summary, the main cause of variability of the charged species of this monoclonal antibody was found to be medium lot-to-lot variability. This enabled us to develop specific mitigation strategies for large scale production to minimize variability and increase process robustness.

Bibliography, Acknowledgements: None.

Disclosure of Interest: None declared





PO101

EVALUATION OF THE GLYCOSYLATION PROFILE OF A MONOCLONAL ANTIBODY PRODUCED IN PERFUSION MODE BY CHO CELLS USING HILIC-HPLC AND MALDI-TOF/TOF MASS SPECTROMETRY

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Background and novelty: Monoclonal antibodies (mAbs) represent the most important class among therapeutic proteins. mAb-based biopharmaceuticals represent the majority of the top 10 drugs worldwide. The glycosylation pattern of mAbs can influence structure, function, pharmacokinetics, pharmacodynamics and immunogenicity. Thus glycosylation can impact product safety and efficacy, and is considered by industry and regulators a critical quality attribute (CQA). In the present work, we investigated the glycosylation pattern of a humanized IgG1 produced in perfusion mode by CHO cells, using both hydrophilic interaction chromatography (HILIC-HPLC) and MALDI-TOF/TOF mass spectrometry.

Experimental approach: The mAb was purified from perfusion culture supernatant using a Protein A affinity chromatography resin and treated with PNGase F to release glycans. For HILIC-HPLC analysis, glycans were labelled with 2-aminobenzamide, separated using a TSKgel Amide-80 column, and glycosylated structures were proposed based on Glycobase 3.2.4. Repeated analysis of samples upon treatments with different exoglycosidases helped elucidating the structure of the oligosaccharide chains.

Results and discussion: The degree of fucosylation was found to be 99%, while mannosylation and sialylation degrees were 0.6% and 0.4%, respectively. The predominant glycoform was G0F (48.6%), followed by G1F (43.3%) and G2F (6.8%). Hybrid glycans represented 0.7% of glycoforms. MALDI-TOF/TOF analysis confirmed this glycoform distribution and showed that the masses of the main glycans were as expected (1485.6 Da for G0F, 1647.6 Da for G1F and 1809.7 Da for G2F). The techniques used in this work allow to comparatively evaluate the impact of different culture conditions and perfusion feeding strategies on glycosylation-related product quality attributes.

Bibliography, Acknowledgements: Special thanks to Dr. Cremata, from CIGB (Cuba). CAPES

Disclosure of Interest: None declared





PO102

STRATEGIES FOR OPTIMIZED CELL CULTURE MEDIA: EFFICIENT DESIGN TO IMPROVE TITER AND INFLUENCE PRODUCT QUALITY

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Background and novelty: Effective biopharmaceutical production is achieved through target specific quality optimized cell culture media. Many factors impact optimal medium performance. Balancing the many individual media components to achieve high titers and support critical protein quality attributes can be challenging. Here we highlight two statistically designed strategies to optimize cell culture media to increase titer and influence product quality.

Experimental approach: Media optimization requires incorporation of progressive DoE models. First, a screening model is used to identify the key components that influence critical culture attributes (e.g., titer, protein quality). Further rounds rely on Response Surface models to optimize levels of the important components previously identified.

Results and discussion: Case Study 1: A six factor D-optimal design targeted the increase of main variant expression and reduction of acidic and basic variants. Results indicate several conditions that improved titer and maximized main variant expression. Subsequent rounds focus on further optimizing key drivers of higher titer and main variant expression.

Case Study 2: Several media intended to support a platform of cell lines were selected from a diverse formulation library and screened. These media exhibited wide concentration ranges of the critical components, intended to maximize titer and impact critical quality attributes. Results showed improvements in quality targets like low man-5 expression, high main variant expression, low aggregation, and high levels of intact protein. Multivariate analysis compared all media components at their respective concentrations across the formulations screened. This information was correlated to titer, critical quality attributes, growth, viability and metabolic parameters. The >15000 correlations were distilled down to reveal 18 factors to evaluate in future rounds.

Bibliography, Acknowledgements: Gerald McEwen and Joseph Camire

Disclosure of Interest: None declared





PO103

NEW PLATFORM FOR THE INTEGRATED ANALYSIS OF BIOREACTOR ONLINE AND OFFLINE DATA

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Background and novelty: More experiments are used to assess bioreactor suitability and stability of clones, to evaluate media and other process parameters, and to start upscaling campaigns. This has resulted in an increase of data and the capturing, processing, aggregation, visualization, and statistical analysis of this data has become a major bottleneck. In addition, the association of the data with the experimental context (e.g., fermentation protocols, media recipes, bioreactor control parameters) is not easily accomplished in high throughput. Therefore, new and highly performant data capture, processing, and analysis systems need to be integrated to enable storage and correlation of experimental context information and various types of analytics data.

Experimental approach: We have developed a new platform for bioprocess development, which enables the automatic capture and visualization of all online and offline data (e.g., pH, O₂, metabolic data), auto-calculations and aggregations (e.g., IVCD, Q_p, consumption rates) and multi-parametric assessment of any type of time-series bioreactor data in the context of experimental protocol data (e.g., process parameters, feeds).

Results and discussion: We present concrete use cases showing the selection of the best producer clones, the identification of optimized media feeding strategies, and the comparison of clone performance across fermentation scales. A special focus is on the analysis of data from mini-bioreactors [such as the ambr15™ systems] operated in parallel (n x 12 reactors). Cross-reactor scale comparisons are a second focus [e.g., ambr15 vs. DasGip bioreactors]. Finally, we show how the platform enables the correlation of process parameters (e.g., fermentation protocols, media recipes, bioreactor control parameters), with key performance indicators of the processes (e.g., Titer, Q_p) and the product quality attributes (e.g., aggregation, glycosylation profiles).

Bibliography, Acknowledgements: Allison Kurz, bioprocess@genedata.com

Disclosure of Interest: None declared





PO104

ACCELERATED PLATFORM PROCESS FOR DEVELOPMENT OF BISPECIFIC ANTIBODY PRODUCTION

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Background and novelty: Bispecific Antibodies (BsAbs) offer great potential to extend the therapeutic spectrum of monoclonal antibody formats. However, generation of producer cell lines for such artificial molecules is often challenging. A well-defined molecular balance between multiple antibody chains is essential to assemble the desired heterodimeric BsAb. In order to obtain the correct heterodimer as predominant species expression of individual antibody chains has to be carefully adjusted. Hence, producer cell line and process development is more complex and requires higher effort as for traditional antibody formats.

Experimental approach: Different steps of cell line development, including vector system, signal peptides, transfection, pool selection and clone screening were optimized to support coordinated and optimal assembling of BsAbs. A new vector design was used that allowed to individually adjust chain ratios. Tailored analytical techniques were established to quantify correct heterodimer content. Up- and downstream development was initiated at clone pool stage.

Results and discussion: Here we demonstrate fast and reproducible generation of high producer clones for different BsAbs with titers up to 3 g/L and 85% heterodimer purity in different bioreactor formats. Expression level and optimal balance of the antibody chains were maintained for more than 70 population doublings. A specifically designed two step downstream process provided 99% BsAb purity.

Our optimized and integrated platform process enables accelerated and highly efficient BsAb production.

Bibliography, Acknowledgements: ProBioGen

Disclosure of Interest: None declared





PO105

A NOVEL APPROACH TO SETUP HYBRID-MODELS IN MAMMALIAN CELL CULTURE

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Background and novelty: Cell culture processes are typically complex systems, for which only limited mechanistic knowledge is available. Semi-parametric hybrid model approaches combine extended extrapolation properties of a strictly mechanistic model and decreased development time of a purely data-driven model. This means, they incorporate the advantages of both modeling strategies [1]. Most of the described hybrid models in literature consist of a material balance as mechanistic model whereas specific rates are approximated with a data-driven part. The novelty of the here presented hybrid model is the application of a verified mechanistic model together with a rationally designed data-driven model, increasing the extrapolation properties of the generated model.

Experimental approach: A mechanistic model for description of the viable cell count in cell culture processes was build according to the workflow described in [2]. Due to the predefined goal of describing influences of typical scale dependent process parameters such as pH and pCO₂ on our objective function the mechanistic model approach was extended by data-driven approaches based on historical data. Different strategies (e.g. MLR, ANN) were evaluated to model those relationships efficiently.

Results and discussion: The developed approach for building a hybrid model minimizes the amount of model parameters, which is in accordance with the modeling principal *as simple as possible, as complex as necessary*. By including an extended mechanistic part, most relationships could be described by meaningful parameters, which cannot be derived from data-driven approaches. The created hybrid model workflow is therefore in alignment with PAT initiatives, as it describes multi-factorial relationships, increases process understanding and allows for real time deployment.

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[2] Kroll et al., Process Biochemistry, submitted, 2016.

Disclosure of Interest: None declared





PO106

ENGINEERING CHARACTERIZATION OF THE ALLEGRO STIRRED TANK REACTORS FOR SUCCESSFUL CELL CULTURE SCALE-UP

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Background and novelty: The core benefit of traditional stainless steel STR systems lies in their direct driven agitation that can deliver a wide range of specific power inputs to the fluid. The Allegro range of single-use stirred tank bioreactors has not only adopted this direct driven impeller technology, but took an innovative step further modifying the geometry of the vessel itself to a cubical design concept.

Experimental approach: Engineering performance characterization of the Allegro STRs; in terms of mixing and mass transfer was carried out using a design of experiments (DoE) approach. Using a media simulant, a pH method was used for mixing time and CO₂ stripping rate, and the gassing out method used with nitrogen/air to determine the $k_{La}O_2$. The engineering characterization was then used to scale-up two CHO processes from conventional glass, cylindrical bench-scale STRs to the Allegro STRs, using a constant power input per volume (P/V) and superficial gas velocity (v_{sg}). The key process indicators for a successful scale-up were the product quality attributes (including N-linked glycans), product titre and cell growth.

Results and discussion: Results showed good mixing and mass transfer properties with no dead-zones and scalable cell culture results in the Allegro STRs. Using the engineering characterization for the cell culture scale-up reduced the need for a large number of small and pilot scale runs. The similarity of the operating window between the Allegro STRs and traditional bench-scale vessels, allows for the application of a simple scale-up strategy.

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Disclosure of Interest: None declared





PO108

IMPLEMENTATION OF A VIRUS BARRIER MEDIA FILTER INTO FED-BATCH BIOPROCESSES

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Background and novelty: Upstream bioprocesses are at particular risk of contamination from adventitious agents. The typical 0.1 µm filters used at this step protect bioreactors from bacteria and mycoplasma but offer no protection from viral contaminations.

A new polyethersulfone virus barrier filter has demonstrated high levels of microorganism retention - full retention for bacteria and mycoplasma (>8.0 LRV - Log Reduction Value) and ~ 5 LRV for small viruses. It also has improved flow and capacity as compared to virus removal filters designed for monoclonal antibody purification. This filter was evaluated for adaptation into upstream bioprocesses.

Experimental approach: Given the small pore size of virus retentive filters, implementing a virus filter upstream of the bioreactor raises the question of whether critical cell culture media components are removed. Therefore, it is important to evaluate the cell culture performance and protein attributes using virus-filtered media to ensure that filtration does not negatively impact the process.

Media and feeds for two CHO upstream fed batch processes were filtered through virus barrier filters or standard sterile filters and evaluated for composition change through several orthogonal methods. Surfactants (essential for shear protection in stirred tank bioreactors) can be difficult to filter. Therefore, fed batch cultures were performed both in shake flasks and bioreactors and were evaluated for differences in cell growth, titer and product quality.

Results and discussion: Media and feed compositions were unaffected by filtration through the virus barrier filter. Cultures showed no differences in cell growth or titer. In addition, the secreted antibodies showed no differences in the glycosylation pattern, amount of aggregates or charge variants.

Bibliography, Acknowledgements: Liu S, et al. Development and qualification of a novel virus removal filter for cell culture applications. *Biotechnol Prog.* 2000;16(3):425-434

Disclosure of Interest: None declared





PO109

HYBRID MODELING OF MAMMALIAN CELL CULTURE BIOPROCESSES

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Background and novelty: Mammalian bioprocesses are complex, since they are subjected to many influences (e.g. biology of the cell, operational input). Consequently, it is important to understand the critical process parameters which affect the critical quality attributes to ensure product quality and gain overall process understanding as highlighted in the Process Analytical Technology initiative. For bioprocess development often design of experiment settings with response surface type models are used, which generally provide a static model. Whereas hybrid models, which take into account existing process knowledge and experimental data have an inherent dynamic structure and can therefore very precisely predict process states. Hybrid models can not only increase process knowledge, but also help integrating and structuring information obtained from different sources. Therefore, we applied this approach aiming for advanced process control and monitoring

Experimental approach: An intensified design of experiment setting was chosen to perform fed-batch cultivations at different scales (shake-flask: 0.3 L and bioreactor: 15 L) using a Chinese hamster ovary cell line, which produced a monoclonal antibody. Enhanced process monitoring was achieved by applying a broad on- and off-line analytical platform.

Results and discussion: We established a hybrid model based on the shake-flask experiments and could demonstrate a good prediction performance for estimating specific process variables that can otherwise not be directly measured (such as viability, total cell concentration or amino acids). The model based on small scale experiments in combination with data from few bioreactor runs could furthermore be transferred to describe the process performance in the bioreactor.

Concluding, the generated models enable process prediction as well as simulation, provide enhanced process knowledge and the basis for advanced bioprocess control.

Bibliography, Acknowledgements: -

Disclosure of Interest: None declared





PO110

EXPANDING PROCESS KNOWLEDGE THROUGH DOE PRINCIPLES: A NOVEL APPROACH TO LATE STAGE CHO CELL CULTURE PROCESS CHARACTERIZATION

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Background and novelty: Design of Experiments (DoE) techniques have been applied throughout the biotechnology industry over the past decade due to their ability to deliver valuable information with speed and efficiency. These methodologies are also capable of enhancing product and process understanding as one is able to develop statistically significant mathematical relationships among process parameters and outputs, such as growth, metabolism and critical quality attributes (CQAs).

Experimental approach: In this work we utilized two different designs— Central Composite and Box-Behnken – for process characterization of a late stage CHO cell culture process and generation of our response surface model (RSM). In addition to the RSM, one factor at a time (OFAT) experiments were conducted to evaluate raw materials and other process parameters.

Results and discussion: With a focus on process intensification, process characterization extended beyond the production bioreactor into the inoculum train. Each phase of the cell culture process was characterized in a modular fashion. Temperature, pH, seed density and their respective ranges were chosen to be included in the design based on process robustness and historical knowledge of our CHO platform process. Statistical significance and practical significance, which was determined via equivalence testing, were assessed to determine the importance of a process parameter and its effect on CQAs. This work emphasizes the importance of selecting the proper factor ranges when conducting these experiments and utilizing relevant statistical methodologies to ensure a comprehensive understanding of your cell culture process.

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Disclosure of Interest: None declared





PO111

BISPECIFIC ANTIBODY – CHALLENGES IN PROCESS DEVELOPMENT

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Background and novelty: In recent years, there has been an increasing demand on novel molecular formats such as bi- and multispecific antibodies and antibody drug conjugates. We have developed an anti-CD3 and anti-CD123 bispecific antibody and investigated challenges of the bispecific antibody in upstream process development.

Experimental approach: In order to improve titer and purity of the bispecific antibody which are major challenges in process development, several different media platforms were tested in the antibody production. The effects of chemical chaperons and temperature shifts on antibody aggregation level were evaluated with a high-throughput 96-deep well system and Ambr250 single-use bioreactors.

Results and discussion: Low titer and high aggregation level of the bispecific antibody were the major issues in the upstream process development. We have achieved an increase in titer via media and process optimization. Although total eleven chemical chaperons and different cell culture temperatures were tested to diminish aggregation, there was no significant improvement on antibody aggregation.

Bibliography, Acknowledgements: We thank Sandrine Perez, Sophie Carayon, Francois Debaene, Catherine Graves, Sara Varricchio, and Thierry Ziegler in CMC team.

Disclosure of Interest: None declared





PO112

DEVELOPMENT AND UPSCALE OF HEK293 TRIPLE TRANSFECTION PROCESS IN SINGLE-USE BIOREACTORS FOR INDUSTRIAL MANUFACTURE OF AAV VECTORS

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Background and novelty: The triple transfection of adherent HEK293 cells is usually not suited for the large scale manufacturing of AAV-based therapeutics. To allow scalability of the triple transfection process, we adapted the adherent HEK293 cells to grow in suspension culture in chemically defined culture medium. The culture parameters were defined in disposable shake flasks and in stirred tank bioreactors. To fulfill the regulatory requirements, a Master Cell Bank of suspension HEK293 cells was manufactured following the good and fully manufacturing practices characterized.

Experimental approach: The transient transfection of 3 plasmids is mediated by Poly Ethylene Imine (PEI). The process was first evaluated in disposable shake flasks, then in 2L and 10L glass bioreactors and was finally scaled up from 50L to 200L in single-use bioreactors. The experiments were performed to produce rAAV8-UGT1A1 vectors for gene therapy of Crigler Najjar Syndrome.

Results and discussion: The results showed that the suspension transfection process leads to robust vector titers with consistent characteristics especially in terms of full/emptycapsid ratio. A batch of 200L provides approximately 8×10^{14} vector genome copies (vg). Therefore, this manufacturing scale will meet the vector amount requirements for phase I/II clinical trial of Crigler Najjar Syndrome. This process was successfully transferred to a CMO facility.

Bibliography, Acknowledgements: na

Disclosure of Interest: None declared





PO113

IDENTIFICATION OF PROCESS PARAMETERS WHICH UNDERPIN ROBUST PLATFORM PRODUCTION PROCESSES

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Background and novelty: Critical objectives for the biopharmaceutical industry are the creation of robust, reproducible processes which result in consistent critical product quality attributes and yields. To meet these requirements within a short time period it is important to apply platform production processes which consist of a common host cell line, expression vector, cell line development process, cell culture media/feed, process control and scale-up methodologies during cell line development, process characterisation and cGMP manufacturing.

Experimental approach: In this study we integrate mathematical based approaches with system characterisation studies across scales to refine both scale dependent (agitation, pH and gassing strategies) and scale independent (medium and feeding strategy) parameters for multiple cell lines and recombinant monoclonal antibodies to identify process parameters which underpin robust platform production processes

Results and discussion: Optimal manufacturing performance involves the optimisation of key bioprocess parameters which traditionally have been optimised in an ad-hoc manner during process development. To increase speed-to-clinic we show that the co-optimisation of both scale dependent and scale independent parameters is key to developing robust production processes which are applicable to multiple cell lines and products.

Bibliography, Acknowledgements: The authors would like to thank the upstream mammalian process development teams in both the UK and the USA for helping to design this study. Also thanks to the analytical development team for the critical product quality attribute analysis

Disclosure of Interest: None declared





PO114

PROCESS INTENSIFICATION TO IMPROVE LENTIVIRAL VECTOR PRODUCTION FROM STABLE CELL LINES

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Background and novelty: Production of lentiviral vectors (LVV) by transient transfection has limited scalability. As such, stable cell line production systems are an attractive and highly pursued alternative for LVV manufacturing. Unfortunately, the development of *high-titer* LVV-producing stable cell lines (PCL) is challenging due to the cytotoxicity of the viral proteins. The PCL used in this study yielded 10 times less LVV in a batch process, compared to a transient transfection process. This gap may be decreased with additional cell line development and/or process intensification. Herein, we describe two upstream process improvements that increase LVV yield in PCLs.

Experimental approach: Our approach focused on increasing the LVV production period and infectious LVV yield in a bioreactor-based LVV production system. To that end, cell separation technologies were evaluated for their ability to both increase active LVV recovery and retain viable cells in the reactor. In conjunction, we devised and tested culture media formulations to improve culture growth, cell specific productivity, and total product yield.

Results and discussion: Using the above approach, we identified a method for continuous harvest and a new production medium. In combination, these process improvements increased average LVV titer by > 3.5-fold, and extended the LVV production period by more than three days. This translated to a 14-fold increase in total LVV yield, which eliminated the gap with the transient transfection process. This work highlights the potential of PCL LVV manufacturing systems.

Bibliography, Acknowledgements:

Disclosure of Interest: None declared





PO115

STRATEGIES TO OPTIMIZE CELL GROWTH AND SCALE UP THE PROCESS USING A NEW SINGLE USE BIOREACTOR SYSTEM, AMBR 250

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Background and novelty: Ambr® 250 is a new bioreactor system for parallel cell culture using 250 mL single-use bioreactors controlled by an automated liquid handling platform. The platform is capable of controlling process parameters such as dissolved oxygen, pH and temperature, performing liquid additions and sampling, and providing up to four continuously pumped liquid feeds. Each bioreactor is fully monitored and controlled by the software and can all be run independently in parallel.

Due to the geometric similarity of the vessels, the scalability from 250 ml to 50L can be an easy strategy for the production process.

The goal of this study is to perform several cell cultures to identify the optimal parameters values using the ambr 250 and to evaluate the scalability of the process. The online measurement of cell growth is also tested.

Experimental approach: For cell culture optimization the ambr 250 (Sartorius Stedim) was used.

To scale-up, the cultures were conducted with Biostat bioreactors.

Cultures samples were regularly collected to determine cell concentration.

Online measurement of viable cell density and total cell density was carried out using the Incyte and Dencytee probes respectively (Hamilton).

Results and discussion: Regarding cell density and viability comparable results between 250 ml, 2L, 5L, and 50L were obtained.

The comparison between online and off-line data of viable and total cells showed similar results.

In conclusion, the Ambr250 Single-Use Bioreactor technology is a good alternative and a suitable system for optimization of cell culture growth conditions involving reduction of costs and timelines. Moreover, excellent scalability to 50L bioreactor was demonstrated.

In addition, the online monitoring of cell growth is a promising tool to reduce laboratory work and to minimize the risk of sample manipulation.

Bibliography, Acknowledgements: We thank Sartorius Stedim and Hamilton for strong support

Disclosure of Interest: None declared





PO116

FLUID DYNAMICS OF THE FLOW FIELD IN A DISPOSABLE 600-ML ORBITALLY SHAKEN BIOREACTOR

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Background and novelty: Orbitally shaken bioreactors (OSRs) have proven to support the efficient cultivation of mammalian cells in suspension. Here we conducted a three-dimensional computational fluid dynamics (CFD) simulation to characterize the flow field in a disposable 600-mL orbitally shaken bioreactor (OSR600), a conical centrifugation container with a ventilated cap.

Experimental approach: CFD models were established for the OSR600 at different filling volumes with a shaking speed of 180 rpm, using the Ansys Fluent software.

The shapes of moving liquid wave in the container were captured using a high-speed camera to validate the CFD models. Volumetric mass transfer coefficients ($k_L a$) were determined using the static gassing-out method and were compared to the values predicted from the CFD results.

Results and discussion: In the model, the shear stress was calculated to be in the range of 0.0-1.0 Pa with the highest shear stress being localized along the lower conical part of the vessel wall.

A distinct increase of the quantity of the liquid affected by high shear stress was observed at filling volumes of 300 mL and 400 mL may have been due to a twisted curvature at the lower end of the observed liquid wave. It is not clear yet how much this elevated stress would affect cells, since overall the range of shear stress in OSRs are considered moderate to low.

As the filling volume was increased from 100 mL to 500 mL, a near constant mass transfer coefficient (k_L) together with the decreasing specific interfacial areas (a) resulted in reduced $k_L a$ values as a function of the filling volume. These results indicate that a smaller filling volume, such as 200 to 300 mL, will be favorable for high-density cell cultivation ($> 10 \times 10^6$ cells/ml) in the OSR600.

Bibliography, Acknowledgements: We gratefully acknowledge Kühner AG for equipment support.

Disclosure of Interest: None declared





PO117

OPTIMIZATION OF DISSOLVED CARBON DIOXIDE LEVEL IN CELL CULTURE TO MAXIMIZE CHO CELL GROWTH AND PRODUCTIVITY

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Background and novelty: The importance of dissolved carbon dioxide on CHO cell growth and productivity is well known. It is generally the detrimental effect of high dCO₂ that is the subject of scrutiny and discussion. In one of the processes transferred by a client, we realized that there needs to be an optima for dCO₂. In this process, it was observed that low dCO₂ (1-2%) at small-scale had adversely affected the cell density and productivity. Experiments performed to evaluate the effect of dCO₂ levels showed a clear impact on cell density and titer.

Experimental approach: As part of tech transfer, the understanding was that the product quality should be comparable between the small-scale runs at the CDMO and 50L runs at client site. In 5L bioreactors, owing to hydrodynamics in the bioreactor, stripping of CO₂ was efficient, thereby decreasing CO₂. Additionally, since pH was not defined, there was no addition of CO₂. Thus, dCO₂ levels were low. While the product quality attributes were comparable to that seen at client site, the cell density and titer were low. Therefore, additional bioreactors were run to investigate the effect of dCO₂. As a benchmark, it was agreed that CO₂ levels seen at the client site will be matched at small-scale. This was achieved by providing pH set point throughout the culture, as well as manipulating the air sparge levels.

Results and discussion: Once dCO₂ matched with high levels seen at client site, cell density and antibody titer were also comparable. Product quality – charge, glycosylation, was unaffected. The high CO₂ levels (~15%) in this process may pose challenges during scale-up. To arrive at an optima, additional bioreactor runs were performed. The results indicated an optima for dCO₂ where growth, titer and quality were unaffected. By defining the dCO₂ operating range for the process, we have increased the probability of a successful scale-up.

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Disclosure of Interest: None declared





PO118

REPURPOSING FED-BATCH MEDIA AND FEEDS FOR HIGHLY PRODUCTIVE CHO PERFUSION PROCESSES

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Background and novelty: Cell culture medium used in perfusion culture needs to support higher cell densities than a medium used in fed-batch culture. Therefore, when switching from fed-batch to perfusion mode, a separate medium that supports high cell density, productivity, and product quality needs to be developed. From a cost and facility design perspective, it is important that the perfusion medium supports these performance attributes at low perfusion rates.

Experimental approach: In this study, we present a fast and convenient approach for developing perfusion media for antibody-producing Chinese hamster ovary (CHO) cells from available fed-batch media and feeds. Applying a design of experiments (DOE) strategy, a base medium was used as a starting point and feeds were added as supplements in various concentrations. The DoE strategy consisted of both screening and optimization designs, and the resulting media prototypes were evaluated in batch cultures. The results show that peak cell density and antibody production could be more than doubled from base medium conditions with the best performing medium in batch cultures.

In the next step, the best performing prototypes were evaluated in small-scale perfusion cultures, using the single-use ReadyToProcess WAVE™ 25 rocking bioreactor system, and the cell-specific perfusion rate (CSPR) was optimized.

Results and discussion: Using the described approach, three base medium/feed combinations for two mAb-producing CHO cell lines were successfully developed. The three best performing prototype media from this study enabled perfusion culturing with a CSPR of less than 25 pL/cell/day.

Bibliography, Acknowledgements: The authors wish to thank the Bioprocess Analytics teams in both companies for in-process sample analysis. Jake Warrington (MedImmune) is acknowledged for medium preparation support. The MedImmune authors thank GE Healthcare for the gift of some of the GE media and feed reagents used in the experiments.

Disclosure of Interest: None declared





PO119

A NOVEL PEPTIDE-BASED PLATFORM FOR THE PRODUCTION OF O-GLYCOSYLATED THERAPEUTIC PROTEINS

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Background and novelty: A current challenge of the biopharmaceutical industry is the improvement of the efficacy and stability of protein-based biotherapeutics, as well as their bioprocess optimization. In this work, we describe a new glycoengineering approach based in O-glycosylated peptide fusion technologies for enhancing the pharmacokinetic and pharmacodynamic properties of protein-based drugs. Moreover, since the peptides comprise a linear epitope recognized by a monoclonal antibody (mAb CC1H7)¹, this strategy could be useful to detect, quantify and purify the fusion protein.

Experimental approach: Two peptide tags derived from the N-terminal end of human granulocyte and macrophage-colony stimulating factor (hGM-CSF) were developed: GMOP (comprising the first 14 amino acids of hGM-CSF and 4 potential O-glycosylation sites) and GMOPm (comprising the first 7 amino acids of hGM-CSF plus 8 additional residues, responsible of 6 potential O-glycosylation sites). Both peptides were fused to the N-terminus of human interferon- α 2b (IFNwt), generating two different analogs that were produced in CHO-K1 cells and thoroughly characterized.

Results and discussion: GMOP-IFN and GMOPm-IFN exhibited increased molecular masses, more glycoforms and sialic acid than IFNwt, indicating that new O-glycans were attached to the IFN. Interestingly, both variants were strongly recognized by mAb CC1H7 in western blot assays. *In vitro* antiviral activity of IFN analogs was not affected, but antiproliferative activity of GMOP-IFN and GMOPm-IFN decreased 70% and 23%, regarding IFNwt. Significant improvements in pharmacokinetics in rats were achieved, showing a 3.3 and 2.8-fold longer elimination half-life and a 3.7 and 4.1-fold decreased plasma apparent clearance in comparison with IFNwt, respectively. Also, O-glycans conferred a notable *in vitro* increment in thermal resistance and stability against plasma protease inactivation.

Bibliography, Acknowledgements: ¹Perotti et al., 2013, Prot Exp Purif 91: 10-19.

Disclosure of Interest: None declared





PO120

IMPACT OF BIOREACTOR DESIGN ON PERFORMANCE OF MICROCARRIER-BASED CELL CULTURE PROCESSES

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Background and novelty: Anchorage-dependent cell (ADC) cultures are used to produce viral vectors, virus-based vaccines and for cell therapies and tissue engineering applications. These applications rely on planar technologies (PT). However, as new cell therapy product candidates move from clinical trials to commercialization, PT have proven to be inadequate to meet large-scale demands. Therefore, new scalable platforms for culturing ADC at high cell concentrations are needed. A promising solution is to grow cells on microcarriers in single-use bioreactors. In this work, a novel bioreactor system using an innovative Vertical-Wheel^(TM) technology (PBS) was evaluated for its potential to support scalable cell cultures.

Experimental approach: Two ADCs were used: human lung carcinoma cells (A549) and human bone marrow-derived mesenchymal stem cells (hMSC). Hydrodynamic parameters such as power input, eddy size and shear stress were estimated. The performance of PBS (2L) was benchmarked against stirred-tank bioreactor (ST) (0.2L) for A549 cell growth and adenovirus production and hMSC expansion.

Results and discussion: For A549 model, higher cell growth and infectious viruses were achieved in PBS when compared to ST. For the hMSC model, higher percentages of proliferative cells were reached in the PBS compared with ST though no significant differences in the cell concentration could be observed. Noteworthy, hMSC population generated in the PBS showed a significantly lower percentage of apoptotic cells and reduced levels of HLA-DR positive cells. These results show that process transfer and scale-up from ST to PBS was successfully carried out in both cell culture models. This work demonstrates the potential of PBS as a new scalable biomanufacturing platform for microcarrier-based cell cultures of biological products.

Bibliography, Acknowledgements: The authors acknowledge Joao Sa and Manuel Garrido for technical support. Marie-Maud Bear, Sebastien Chauvel and Stephen Caracci (Corning Inc.).

Disclosure of Interest: None declared





PO122

HIGH YIELD PROCESS FOR THE PRODUCTION OF ACTIVE HUMAN ALPHA-GALACTOSIDASE A IN SUSPENSION CHO K1 CELLS

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Background and novelty: Fabry disease is an X-linked recessive disorder caused by deficient or absent lysosomal α -Galactosidase A. Currently, two enzyme replacement therapies are commercially available. Recombinant human α -Galactosidase A (rh α GAL) has been successfully expressed in adherent CHO-K1 cells by a stable amplification method, achieving productivities up to 7.5 pg.cell⁻¹.day⁻¹ [1]. We replaced the amplification strategy by lentiviral transduction of suspension cells, obtaining productivities up to 59 pg.cell⁻¹.day⁻¹.

Experimental approach: Suspension CHO-K1 cells were serially transduced with third generation lentiviral vectors (LVs) containing rh α GAL sequence. Fifteen clones were obtained by limit dilution method. Two purification steps by anionic exchange and hydrophobic chromatography were developed and *in vitro* enzymatic properties were characterized. Monosaccharide compositions and 2-AB labeled glycans were analyzed. The properties of rh α GAL were compared to the commercial enzyme (Fabrazyme®).

Results and discussion: LV transduction improved the global production process, as clones with high productivities (3.5 to 59.4 pg.cell⁻¹.day⁻¹) and enzyme activities (2.9E10³ to 4.1E10⁴ IU.mg⁻¹) were obtained. After two purification steps, the active enzyme was recovered (1.5E10⁶ IU.mg⁻¹) with 90% purity and 76% overall yield. Michaelis Menten analysis demonstrated that rh α GAL was capable of hydrolyzing the synthetic substrate (4 - MUG) at a comparable rate to Fabrazyme® (V_{max} 60.1 \pm 12.4 nM.min⁻¹ and 63.2 \pm 25.3 nM.min⁻¹; K_M 1.6 \pm 0.6 mM and 1.2 \pm 0.2 mM for rh α GAL and Fabrazyme®, respectively). Although glycosylation pattern and *in vitro* stability in plasma of both molecules were similar, rh α GAL contained 40% higher level of sialic acid. In summary, our process achieves the highest rh α GAL productivity reported to date, maintaining the biochemical properties of the commercial product.

Bibliography, Acknowledgements: 1. Ioannou *et al.*, (1992). *J. Cell Biol.*

Disclosure of Interest: None declared





PO123

DESIGN AND EVALUATION OF NEXT-GENERATION BIOLOGICS FOR CANCER IMMUNOTHERAPY

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Background and novelty: Bi- and multi-specific antibodies, Antibody-cytokine fusion proteins, non-immunoglobuline scaffolds, chimeric antigen receptors (CARs), engineered T-cell receptors (TCRs) and TCR-based bispecific constructs can provide significant advantages for use in cancer immunotherapy. However, as highly engineered molecules they pose new challenges in design, engineering, cloning, expression, purification, and analytics.

Experimental approach: In close collaboration with leading biopharmaceutical companies, we have developed end-to-end platforms for biologics discovery and bioprocess development [Genedata Biologics™ and Genedata Bioprocess™]. We implemented workflow support systems enabling the automated design, screening, and expression of large panels of these candidates. In addition, screening for the best-producer cell line is supported. We also have built in tools for developability and manufacturability assessments of these complex molecules.

Results and discussion: We will present how these complex next-generation biologics molecules are being designed, screened and analyzed as well as the corresponding production cell lines are being tracked enabling highly parallelized and high-throughput workflows and a systematic evaluation of protein production and cell line development.

Bibliography, Acknowledgements: Allison Kurz, Genedata, Basel, Switzerland; email: biologics@genedata.com

Disclosure of Interest: None declared





PO124

CONTINUOUS GLUCOSE MONITORING AND CONTROL IN BIOREACTORS WITH A DISPOSABLE OPTICAL BIOSENSOR, A NEW APPLICATION FOR AN OLD CONCEPT

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Background and novelty: Physicochemical parameters such as pH and temperature are typically monitored and controlled continuously during a bioprocess. However, real-time measurements of nutrients like glucose remain challenging. Here, we present a robust glucose biosensor that was optimized for mammalian cell culture in bioreactors. We demonstrated that the sensor has a long functional stability, is sterilisable and applicable for a bioprocess without displaying signs of biofouling.

Experimental approach: The biosensor is based on a commercially available optical oxygen sensor (PreSens GmbH), coated with a glucose oxidase containing layer. The enzyme layer is covered by a perforated hydrophilic membrane, rendering the sensor diffusion limited. By means of optimizing the enzyme layer composition and the diffusion membrane, we were able to generate sensors with varying dynamic ranges (0.5-45 mM glucose) and decent response times suitable for cell culture purposes.

Results and discussion: We implemented the glucose sensors in 125 mL cell culture flasks and demonstrated a tight control of glucose levels. We were able to run fed-batch cultures in flasks for one week and verified glucose levels by periodical offline measurements. The implementation of the biosensor in a continuous stirred-tank reactor and a single-use wave bag bioreactor (2 L) was also feasible.

The sensor described here, allows an advanced process control, which cannot only help to improve batch to batch consistency, but also increase the product quality and yield. Since glucose is a major nutrient for mammalian cells, its tight regulation during cell culture will support the optimization of bioprocesses.

Bibliography, Acknowledgements: We gratefully acknowledge the financial support from the Bmbf program of the German Science & Engineering Foundation (0101-31P7809) and the support from Steinbeis Zentrum and PreSens GmbH.

Disclosure of Interest: None declared





PO125

DEVELOPMENT OF SIMPLE AND EFFICIENT CELL HARVEST METHODS FOR MICROCARRIER CULTURES

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Background and novelty: The realization of cell therapies and regenerative medicine applications is dependent upon development of cost-effective, robust and reproducible technologies for generation of high quality cells. Microcarriers employed in single use bioreactors provide a system for cell expansion however, in order to use microcarriers for these purposes, cells attached to microcarrier surfaces must be retrieved in an acceptable state. Pall SoloHill microcarriers are rigid spheres that support rapid and efficient cell attachment and growth of cells in bioreactors. The physical characteristics allow users to employ standard cell culture enzymatic harvest methods for detachment of cells from microcarriers but techniques used in small scale must be adapted and optimized for use in larger platforms

Experimental approach: Human mesenchymal stem cells (hMSCs) and vero cells were expanded in PadReactor bioreactors in culture volumes that ranged from 6 L to 50 L and cells were detached from microcarriers using enzymatic digestion. A single-use closed system composed of multiple continuous unit operations was designed and manufactured. Cell/microcarrier slurries were processed and cell yield was quantified. Viability of resultant cell slurries and characterization of hMSC identity and function was performed.

Results and discussion: Results demonstrate the ability to rapidly and efficiently harvest cells grown on rigid microcarriers in bioreactors using a single-use closed harvest system. Protocols to effectively harvest Vero cells and hMSCs were developed. Harvested cells were highly viable and retained functional attributes. This scalable system and associated protocols simplifies manufacturing processes for adherent cells in suspension cultures and makes the process of generating billions to trillions of high quality cells from a single bioreactor possible.

Bibliography, Acknowledgements: none

Disclosure of Interest: None declared





PO126

SCALABLE STEM CELL EXPANSION BIOPROCESS USING SINGLE-USE, FED-BATCH REACTOR ENGINEERED FOR HIGH PRODUCTIVITY

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Background and novelty: Human mesenchymal stem cells (hMSCs) are extensively utilized in regenerative medicine. Most applications require large numbers of high quality cells, robust media and large surface area for cell expansion. Microcarrier cultures performed in closed single-use systems provide a platform for hMSC expansion. High quality cell banks and bioprocess medium used in these platforms positively impacts performance. The goal of these studies was to develop a simple and robust process utilizing microcarriers, cells, media and single use bioreactors which is accompanied with associated protocols for generation of large quantities of high quality hMSCs.

Experimental approach: Conditions for proliferation and expansion of human bone marrow mesenchymal stem cells (Allegro™ Unison hBM MSC) on microcarriers (Pall SoloHill) were identified at small scale and employed in single-use bioreactors (PadReactor® Mini Bioreactor). Cryopreserved hMSC cell banks were thawed and directly seeded onto microcarriers in hMSC cell culture media (Allegro Unison) in PadReactor Mini bioreactors and expanded for three days. Cells harvested from this bioreactor were seeded on microcarriers in a larger single use PadReactor system, cultured for 5 days using a fed-batch process, and were harvested and characterized.

Results and discussion: A rapid and robust process for generation of up to 35 billion hMSCs from fed-batch microcarrier cultures in a single use reactor seed train was developed. hMSCs proliferated on microcarriers directly from cryopreserved cell banks in high performing hMSC medium utilizing a fed-batch approach. Our system achieves > 500M hMSCs/L and cells maintained cell identity markers, angiogenic cytokine secretion profile, multi-lineage differentiation, and inducible immunomodulatory functions. This system can be rapidly implemented, enabling production of billions of hMSCs for product development programs.

Bibliography, Acknowledgements: none

Disclosure of Interest: None declared





PO127

GLYCOSYLATED HUMAN CCBE1 PROTEIN: FROM RECOMBINANT PROTEIN PRODUCTION TO PROTEOMIC CHARACTERIZATION

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Background and novelty: Collagen and calcium-binding EGF domain-1 (CCBE1) protein is essential for lymphatic vascular development. Its expression was also observed in human cardiac progenitors, suggesting a potential involvement in heart morphogenesis. However, the ineffectiveness in obtaining sufficient amounts of well-characterized CCBE1 has hampered its study as a secreted factor in cardiac commitment. Here, the main goal was to establish the production and purification processes of full-length human recombinant CCBE1 (rCCBE1) to further evaluate its biological activity.

Experimental approach: rCCBE1 was produced by HEK293-E6 transient transfection. Different bioprocess strategies were exploited to improve rCCBE1 production and secretion, such as high-density transfection (Backliwal G. 2008), mild hypothermia (33 °C) and fed-batch mode. Mass spectrometry (MS)-based tools were implemented for in-process control and final product characterization.

Results and discussion: Small scale experiments were initially performed for DNA concentration and transfection reagent screening, showing higher rCCBE1 secretion using 1 µg/mL and polyethylenimine. Immunofluorescence microscopy revealed high rCCBE1 retention in the ER. Employing high-density transfection protocol and 33 °C, 4-fold increase in rCCBE1 yield and secretion was observed. Further improvements were achieved with glucose feeding. Interestingly, mature glycosylated profile of secreted rCCBE1 was mainly observed in cultures with high cell viabilities (> 90 %), whereas lower cell viability translated in high-mannose glycoprotein enrichment. rCCBE1 purified through affinity chromatography was characterized by MS and its functionality was assessed during cardiac commitment of pluripotent stem cells.

Bibliography, Acknowledgements: This work was supported by iNOVA4Health and CardioRegen (HMSP-ICT/0039/2013) projects.

Disclosure of Interest: None declared





PO128

SEED TRAIN CULTURE CONDITIONS CAN AFFECT PRODUCTION CULTURE PERFORMANCE: A CASE STUDY FOR A CHO CELL CULTURE PROCESS

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Background and novelty: A pattern of slow growth behavior was observed in inoculum train and subsequent production cultures of a CHO cell line expressing a recombinant mAb. The affected runs had progressive slowdown in growth from inoculum train through production, smaller cell sizes, low viability in production culture, and low titer (60-85% decrease). Cultures exhibiting this slow-growth phenotype can rapidly switch from slow to normal growth behavior within a single seed train passage. When investigating the potential root cause it was determined that seed train culture conditions correlated with the observed phenotype in the subsequent inoculum train and production culture.

Experimental approach: Numerous cell culture experiments were conducted in controlled bioreactors to identify which culture parameter(s) could affect inoculum train and production culture performance. In addition, different cell biology assays, including cell cycle, apoptosis and ER size analysis by FACS, mRNA transcript level analysis by PCR and, Western blotting for analysis of proteins involved in protein secretion/folding, were applied in an effort to better understand the biology of this slow-growth phenotype.

Results and discussion: Bioreactor studies demonstrated that a slightly lower seed train culture pH could trigger the observed slow growth phenotype in the subsequent inoculum train and production cultures. Of the cell biology assays evaluated, Western blot analysis of BiP, an ER chaperone and part of the Unfolded Protein Response pathway, was found to be a reliable biomarker for the slow-growth phenotype. Higher intracellular BiP levels in seed train cultures correlate with subsequent poor performance in inoculum train and the production cultures, leading to the hypothesis that lower pH in seed train cultures increases the level of ER stress. Study and optimization of seed train pH resulted in mitigation of this phenotypic behavior.

Bibliography, Acknowledgements: .

Disclosure of Interest: None declared





PO129

A METHODOICAL AND SYSTEMATIC INTEGRATION OF UPSTREAM AND DOWNSTREAM PROCESSING OF BIOPHARMACEUTICAL PROTEINS

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Background and novelty: Based on their broad scope of application, monoclonal antibodies (mAb) and upcoming more-often fragments are being used as therapeutic agents and for diagnostics. Optimizations in upstream processing concepts have led to increasing product titers and raised impurity profiles (Jain & Kumar 2008; Shukla & Thömmes 2010). Various compositions of the cultivation broth present challenges in the downstream processing of biotechnological produced proteins.

Experimental approach: The integration of upstream and downstream process development in combination with alternative cell harvest operations and product purification processes favors the overall process. Based on the cultivation of a representative CHO cell line a medium was developed, which generates higher titers and cell numbers as well as lower amounts of host cell proteins.

Results and discussion: The obtained medium led to an increase of product concentration by a factor of up to 2.5, cell concentration by a factor of 2.3 and a IgG/protein ratio of 41 ± 2 %. In addition to media development, there exist possible alternatives and expansions in order to alter the upstream process characteristics with regard to downstream processing efficiency and product quality. Bioprocess engineering will probably focus in regulated industries on quality by design and process analytical technology mechanisms, in order to design, analyze and control manufacturing processes. Defining Critical Process Parameters for each process unit enables the identification of parameters, which can be varied and those, which are difficult to control. These can be presented in Ishikawa-diagrams.

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Disclosure of Interest: None declared





PO130

OPTIMAL SELECTION OF THERAPEUTIC ANTIBODIES AND PRODUCTION CELL LINES BY ASSESSMENT OF CRITICAL QUALITY ATTRIBUTES AND DEVELOPABILITY CRITERIA

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Background and novelty: When developing novel biologics, the continued assessment of quality and developability is crucial. More product analytics methods are being applied early on in biopharma R&D, including cell line development. In parallel, systems are needed that comprehensively integrate molecule and sample information, as well as manufacturing cell lines and process parameters. Critical quality attribute assessment can be enabled along the whole bioprocess development workflow, including cell line development, upstream and downstream process development, as well as analytical and formulation development.

Experimental approach: We have developed a new platform for bioprocess development, which structures the analytics data and process parameters generated during bioprocess development. The analytics parameters being tracked include biological data, such as bioactivity, and physicochemical data, such as glycosylation, chemical liabilities (PTMs and other factors introducing heterogeneity into the molecule), aggregation, stability under different conditions (low pH, low and high temperature), solubility, impurities. Included are decision support tools for molecule, clone and process selection, e. g., in cell line and upstream process development.

Results and discussion: We present concrete use cases showing how to identify and monitor critical quality attributes in dependence of molecules, cell lines, and process parameters, to track the quality of the produced materials and to facilitate process and developability assessments. We show product analytics data being used to identify the optimal producer cell line. We also show how the system tracks CQA, such as aggregation and host cell protein content, during a media optimization campaign. Finally, we demonstrate developability assessment of various antibodies or the same antibody at different stages in development.

Bibliography, Acknowledgements: Allison Kurz, Genedata

Disclosure of Interest: None declared





PO131

**COST MODELLING OF UPSTREAM PRODUCTION PROCESS OF LENTIVIRAL VECTORS IN HEK-293 CELLS
COMPARING MULTI-TRAY 10 STACKS AND FIXED-BED BIOREACTOR**

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Background and novelty: Use of lentiviral vectors (LVV) for gene therapy of genetic disorders, infectious diseases or cancer is increasing since their first use in clinical trial in 2003. For upstream process (USP), most of published protocols for generation of LVV compliant with Good Manufacturing Practices (cGMP) rely on transient transfection of adherent HEK293(T) cultured in multi-tray 10-stacks (MT10). Downstream processing (DSP) varies greatly depending on mode of gene delivery (*in/ex-vivo*) and required purity of LVV. Recent publications reported use of fixed-bed bioreactors (FBB) as alternative USP method for viral vectors manufacturing. This *in-silico* study compares MT10 and FBB methods from cost of goods (CoGs) perspective.

Experimental approach: LVV cGMP production processes were extracted from literature to model typical MT10 and FBB processes in BioSolve cost modelling Software (BioPharm Services). Both processes were simulated in a factory relying mostly on single-use technologies and closed systems in USP. DSP was not included in models. Operating expenses (OPEX) and capital expenses (CAPEX) were simulated for different batch scales (from 100 L to 3000 L) and different scenarios (harvest number, reagents optimization) over a 47 weeks campaign.

Results and discussion: Comparison shows a reduction of CoGs of up to 50% when using FBB over MT10. CoGs structure highlights the impact on both CAPEX, with smaller footprint of FBB, and OPEX, with significant decrease of labor, plastic-ware and QC costs. More importantly, process time is 2-fold decreased with FBB, potentially doubling LVV throughput and producing more than 50,000 L of bulk per year with a single production line.

Bibliography, Acknowledgements: None

Disclosure of Interest: None declared





PO132

HIGH GLUCOSE CONCENTRATION AND LOW SPECIFIC CELL GROWTH RATE IMPROVE SPECIFIC R-TPA PRODUCTIVITY IN CHEMOSTAT CULTURE OF CHO CELLS

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Background and novelty: Different strategies have been used to increase the specific productivity (q_P) of r-proteins in CHO cell culturing, including the application of mild hypothermia (MH) (28-35°C) and glucose concentration. These strategies involve a concomitant variation in the specific cell growth rate (μ) in batch culture. Recent research has shown a differential effect of MH and μ on production of r-tPA, increasing q_P when decreasing μ is applied jointly with MH, this at very low glucose level. How this relationship between MH and low μ is affected by increased glucose concentration remains unknown.

Experimental approach: In order to evaluate the effect of these variables, chemostat cultures were performed at two μ (0.010 or 0.018(h⁻¹)), two temperatures (33 or 37°C) and three glucose concentrations (20, 30 or 40mM) in feed. The analysis of cell growth, r-protein production and key metabolites were done.

Results and discussion: A decrease in cell density was observed in response to increasing glucose concentration in feed, regardless of MH or μ evaluated. At 20mM the maximum cell densities were reached which were 1.50 and 1.65 x10⁶ cells/ml at 37/33°C and 0.018(h⁻¹) respectively; and 1.10 and 1.33 x 10⁶ cells/ml at 37/33°C and 0.010(h⁻¹) respectively. The increase in glucose concentration of 20 to 40mM resulted in an increase in q_P of 3 and 3.3 times at 33°C/0.018(h⁻¹) and 37°C/0.018(h⁻¹) respectively. A slightly lower increase of 2.4 and 1.8 times was reached at 33°C/0.010(h⁻¹) and 37°C/0.010(h⁻¹) respectively. The highest q_P s were reached at 0.010(h⁻¹), however it was not observed a positive effect of MH. Contrary to what happens in batch culture, MH does not improve the q_P of r-proteins under the different conditions evaluated (increasing glucose concentrations; low μ), but low μ at high glucose concentration has a positive effect on q_P of r-proteins and metabolism

Bibliography, Acknowledgements: Fondecyt 3150373
Fondecyt 1161452

Disclosure of Interest: None declared





PO133

UNDERSTANDING GLYCOSYLATION VARIABILITY IN CHO CELL CULTURES

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Background and novelty: Control of glycosylation of recombinant monoclonal antibodies is important to maintain consistent effector function and therapeutic efficacy. Multiple cell culture factors can impact glycosylation, including many trace metals, which are co-factors for enzymes in the glycosylation pathway. In this case study, variation in glycosylation was observed across runs for a single cell line. Variation in manganese levels was identified as a contributing factor. Manganese is a co-factor of β -1,4-galactosyltransferase, which catalyzes the addition of terminal galactose to N-acetylglucosamine (Witsell et al. 1990), and can impact galactosylation levels in combination with other components involved in these processing steps (Gramer et al. 2011).

Experimental approach: To understand the sources of manganese variation in the process, lot-to-lot variability was examined for several raw materials used in the cell culture media, and different media preparation procedures were compared to quantify manganese loss. Small-scale studies were also performed to evaluate the effect of manganese on glycosylation both alone and in combination with galactose.

Results and discussion: Manganese levels in several media components, including hydrolysate, were found to vary across lots. Some manganese loss was also observed during media preparation operations. Together, these factors account for the observed variation in manganese levels in the production culture. Titration of manganese at targeted levels confirmed the previously observed trends of increased galactosylation at higher manganese levels. An interaction effect was also observed between manganese and galactose, with a more dynamic change in galactosylation with varying manganese at lower levels of galactose. These learnings contribute to our overall understanding of cell culture factors that impact glycosylation.

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Disclosure of Interest: None declared





PO134

ONLINE AND REAL-TIME CONTROL OF CHO CELL SPECIFIC GROWTH RATE THROUGHOUT CULTURES IN BIOREACTOR

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Background and novelty: The optimization of CHO cell culture processes requires a real-time control of some critical parameters of cell physiological state, which meets the growing interest of the PAT initiative in pharmaceutical industries. One essential parameter is the cell specific growth rate (μ), which can rapidly decrease and then affect the production and quality of products. Classically, the time profile of μ is only calculated at the end of culture from a limited number of off-line cell density measurements. It is thus inadequate for a control strategy. This work presents an innovative approach, which allows a real-time evaluation of μ .

Experimental approach: Antibody-producing CHO cells were cultured under various operating conditions in a 2L stirred bioreactor equipped with an *in situ* capacitance probe (Hamilton). Viable cell density (VCD) was predicted from permittivity measured every 12 minutes. Then, based on VCD prediction and cell mass balance equations, a model was developed for real-time μ calculation. Several signal noise filters and various calculation methods were applied to reach better model stability. The model was validated by comparing the online estimated values to experimental ones, calculated at the end of the culture.

Results and discussion: This software sensor of μ was used to monitor online cell physiological state throughout various batch and feed-harvest cultures. The decrease of μ has been rapidly detected, which allowed us to maintain μ close to its maximal value by medium renewal at the beginning of the decrease of μ to avoid intracellular nutrients depletion. A more efficient control of the culture could then be achieved with a better anticipation than with monitoring simply cell density or nutrient concentrations. Moreover, as μ greatly influences the production and quality of antibody, its online monitoring and control show great potentials to animal cell culture bioprocesses.

Bibliography, Acknowledgements: /

Disclosure of Interest: None declared





PO135

SOLID PHASE ENZYMIC RE-MODELLING TO PRODUCE SINGLE GLYCOFORM ANTIBODIES

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Background and novelty: Monoclonal antibodies are normally synthesised as heterogeneous mixtures of glycoforms but clinical efficacy may depend upon single glycoforms which have been difficult to isolate. We have now developed an efficient method for isolating single glycoforms by solid phase re-modelling which is superior to previous methods because it allows a sequential series of enzymatic changes without the need for intermediate purification of the antibody. Solid-phase binding exposes the antibody glycans to enable easier access of the transforming glycosylation enzyme.

Experimental approach: Antibodies (camelid, Cetuximab and polyclonal human) were isolated on a solid-phase lectin column and their glycans were modified by a sequential addition of enzymes for a desired transformation.

Results and discussion: Galactosylated antibodies (>95% yield) were produced by a two stage reaction involving sialidase followed by galactosyltransferase with UDP-Gal. Sialylated antibodies (>95%) were produced by a 3 stage reaction involving sialidase, galactosyltransferase and finally treatment with the 2,6 sialyltransferase in the presence of CMP-NANA. The latter reaction produced equimolar quantities of monosialylated and disialylated Cetuximab and polyclonal antibodies. The results suggest that for human antibodies (150 kDa) this is the maximum level of sialylation possible given the steric constraints between the two CH2 domains of the dimeric structure. The ability to sialylate the smaller camelid antibody (80 kDa) was much greater with a resulting high (>90%) level of the disialylated glycan structures, indicating that the steric constraints for glycosylation are much less. These sialylated antibodies have significant potential clinical importance for their anti-inflammatory activities.

Bibliography, Acknowledgements: Financial support is gratefully acknowledged from NSERC, Canada ((MabNet).

Disclosure of Interest: None declared





PO136

EFFICIENT AND INTENSIFIED BIOPROCESS DEVELOPMENT BASED ON HIGH THROUGHPUT AND HIGH AUTOMATION TOOLS COUPLED WITH MULTIVARIATE DATA ANALYSIS

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Background and novelty: Therapeutic proteins feature tens of critical quality attributes (CQAs), all which are highly important for the efficacy, potency and safety of the drug. For process development a deep understanding of the dynamic interrelationship of the potentially influential process parameters and the CQAs is required. For this purpose, a large number of experiments is usually performed at multiple scales to eventually define optimal operation conditions.

This work analyzes the information hidden in the 'big data' produced throughout an entire cycle of cell culture process development, from high throughput screening at microliter scale, through process optimization and scale-up to monitoring of the validated process at pilot scale. With particular focus on product quality, a novel toolset attractively combining high throughput technologies, highly automated PAT solutions and advanced process data analytics is presented for each upstream process development step.

Experimental approach: This work is based on many hundred experiments testing the effect of multiple CHO-derived cell lines, various media supplements and operation conditions to produce a biosimilar monoclonal antibody. The experimental scales range from microliter 96-deep-well plates and milliliter-reactors (ambr-15) through 3 liter bench scale to 200 liter pilot scale bioreactors. Besides the central role of small scale high throughput experiments, Raman spectroscopy as an online information source is investigated.

Results and discussion: The integration of the versatile data analysis toolbox provides a solid basis to predict the process outcome, to make decisions and to sequentially define the subsequent set of experiments to be performed at larger scale. The data- and knowledge-driven toolbox enables to substantially reduce the experimental effort as well as the risks in process development.

Bibliography, Acknowledgements: Michael Sokolov et al., 2015-2017

Disclosure of Interest: None declared





PO137

ULTRA SCALE-DOWN MIMICS FOR PERFUSION CULTURE: EXPERIMENTAL AND MODELING OPTIMISATION STUDY FOR RAPID BIOPHARMACEUTICAL PROCESS DEVELOPMENT

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Background and novelty: The implementation of perfusion culture in place of fed-batch approaches has the potential to reduce manufacturing costs associated with the production of historically expensive products such as mAbs, and industry interest in the technology is steadily increasing. This work aims to develop a perfusion scale-down system capable of reproducing the specific characteristics of the perfusion culture process, namely cell retention capabilities, the ability to support high cell densities and operate for extended periods compared to fed-batch cultures. Cell culture in microwell plates in fed-batch mode is well defined; however to the best of our knowledge this represents the first attempt at the development of quasi-perfusion cell culture at this scale.

Experimental approach: Cultivation approaches in the microscale have been developed using a GS-CHO cell line in 24 well microwell plates, with a working volume of 1.2mL. Quasi-perfusion was achieved via sedimentation or centrifugation of the plate to mimic cell retention, generating separation efficiencies higher than 98%. Media exchanges commenced on day 3 at a rate of 1 vessel volume per day (VVD).

Results and discussion: The use of the quasi-perfusion approach generated improvements in cell densities of up to 2.5 fold in comparison to fed-batch studies. Comparison of metabolic profiles, volumetric productivities and final yields of fed-batch and quasi-perfusion highlights the advantages of perfusion culture over alternative operational modes and demonstrates excellent comparability between the results obtained in this study using microwell plate format and larger scale perfusion cultures. The results obtained demonstrate that quasi-perfusion in microwell plates has potential for use early-phase development of perfusion culture processes, with the potential to generate a considerable cost and time saving.

Bibliography, Acknowledgements: -

Disclosure of Interest: None declared





PO138

DESIGNING A CAMELID/HUMAN HEAVY-CHAIN ANTIBODY WITH ENHANCED ANTITUMOUR ACTIVITY

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Background and novelty: Therapeutic antibody research has expanded from full-sized IgGs to smaller versions involving single-domain antibodies (sdAbs) which are robust and bind selectively to a defined epitope. In cancer applications, it is advantageous to fuse these sdAbs to a human Fc region such that they are able to exert anti-tumour activity through antibody dependent cell-mediated cytotoxicity (ADCC) without being easily cleared from the renal system. EG2-hFc (80 kDa) is an example of a chimeric heavy-chain antibody (chcAb) composed of a human IgG1 Fc region bound via a hinge to a camelid-derived, variable domain (V_{HH}) specific for the human epidermal growth factor receptor 1 (EGFR1). The goal of this study was to explore the design of EG2-hFc through hinge and Fc-glycan modifications to improve its therapeutic efficacy.

Experimental approach: Engineering strategies were implemented to modify the hinge sequence and length, as well as the conserved *N*-linked glycan. Spectropolarimetry and hydrodynamic studies were initially used to determine the effect of these modifications on EG2-hFc behaviour in solution. Following that, binding affinities to EGFR1 and Fc γ R11a were determined by MirrorballTM cell cytometry and micro-scale thermophoresis, respectively. Finally, an *in vitro* ADCC assay was performed on EGFR⁺ breast cancer cells to correlate the engineered modifications to the tumour-killing capability.

Results and discussion: These experiments determined that with an extended hinge, EG2-hFc has unique hydrodynamic behaviour when compared to the wild-type and other variants. This extension relieves any steric hindrance of the V_{HH} , increasing the affinity to EGFR1. This translates to an augmented ADCC response, comparable to that of afucosylated EG2-hFc. These findings contribute to the growing body of research around chcAbs, suggesting an alternative and more straightforward method to increasing the ADCC response.

Bibliography, Acknowledgements: MabNet NSERC network

Disclosure of Interest: None declared





PO139

IDENTIFICATION OF PARAMETERS INFLUENCING ANTIBODY HEAVY CHAIN DIMER FORMATION IN MAMMALIAN CELL CULTURE.

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Background and novelty: Therapeutic monoclonal antibodies are the best selling class of biologics on the market. The most common sub-type of monoclonal antibodies, immunoglobulin G (IgG), is composed of two identical light chains (LC) and two identical heavy chains (HC) linked by disulphide bonds. It has been reported that higher relative concentration of heavy chain is directly linked with antibody aggregation (1). We describe here the effect of various parameters on heavy chain dimer (HC dimer) formation and a method to reduce HC dimers resulting in increased antibody quality and productivity.

Experimental approach: While developing three different IgG₁ producing stable cell lines we observed HC dimers in two out of three cell lines. To investigate the formation of HC dimers we evaluated various parameters including HC to LC DNA ratio, culture temperature, culture agitation and culture media. We experimentally examined the effect of physiological/hypothermic temperature on CHO stable cell lines secreting two IgG₁ monoclonal antibodies. The effect of different HC to LC DNA ratio was determined by transient transfection of CHO cells expressing different antibodies. Antibody productivity was determined by SPR. HC dimer formation was confirmed by Western Blot.

Results and discussion: We observed that the different parameters evaluated, hypothermic temperature and HC to LC DNA ratio directly affect HC dimer formation. Hypothermic temperature and higher HC to LC DNA ratio increases HC dimer formation in two of the three IgG₁ secreting cell lines. In conclusion our work demonstrates that the occurrence of HC dimers secreted by CHO cells can be reduced by increasing the antibody LC to HC DNA ratio. Excess antibody LC concentration increased antibody productivity approximately by 3 fold and improved product quality attributes by decreasing HC dimer formation.

Bibliography, Acknowledgements: Oh.S et al. Journal of Biotechnology 165 (2013) 157-166

Disclosure of Interest: None declared





PO140

DATA MANAGEMENT IMPLEMENTATION TO SUPPORT HIGH THROUGHPUT MAMMALIAN CELL PROCESS DEVELOPMENT

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Background and novelty: During the 3 past years, USP process development and associated analytical support has been successfully automated, reducing development cost and timelines. Thus, the large amount and variety of data generated has become increasingly challenging to process and rapidly became a bottleneck.

Experimental approach: As a consequence custom solutions have been developed and are on going implementation in the Sanofi Pasteur Bioprocess R&D function to easily aggregate, store, mix and match data sources, join tables in a simple visual canvas, create and edit metadata coming from process robots

Results and discussion: This data management Infrastructure permits instant visualization, organization and sharing of data from few μ L to large scale processes, and will allow user friendly analysis for further initiatives (statistical/trend analysis, QbD, process transfer).

A Harmonization project is currently on going to connect databases from USP to Formulation & Stability platforms, in order to have single data mining of all manufacturing steps from cell bank thawing to final product stability

Bibliography, Acknowledgements: Thanks to all the persons involved in Data Management implementation in BRD.

Disclosure of Interest: None declared





PO141

IMPACT OF MICROVESICLES OVER CELL GROWTH AND RECOMBINANT PROTEIN PRODUCTION FROM CHO CELLS

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Background and novelty: CHO cells are the main workhorse for production of recombinant proteins at commercial scale. Further improvements in production are of eminent importance to meet global demand at affordable cost.

Microvesicles released by cells into culture media may have impact on various bioprocess-related phenotypes such as cell growth, cell death, product degradation and/or host cell proteins composition. However their impact on CHO-based bioprocess has not been evaluated yet.

Experimental approach: In this investigation, we evaluated the impact of microvesicles on regulation of cell growth and recombinant protein production. The cells were supplemented with microvesicles collected from different phases of culture (lag, log, stationary and death) and their impact on growth was observed. The microvesicles from different phase of cultures were also evaluated for specific enrichment/ deprivation of cell growth regulation-associated targets using Western blotting. The microvesicles were also profiled for the presence of proteolytic enzymes using gelatin zymography.

Results and discussion: The growth of cultures supplemented with microvesicles collected from log phase had shorter lag phase and achieved 1.2 fold higher cell density without having significant impact on the culture viability over time. The cultures supplemented the microvesicles from lag, stationary and death phase remained unaffected. Microvesicles from log phase were observed to be enriched with cyclin-D1 compared to microvesicle-free fraction and intracellular level at lag, stationary and death phase of culture. The microvesicles were also observed to be enriched with proteolytic enzymes and hence may contribute to product degradation during production and/or storage.

Therefore strategies needs to be developed that provides precise control over microvesicles associated regulation of cell growth and recombinant protein production.

Bibliography, Acknowledgements: Department of Biotechnology, India

Disclosure of Interest: None declared





PO142

AUTOMATED HIGH THROUGHPUT CELL CULTURE METHODS FOR THE DEVELOPMENT AND INVESTIGATION OF LARGE SCALE PERFUSION PROCESSES

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Background and novelty: With the renewed interest in perfusion cultures, a scale down High Throughput (HT) method to investigate such cultures is needed. Perfusion cultures, operating at higher viable cell densities (VCD) and for longer durations, require more resources and planning when compared to their fed-batch counterparts. To address these issues, a reliable HT platform would help improve the throughput and experimental power of small scale perfusion studies.

Experimental approach: Two HT methods to mimic large scale cultures were evaluated using an AMBR15. The first method (i.e. Perfusion Mimic) required cells to settle prior to cell free harvest collection. The second method (i.e. Chemostat) operated such that cells were collected along with the harvest. ViCell integration enabled on-line measurement of VCD, facilitating the automation of cell bleed and harvest collection. Given discontinuous harvest collection (i.e. batch harvest), a mathematical framework was also developed to ensure dilution rates matched Cell Specific Perfusion Rates used in continuous perfusion bioreactors.

Results and discussion: The Perfusion Mimic method successfully achieved long term, high VCD cultures, with process trends consistent to those at large scale. VCD set-point in the AMBR system was reduced to accommodate a lower vessel $k_L a$. The Chemostat method exhibited comparable process trends to large scale cultures. The mathematical framework was successful in ensuring rates of nutrient supply and product/waste removal were consistent to those of continuous perfusion cultures. Compared to the Perfusion Mimic method, the Chemostat method achieved superior VCD control and required less resources. This resulted in a reliable HT system capable of mimicking large scale perfusion processes, while providing a powerful tool for continuous bioprocesses development and troubleshooting of large scale perfusion cultures.

Bibliography, Acknowledgements: .

Disclosure of Interest: None declared





PO143

KINETIC STUDIES OF CO-INFECTION OF SF9 CELLS BY RECOMBINANT BACULOVIRUSES AT LOW MOI – IMPLICATIONS FOR VLP AND AAV VECTOR PRODUCTION

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Background and novelty: Baculoviruses are widely used to express heterologous genes in insect cells. The baculovirus expression vector system is particularly expedient for large-scale applications, such as the production of virus like particles (VLPs) or adeno-associated viral (rAAV) vectors (Galibert & Merten 2011). In both cases at least two different baculoviruses are required for the generation of VLP or AAV. Concerning AAV, the most advanced system is based on the use of two different viruses providing the AAV functions rep-cap and the AAV vector sequence flanked by the ITRs (Smith et al. 2009) to infect Sf9 cells at low MOI (multiplicity of infection).

Experimental approach: This study deals with the kinetics of infection of Sf9 cells by several baculoviruses. Baculoviruses expressing genes coding for different fluorescent proteins were used to co-infect Sf9 cells and the resulting fluorescence was monitored by spectral flow cytometry.

Results and discussion: The co-infection kinetics gave insights on cells being infected by either a single or several baculoviruses. Moreover, we have determined the impact of variability in the titration process of infectious baculovirus particles and the potential disequilibrium between both baculoviruses in the case of co-infection at low MOIs. Even in the case of using exactly identical low MOIs (i.e. 0.05 per baculovirus species) for the two baculoviruses we could show that only 60-70% of the cells have been infected by both baculoviruses and 30-40% only with one baculovirus species. This information is critical to understand and improve the production process of VLPs or rAAV. Based on these results and kinetics related to baculovirus release, to baculovirus superinfection, etc., a model has been developed describing co-infection kinetics at low MOI.

Bibliography, Acknowledgements: Galibert L & Merten O-W (2011) *J. Invertebrate Pathol.* 107, S80.

Smith RH et al. (2009) *Mol. Ther.* 17, 1888.

Disclosure of Interest: None declared





PO144

GENERIC WORKFLOW FOR THE SETUP OF SUBSTANTIAL TARGET-ORIENTED MECHANISTIC PROCESS MODELS FOR MAMMALIAN PROCESSES

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Background and novelty: A multitude of new applications in bioprocess technology strongly depends on model-based methods as they feature prediction and control capabilities. The critical path is usually the availability of suitable, reliable models. Therefore, a novel workflow was developed in order to handle typical modeling issues such as: i) a lack of knowledge, ii) the absence of information and iii) a lack of qualified personal with modelling skills. Following the workflow, a target-oriented dynamic process model can be generated.

Experimental approach: This workflow is based on backpropagation starting from a material balance for a certain target variable. Iteratively, necessary states as well as mechanistic links are included in the model using a model library reducing the computational effort. The parameters of these links are estimated using a simplex algorithm whose objective function depends on the target variable only. Practical identifiability analysis is used for the assessment of the need of further iterations and for the validation of the mechanistic model.

Results and discussion: To demonstrate the applicability of the approach and the benefits deriving from the workflow, a model aiming at modeling the viable cell count in mammalian cell culture process is used as example. The generated model satisfies the predefined requirements and is very simple in its structure, consisting of three states and seven model parameters. The presented workflow is easy to use, generic and transparent, so that applications in a regulatory environment should be possible. Hence, the here presented approach provides additional process knowledge that can be used in bioprocess development and optimization.

Bibliography, Acknowledgements: We thank the Austrian Federal Ministry of Science, Research and Economy in course of the Christian Doppler Laboratory for Mechanistic and Physiological Methods for Improved Bioprocesses and Sandoz GmbH for financial support.

Disclosure of Interest: None declared





PO145

LEACHABLES FROM SINGLE-USE DISPOSABLE BIOREACTORS – MAKING BETTER BAGS WORSE

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Background and novelty: The CHO cell has been the bedrock of the biopharmaceutical industry for the production of high quality recombinant therapeutic proteins. Despite g/L titres being routine, the cost of drug production is at an all-time high. Minimizing the initial capital investment associated with hard-piped stainless steel facilities is currently being explored through the implementation of single-use (SU) disposable systems. However, the introduction of new materials into the bioprocessing pipeline comes with it a cohort of unknown compounds, the majority of which remain unidentified.

Experimental approach: By applying a comprehensive discovery phase including GC-MS, UHPLC, LC-MS and ICP-MS, sampled from 37 SU disposable plastic bioreactor bags supplied by industrial partners covering a range of vendors and manufacturing dates, a comprehensive list of leachable compounds have been identified.

Results and discussion: We have demonstrated that a leachate from polyethylene-based films, bis(2,4-di-tert-butylphenyl)phosphate or bDtBPP reduces CHO cell growth at concentrations found to accumulate in culture media (0.035-0.1 mg/L)¹. Cytotoxic evaluation has revealed that these never seen before compounds are toxic to CHO cell growth at concentrations comparable to bDtBPP. This further raises the concern that the cocktail of leachates accumulated over the course of a bioprocess will have a synergistic effect on growth. Similar to bDtBPP originating from the anti-oxidant Irgafos-168 required for manufacturing, these novel leachates also originate from materials selected to “enhance” the performance of these systems as bioreactors. Finally, incomplete removal of these contaminants during downstream processing poses a serious risk to the patient to the extent of being endocrine disruptors.

Bibliography, Acknowledgements: 1. **Kelly PS, et al.** Process-relevant concentrations of the leachable bDtBPP impact negatively on CHO cell production characteristics. *Biotechnology Progress* 2016 Aug.

Disclosure of Interest: None declared





PO146

PERFORMANCE EVALUATION OF A CHEMICALLY DEFINED FEED IN TERMS OF CELL GROWTH AND PRODUCTIVITY IN CHO DG44 DHFR- CELLS

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Background and novelty: In a time of rapid growth within the biopharmaceutical industry particularly in the development of monoclonal antibodies and biosimilar, the availability of high performing chemically defined cell culture medium and feed for mammalian cell culture is crucial. Chemically defined medium and feeds are in high demand due to the avoidance of lot to lot variability and regulatory concerns. Kerry has developed a CHO cell specific chemically defined (CD) feed, which works synergistically with our AmpliCHO CD medium to improve viable cell density, cell viability and recombinant protein production.

Experimental approach: This efficacy of the CD feed was demonstrated by performing multiple fed-batch experiments in shake flasks and in bioreactors using AmpliCHO CD medium with Kerry's CD feed and compared with performance of a competitor CD feed with their CD medium. Samples were collected routinely and analyzed for viable cell density, cell viability, nutrient and metabolite profiles, recombinant protein concentration and glycosylation heterogeneity of the final product.

Results and discussion: The combination of Kerry's CD feed with AmpliCHO CD medium resulted in significantly higher IgG concentration and increased specific productivity compared to competitor products. Furthermore, glycosylation heterogeneity of the final product, growth profile, in terms of viable cell density and cell viability, were comparable with that of the competitor feed in their companion mediums. The availability Kerry's CD feed along with AmpliCHO CD medium provides a viable option for overall improvement in CHO cell culture processes.

Bibliography, Acknowledgements: n/a

Disclosure of Interest: None declared





PO147

A PH CONTROLLED HIGH-THROUGHPUT SYSTEM FOR CELL CULTURE PROCESS DEVELOPMENT

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Background and novelty: For therapeutic monoclonal antibodies, there are advantages to go as quickly as possible from DNA to Phase I clinical trials. However, moving quickly carries the risk that the initial cell line will be unsuitable for commercial production. In that case, a cell line change would be beneficial. The new cell line should offer advantages, such as higher productivity, but should ideally have similar product quality to Phase I. Identifying the best clone can require testing a large number of clones and conditions, so a high throughput system was developed to handle the required throughput. The initial system used shaken tubes and gave results comparable to flasks. The system has been recently enhanced to include pH control, which provides more and better data on clone performance.

Experimental approach: CHO cells are cultured in batch or fed-batch mode in shaken 50mL conical tubes, with 96 tubes per rack and up to 4 racks. Optical pH sensors were integrated into the tube bioreactors so pH could be measured every few minutes, using PreSens ITR iTube96Readers installed inside of CO₂-controlled incubator shakers. A device was constructed that takes on the pH sensor readings by delivering a 20µL dose of base to individual reactors if the pH fell below the process setpoint. Two-sided control was achieved by adding small amounts of acid to the cultures.

Results and discussion: The system controls cell culture pH to within ±0.1 pH units. Base was typically added every 2 hours during the lactate production phase, explaining why daily pH interventions are insufficient to effectively control pH in shake flask cultures. Comparability between tubespins and benchtop reactors was significantly improved by the addition of pH control. Tubespins with pH control are an efficient tool for screening multiple clones against multiple cell culture conditions, and generate useful data for clone selection including titer, product quality, and metabolic liabilities.

Bibliography, Acknowledgements: .

Disclosure of Interest: None declared





PO148

OVERCOMING THE CHALLENGES IN DEVELOPING A HIGH CELL DENSITY PERFUSION CELL CULTURE PROCESS FOR A RECOMBINANT ENZYME

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Background and novelty: Advancements in cell retention systems, stable cell lines and cell culture media have made high cell density perfusion processes possible over the recent years minimizing the manufacturing facility footprint, lowering overall investment and operating costs. In this presentation, we describe a case study on the development of a late phase high cell density perfusion process for a CHO cell line expressing a recombinant enzyme. For this product, an early stage perfusion process using hydrolysate-containing media was rapidly developed to supply initial clinical trials. A decline in cell viability towards later harvests and subsequently variable product quality over time was observed in the bioreactor process. In order to meet commercial needs, a robust and steady state high cell density perfusion process was developed during late phase development.

Experimental approach: Development studies were carried out using a high throughput bioreactor system and a traditional 10L stirred tank bioreactor system equipped with an alternating tangential flow device. Development costs and timelines were significantly reduced due to the usage of high throughput bioreactor systems. The process optimization studies focused on screening several chemically defined cell culture media, optimizing bioreactor process parameters and process control schemes.

Results and discussion: The optimization of bioreactor process parameters, process control schemes and cell culture media resulted in a robust and scalable high cell density perfusion process. Viable cell densities as high as 90E6 vc/mL and viabilities >95% were achieved with the new process. A robust steady state cell culture process was achieved which resulted in an improved product quality profile over the course of the production run. In this presentation, we will discuss the challenges encountered during the development of the process and strategies to overcome them.

Bibliography, Acknowledgements: na

Disclosure of Interest: None declared





PO149

PROCESS INTENSIFICATION GUIDED BY SYSTEMS BIOTECHNOLOGY: ANALYSIS OF A CHO FED BATCH PROCESS WITH ULTRA-HIGH SEEDING AFTER PERFUSION PRESTAGE FOR THE PRODUCTION OF MONOCLONAL ANTIBODIES

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Background and novelty: Constant growth of biopharmaceutical drugs raises an urgent need for highly efficient production processes of therapeutic proteins. The application of continuous and semi-continuous cell culture processes, applying perfusion technologies, offers a way to increase process performance by making advantage of higher cell densities.

Experimental approach: We present an extensive analysis of a process intensification based on prestage perfusion and production cultures with ultra-high seeding cell densities (uHSD, up to 10e6 cells/ml). The process outline effectively shifts biomass production to the prestage and uses full time in the larger bioreactor for production. This increase was facilitated by adaptations of both media and process parameters and was guided by mechanistic modeling as well as NGS analysis of both cultivation steps. Finally, the process was successfully transferred from lab scale glass bioreactors to representative stainless steel bioreactors (20L perfusion r. and 80L production r.) for evaluation of further scale up potential.

Results and discussion: Our setup allowed increasing the product titer of a platform process from 2.5 g/L to 4.8g/L I in the same runtime and with comparable product quality. During the NGS analysis we detected very stable gene expression levels in the perfusion cultures. On the other hand, transfer to the production bioreactor initiated system wide alterations of gene expression in both low and ultra-high seeding cultures. These changes could be minimized by alternations of the production bioreactor medium that preserved conditions of the perfusion prestage. Moreover, we could identify an earlier shift from growth associated gene expression patterns to production stage associated gene expression in uHSD cultures, which could be further supported by feeding lactate.

Bibliography, Acknowledgements: none

Disclosure of Interest: None declared





PO150

SELECTION, OPTIMISATION AND RAW MATERIAL VARIABILITY PREDICTION FOR HYDROLYSATE-BASED BIOLOGICAL ADDITIVES USED TO SUPPLEMENT BIOPHARMACEUTICAL GROWTH MEDIA.

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Background and novelty: Biological additives (BAs) such as yeast extracts & peptones, are important raw materials used to supplement mammalian cell culture and microbial growth media. For mammalian cell culture, BAs typically replace many of the nutritional factors historically supplied by animal-derived serum albumin. BAs are subject to raw material variability as a result of genetic, environmental & processing variation. The potential effects of this raw material variability is difficult to characterise during early development. This contribution presents a method that improves screening, selection & optimisation of BA for media feeds & allows prediction of the potential effects of raw material variability on mammalian cell line performance & productivity.

Experimental approach: A chemometric technique, based on quantitative structural activity relationship modelling (QSAR) is demonstrated as an effective tool to characterise BA composition variability & link this variability to cell culture performance. A database of chemical descriptors for > 180 commercially available BAs was compiled from vendor data.

Results and discussion: Principal component analysis (PCA) is used to summarise the BA database explaining over to 70 % of BA compositional variability in four principal components, termed principal properties (PPs) . The PPs summarise the multivariate design space for BA composition & are used for BA selection & to optimise BA formulation to improve cell culture performance & productivity. PPs can be used as design factors in an DOE experimental design & are used to link BA composition with cell culture performance. The resulting BA-QSAR models will be used to demonstrate applications such as BA screening and selection, formulation optimisation & prediction of the effect of raw material manufacturing lot variability on culture performance.

Bibliography, Acknowledgements: ROK Bioconsulting

Disclosure of Interest: None declared





PO151

UNDERSTANDING THE EFFECTS OF UTILIZING A COMPLETE FEEDING SUPPLEMENT TO MODULATE GLYCOSYLATION PROFILES

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Background and novelty: Chinese Hamster Ovary (CHO) cells are the primary expression system used in the biopharmaceutical industry for the production of therapeutic glycoproteins. The glycosylation profiles of proteins in CHO cells are critical parameters that need to be extensively studied to ensure effective, consistent and high-quality protein products. N-linked glycans can display a wide range of heterogeneity, the degree of which is dependent on several factors including cell line, media, feeding supplements, and process. The effects of these variables have made it challenging to specifically target and maintain desired glycosylation profiles.

Experimental approach: In order to address these challenges, we explored the effects of a complete glycosylation modulating feed supplement along with a unique fed-batch process that together have demonstrated the ability to modulate glycan profiles while maximizing protein titers.

Results and discussion: We performed studies using an IgG-expressing CHO DG44 cell line. Utilizing glycosylation enhancing GlycanTune™ Total Feeds in combination with the complimentary EfficientFeed™ + Supplements, we demonstrated a progressive series of adjustments of G0F glycan proportion from 30% to 80% while still maintaining growth and productivity. Additionally we tested this ability to target specific glycosylation profiles with other commercially available media and feed supplements, confirming that GlycanTune can modulate glycan profiles, although with a narrower range of adjustability of 20% compared to 50% with the recommended EfficientFeed+ Supplements. GlycanTune when paired with EfficientFeed+ Supplements or other commercially available media and feed combinations can result in more specific and predictive glycan profiles compared to basic supplementation protocols.

Bibliography, Acknowledgements: Shaheer Khan, Bharti Solanki

Disclosure of Interest: None declared





PO152

EVALUATION OF A CHEMICALLY DEFINED MEDIA DESIGNED FOR BATCH, FED-BATCH AND PERFUSION PROCESSES WITH CHO CELLS

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Background and novelty: Biopharmaceutical companies routinely utilize fed-batch processes for maximizing recombinant protein yields in Chinese Hamster Ovary (CHO) cells. Traditionally to meet higher demands, a fed-batch process requires capital investment in building/expanding facilities. Any decrease in demand leads to underutilization of the facilities. Furthermore, utilizing perfusion processes for cell culture offers the advantages of lower capital costs, scalability, smaller footprint, same yields as fed-batch and consistent product quality. Our work outlines the comparison of commercially available chemically defined (CD) medium for CHO cells in either a batch, fed-batch or perfusion CHO-cell based process.

Experimental approach: An IgG expressing CHO DG44 dhfr- cell line was used to perform experiments in shake flasks and in bench-top bioreactors. At regular intervals, samples were assessed for viable cell density, viability, nutrient profiles, titer and product quality of the different CD medium. To analyze the applicability of the different CD medium in a perfusion processes, multiple experiments were performed using the same CHO line. Initially, shake flask experiments with fixed volume medium replacement at fixed intervals were performed, followed by experiments with perfusion culture of cells in hollow fiber bioreactors with sample analysis at regular intervals.

Results and discussion: AmpliCHO CD medium demonstrated its ability to substantially improve cell growth/viability, enhance recombinant protein titers as compared to commercially available CD medium without affecting key quality attributes like glycosylation in batch, fed-batch and perfusion CHO-cell based processes. This demonstrates the versatility of the AmpliCHO CD medium to be used in either batch, fed-batch or continuous CHO-cell based applications without affecting the key quality attributes of recombinant proteins.

Bibliography, Acknowledgements: n/a

Disclosure of Interest: None declared





PO153

SURFACTANTS IN CELL CULTURE MEDIA: IMPACT ON HEK AND CHO CELLS IN CULTIVATION AND TRANSFECTION

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Background and novelty: Surface active agents (surfactants) are commonly used cell culture medium components for reducing shear stress in non-static suspension culture. Despite the preferred application of poloxamer 188, there is an ongoing discussion within the cell culture community about surfactant-related process deviations. Furthermore, surfactants have shown to interact with polyplexes as well as polymer nanoparticles within various applications (e.g. transfection, encapsulation). Better understanding the related mechanisms of action will facilitate finding alternative components for progressive cell culture media formulations.

Experimental approach: HEK 293-F and CHO-K1 cell lines were cultivated in plain and baffled shake flasks to evaluate the impact of shear stress. The surfactants were also evaluated regarding their impact on transient GFP expression. The localization of poloxamers in HEK 293-F culture was investigated using fluorescein-labeled Pluronic[®] F-68 and F-127 in flow cytometry and confocal microscopy.

Results and discussion: Growth comparison of HEK and CHO cultures with varying shear stress showed distinct differences depending on the poloxamer used. Low shear stress conditions led to comparable growth of HEK 293-F cells with a maximum viable cell density (vcd) in the range of $1 \cdot 10^7$ cells/mL, independent from respective surfactant. In contrast, experiments in baffled shake flasks revealed differences in peak vcd within a range of $0.2 - 1 \cdot 10^7$ cells/mL. Results from transient transfection indicated comparable performance for Pluronic[®] F-127 and Kolliphor[®] P 188 (> 95% GFP-positive) while Pluronic[®] F-68 showed slightly lower GFP expression. Tracking of poloxamers in the culture revealed a time-dependent uptake by cells (> 10-fold increase in signal after 96 h) and a colocalization with cell membrane and lysosomes.

Bibliography, Acknowledgements: We would like to thank the German Federal Ministry of Education & Research (BMBF # 031A518B Vectura) for funding.

Disclosure of Interest: None declared





PO154

OVERCOMING THE MEDIA DESIGN CHALLENGES IN TRANSIENT GENE EXPRESSION WITH CHO CELL LINES

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Background and novelty: While transient gene expression (TGE) is routinely performed in HEK cell lines, high transfection of CHO cell lines proves to be somewhat more complex. For modern TGE procedures, a one-step solution and scalability is mandatory. Media components such as iron, which is essential for culture growth, chelators, etc. can inhibit polyethylenimine (PEI)-mediated transfection [1], while polymers have shown to enhance efficiency in e.g. electroporation. In this study, various media components were investigated to allow efficient TGE with CHO.

Experimental approach: CHO-K1 and CHO-T suspension cell lines were investigated regarding growth and TGE in different medium variants. For determination of transfection efficiency, linear PEI was used with a GFP plasmid and flow cytometric analysis after 48 h. Protein expression in batch processes was monitored after transfecting two plasmids carrying heavy and light chain genes for IgG expression.

Results and discussion: While ammonium iron(III) citrate and iron(III) chloride are known for their inhibiting effect [1], iron(II) sulfate heptahydrate and ammonium iron(III) sulfate dodecahydrate also reduced or completely prevented transfection in this study. Aurintricarboxylic acid (endonuclease inhibitor; enhancing component in e.g. salivary gland transfection) and polyvinylpyrrolidone (polymer; beneficial in electroporation) were both found to negatively impact PEI-mediated transfection of CHO cells, while another tested polymer enhanced growth and transfection efficiency. Using optimized medium variants, cell density in batch shake flask cultivations was well above $1 \cdot 10^7$ cells/ml with a culture duration of 8-10 days. Transfection efficiency 48 h post-transfection was well above 90%, depending on the CHO cell line. This proves feasibility of the medium for application in both growth and transfection.

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Disclosure of Interest: None declared





PO155

IMPACT OF PH ON MONOCLONAL ANTIBODY PRODUCTIVITY AND PRODUCT QUALITY OF A BIOSIMILAR CLONE IN A MICRO BIOREACTOR SYSTEM

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Background and novelty: The application of design of experiments (DOE) methodology as a means of experimental analysis during process development is a key tool to determine whether a biosimilar is economically viable while providing valuable information about commercial scale-up. Understanding and controlling cell culture process parameters is of vital importance for maximising monoclonal antibody (mAb) expression and ensuring product quality. Parameters include, but are not limited to: pH; dissolved oxygen; cell culture (including concentrated feeds); agitation speed; and temperature control. This study explores the effect of pH on mAb expression and product quality involving biosimilar production by Chinese Hamster Ovary (CHO) cells in an ambr® 15 cell culture micro bioreactor system (Sartorius Stedim Biotech)

Experimental approach: A single clone was selected and seeded in duplicate conditions into an ambr® 15. To eliminate bias, well locations were randomised within the culture station. The study duration was 17 days. The product was tested for potency, kinetics, SDS-PAGE and glycan analysis to confirm biosimilarity to the originator

Results and discussion: An 84% increase in mAb expression at harvest was observed from pH 7.1 to 7.5, without an adverse effect on critical quality attributes. Having an understanding of how parameters are interdependent can improve final processes. This experiment focuses on a single parameter change (pH) which significantly impacts mAb production and cell viability. Higher pH correlates with higher viability, resulting in increased mAb concentration

Bibliography, Acknowledgements: Mrs Neelam Bhandarkar, Dr Vitri Dewi, Mr Andrew Wu, Mr Adrian Cawley, Ms Hollie Barret, Dr Caroline Ferrari

Disclosure of Interest: None declared





PO156

ANTIOXIDANT EFFECT OF THIAZOLIDINE MOLECULES IN CELL CULTURE MEDIA IMPROVES STABILITY AND PERFORMANCE

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Background and novelty: For production of biopharmaceutical proteins, complex cell culture media and feeds are used. Some of these media compounds are sensitive to oxidative degradation, affecting overall stability of the formulation. To ensure consistent performance during manufacturing, this study deals with the antioxidative capabilities of thiazolidine molecules in chemically defined cell culture formulations.

Experimental approach: 2-methyl-1,3-thiazolidine-2,4-dicarboxylic acid and 2-(2-carboxyethyl)-1,3-thiazolidine-2,4-dicarboxylic acid, obtained by condensation of cysteine/pyruvate and cysteine/alpha-ketoglutarate respectively, were tested in stability studies and fed-batch experiments. Antioxidative capabilities and influences on CHO growth and productivity were determined in small scale and bioreactor experiments. Additionally, fluorescence based assays, gene expression arrays, western blot analysis and quantitative/qualitative LC(-MS) based methods were applied, to elucidate the molecules mechanism of action.

Results and discussion: Stabilizing effects on media compounds like folic acid, thiamine, methionine and tryptophan and a decrease in the formation of oxidation products was observed. Besides, thiazolidine containing feeds were found to prolong growth of a recombinant CHO cell line and to increase IgG1 mAb productivity. The improved performance was correlated to lower reactive species generation, extracellularly and intracellularly. Moreover, an antioxidative response was triggered via induction of superoxide dismutase and an increase in intracellular total glutathione pool, the major intracellular antioxidant. Concluding, thiazolidine molecules are an interesting class of antioxidant molecules and their application to cell culture media and feeds results in an increased stability and performance. Thus, we believe that our results will contribute to further improve the production process of biopharmaceutical proteins.

Bibliography, Acknowledgements: -

Disclosure of Interest: None declared





PO157

SILK PROTEIN SERICIN PEPTIDE AS GROWTH FACTOR IN MAMMALIAN CELL CULTURE

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Background and novelty: In mammalian cell culture for regenerative medicine, as well as biotherapeutics production, various mammal-derived factors are supplemented to the culture media. But these mammal-derived factors must be replaced by non-mammal factors because mammal-derived factors have the risk of zoonotic disease. Previously, we reported that peptides from sericin, a natural protein from cocoon of silkworms, supported the proliferation and survival of various cell lines and successfully replaced mammal-derived factors such as BSA.

In this study, we aimed to elucidate the mechanism how sericin promotes the proliferation of mammalian cells. For the purpose, we focused on a human keratinocyte cell line, highly depend on sericin, as well as on EGF. Using this cell line, the proliferation signal pathway from sericin was compared with that of EGF.

Experimental approach: Human keratinocyte PHK16-0b cells were sub-cultured in Keratinocyte-SFM medium supplemented with 5 ng/mL of EGF and 50 µg/ml BPE. One day later, the medium was replaced to that without both EGF and BPE. After additional 24 hours, the medium was replaced to those supplemented with EGF or sericin and any of signal inhibitors, and the cells were cultured for three days.

Results and discussion: Both EGFR inhibitor and EGFR-neutralizing antibody inhibited the proliferation in the culture with sericin and EGF, implying that sericin promotes the proliferation through EGFR as well as EGF. MAPK pathway inhibitor and JAK/STAT pathway inhibitor inhibited proliferation of both conditions, but PI3K pathway inhibitor inhibited only sericin condition and failed EGF condition, implying that sericin promotes the proliferation partly through other pathway different from EGF.

We found that sericin promotes the cell proliferation through EGFR-MAPK and EGFR-JAK/STAT signaling, as well as PI3K signaling independent of EGF.

Bibliography, Acknowledgements: This work was partly supported by Manufacturing Technology Association for Biologics, Japan.

Disclosure of Interest: None declared





PO158

DISACCHARIDES AS ENERGY SOURCE IN PROTEIN-FREE MAMMALIAN CELL CULTURES

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Background and novelty: Mammalian cells are generally considered to be unable to utilize polysaccharides for cell growth. The recent discovery of the only known animal sucrose transporter suggested that understanding in mammalian polysaccharide metabolism may still be enhanced. In addition, the potential use of polysaccharides as energy source can have practical implications in biopharmaceutical manufacturing, because it can increase the carbohydrate loading and decrease lactate accumulation.

We evaluated the use of disaccharides to support the growth of a CHO-K1 cells in a serum-free protein-free culture. We found that CHO-K1 cells can utilize maltose for growth in the absence of glucose. To our knowledge, this is the first report of a serum-free protein-free mammalian cell culture using a disaccharide as the energy source.

Experimental approach: CHO-K1 cells were first tested for adaption to glucose-free disaccharide-containing serum-free protein-free culture medium. To determine the utilization of maltose, an antibody-production CHO-K1 cell line was cultivated in a chemically defined medium containing limiting concentration of glucose supplemented with maltose. Intra-cellular and extracellular maltose profiles were then obtained.

Results and discussion: CHO-K1 cells adapted to glucose-free maltose-containing protein-free medium. CHO-K1 production cells utilized maltose after glucose depletion in a biphasic manner. Maltose was internalized by the cells and did not hydrolyze spontaneously in the conditioned culture media. Maltose supplementation led to 15% improvement in the recombinant monoclonal antibody titer from batch culture.

Bibliography, Acknowledgements: This work was supported by the Biomedical Research Council of the Agency for Science, Technology and Research (A*STAR), Biological Design Tools and Applications Funding scheme funded by the National Research Foundation (NRF2013-THE001-093), and Joint Council Office Grant funded by A*STAR (15302FG147).

Disclosure of Interest: None declared





PO159

DEVELOPMENT OF A CHEMICALLY DEFINED MEDIUM FOR OPTIMAL GROWTH AND RECOMBINANT PROTEIN EXPRESSION IN HEK293 CELLS

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Background and novelty: Mammalian cell lines have gained an increased role in the biopharmaceutical industry, with applications such as cell-based therapies or recombinant protein expression for biologics and vaccines becoming more prevalent. This has created a demand for cell culture media that can support higher cell growth and increased protein expression while maintaining high viability. Another factor that has emerged from the use of mammalian cell lines in industry is the need for animal component free (ACF) and chemically defined (CD) medium. Chemically defined media have been developed to eliminate the risk of adventitious agents in the final therapeutic product from animal-derived components and to ensure more consistent results in cell growth and protein production.

Experimental approach: HEK293 is a human cell line that is used for both transient and stable recombinant protein production, as well as for vaccine production due its ability to be grown in suspension. To address the need for cell culture media to support these applications, Kerry has developed an ACF, chemically defined cell culture medium to support the growth of HEK293 cells in suspension. This medium was designed from the knowledge gained in developing a medium for Chinese hamster ovary cells, and was refined by analysis of spent media after growth in shake flasks to determine the nutrients needed for optimal growth. Transient transfections were also performed to confirm that no components in the medium would inhibit the production of recombinant proteins.

Results and discussion: The Kerry medium formulation shows similar growth and viability profiles compared to competitor CD media. In addition, it has been shown that Kerry HEK293 medium supports the expression of recombinant secreted embryonic alkaline phosphatase (SEAP) in transient transfection. These results show that this medium can be used for industrial production with HEK293 cells.

Bibliography, Acknowledgements: n/a

Disclosure of Interest: None declared





PO160

IDENTIFICATION OF CELL CULTURE MEDIA COMPONENTS THAT PREVENT AGGREGATION OF AN FC-FUSION PROTEIN

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Background and novelty: In the past several years the biotherapeutic industry has been thoroughly characterizing protein quality attributes (PQAs) including protein aggregation. Environmental factors (i.e temperature and pH) can increase amino acid oxidation within a protein leading to scrambling of disulfide bonds. These reactions increase protein aggregation resulting in a final drug product with immunogenicity concerns and loss of efficacy.^{1,2} Therefore, it is important to understand cell culture media's impact on aggregation.

Experimental approach: A MilliporeSigma [CHO ZN™] cell line generating an Fc-fusion protein was used to evaluate impact of 80 media formulations on protein aggregation. Data was then analyzed using Umetrics SIMCA MVA software to determine the correlating raw materials. Proteins were screened for aggregation on a Waters UPLC BEH200 SEC column. Design Expert models were used to confirm initial component correlations as well as optimize component concentrations resulting in the desired protein aggregation profile.

Results and discussion: Initial media screening identified variation in protein aggregation ranging from 10%>40% aggregates implying different media will impact aggregation. MVA identified seven components that correlated to the decreased aggregation. Using factorial designs it was determined three of the seven had a significant impact on aggregation. Evaluating them individually lead to a 40% decrease in aggregation, while increasing the native structure in the Fc-fusion protein. When adding these components together at an optimal concentration, a larger decrease in protein aggregation was observed.

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Disclosure of Interest: None declared





PO161

BIOREACTOR SCALING THOUGHT NEW – FROM 0.25 TO 2000 L WITH UTILITY FUNCTIONS

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Background and novelty: There is a broad range of single-use bioreactor scales on the market from small scale systems up to 5 L that are used for process development, to production systems up to 2000 L. Successful process transfer requires that all scales result in comparable cell growth and productivity. To achieve that, stirred single-use bioreactor family scales from 0.25 L to 2000 L were tested using a scaling algorithm based on known process engineering parameters of each bioreactor system.

Experimental approach: A CHO fed-batch process was used with a duration of 12 days, a peak viable cell density of $19 \cdot 10^6$ cells/mL, and a product concentration of 3-4 g/L at the point of harvest. Parameters considered for the scaling algorithm included Reynolds number, kLa-value, tip speed, and specific power input. Cell culture cultivations were performed in a range of bioreactor scales, and results were analyzed and compared.

Results and discussion: Comparable results for viable cell density, product concentration, and product quality were achieved in the bioreactor scales tested. A CHO fed-batch process performed in a range of bioreactor scales from 0.25 L to 2000 L confirms the scaling algorithm strategy for successful process transfer.

Bibliography, Acknowledgements: Thanks goes to the R&D departments world wide who were contributing to these studies.

Disclosure of Interest: None declared





PO162

SCALE-UP OF A STIRRED SINGLE-USE BIOREACTOR FAMILY

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Background and novelty: Single-use bioreactors are increasingly used over the past decades encouraged by the advantages over multi-use systems, including reducing lead times and the risk of cross-contaminations. Scale-up and scale-down of these systems can be challenging due to differing shape, agitation, and gassing, therefore stirred multi-use bioreactors remain the gold standard. To aid in the scale-up of single-use bioreactors, a range of scales with similar geometric ratios to multi-use systems were developed and evaluated.

Experimental approach: Single-use bioreactors in a range of 0.25 L to 2000 L with similar geometric ratios to multi-use systems were evaluated and characterized according to DECHEMA guidelines to allow for a quality by design approach. Process characterization of the bioreactors includes oxygen transfer, mixing, and power input studies for the generation of utility functions for cell culture cultivations.

Results and discussion: The process engineering results are suitable for mammalian cultivations and comparable with established systems for cell cultivation. On the basis of the generated utility functions process scaling can be performed smoothly. Concluding the advantage of the stirred single-use bioreactor family is shown.

Bibliography, Acknowledgements: Thanks goes to the R&D departments world wide who were contributing to these studies.

Disclosure of Interest: None declared





PO163

STRATEGIES FOR OPTIMIZING UPSTREAM PROCESSES

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Background and novelty: The cell culture process is crucial for maximizing the production of biologics and vaccines, as well as optimizing product quality attributes, minimizing impurities, and simplifying operations. Companies employ different strategies based on the business and technical requirements for their desired products.

Experimental approach: Case studies were used to evaluate the effectiveness of the different strategies in achieving the desired goals.

Results and discussion: Achieving high cell densities and titers are common goals, but companies producing biosimilars may engage in a strategy that focuses on matching specific product quality attributes, while companies producing a viral vaccine may leverage a strategy that minimizes impurities. A well-defined sourcing strategy that includes use of high quality/purity animal-origin-free components can improve performance and reduce risk. Additionally, companies are seeking to identify opportunities to streamline operations and reduce cost of goods, regardless of the type of product they are developing. This presentation will highlight case studies to demonstrate how optimizing the composition and format of media supports all of these upstream strategies.

Bibliography, Acknowledgements: Scott Jacobia, Andy Campbell, Steve Gorfien, and Mark Stramaglia

Disclosure of Interest: None declared





PO164

COMPARISON OF BOLUS AND CONTINUOUS FEED STRATEGIES FOR A CHO-K1 CELL LINE

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Background and novelty: Recombinant enzyme production in mammalian cells has a high level of complexity due to the criticality of product stability and activity. A process parameter that is easily accessible for optimization is the feeding strategy. A balanced nutrient supply has been shown to increase the cell specific productivity (qP) while maintaining the product quality. Our process uses Chinese hamster ovary (CHO) cells in a bi-phasic fed-batch process with bolus feeds in exponential growth phase as well as at early and late stationary phase. Amino acid (AA) analysis of the current process showed the depletion of some essential amino acids early in the culture and specifically an increase in glutamate consumption triggered by asparagine depletion in the media. For other amino acids excessive concentrations were observed, which can impact cell metabolism by the imbalance in the nutrient supply.

Experimental approach: In this study we tested different feeding regimes by shifting the sequence of the bolus feeds and using continuous feed strategies. Continuous feeding was used at a pre-determined amount or adjusted to maintain a constant residual glutamate concentration. An on-line biomass sensor was incorporated for measuring cell growth and correlation of the signal to the cell metabolic state.

Results and discussion: The results provided insights into the cell specific nutrient requirements and allowed us to establish a balanced feed strategy.

Bibliography, Acknowledgements: -

Disclosure of Interest: None declared





PO165

INLINE SENSORS AND A PREDICTIVE CONTROLLER TO OPTIMALLY CONTROL CHO CELL CULTURE PROCESSES

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Background and novelty: In the biopharmaceutical industry, optimal control of cell culture processes can deliver high titer without impacting product quality. ^{1,2} As key technologies for optimal control, we have developed integrated inline sensors and a predictive controller for bioreactors. Inline NIR and capacitance sensors monitor the glucose / lactate concentration and viable cell density respectively. The predictive controller predicts future glucose consumption by the estimation of specific rate profiles.

Experimental approach: To evaluate the inline monitoring precision, the glucose bolus culture of CHO cells producing a monoclonal antibody was carried out using our bench scale bioreactor. NIR sensor coupled to an optical probe acquired spectrum data, and the glucose / lactate concentration was extracted by chemometrics technology. The capacitance sensor coupled to a dielectric probe measured capacitances, and the viable cell density was calculated by our mathematical model. The inline monitoring precision was evaluated as the differences between inline values and offline values. In another cell culture to evaluate glucose control precision, glucose was continuous fed to maintain 2 g/L setpoint by adjusting the feed rate according to the difference between inline value and predicted future consumption.

Results and discussion: In the cell culture with several boluses of glucose feed to keep > 0.5 g/L (bolus target was > 5 g/L), the glucose monitoring precision was ± 1 g/L of offline values. This cell culture reached 0.9×10^7 cells/mL, and the viable cell density monitoring precision was $\pm 10\%$ of offline values. In another cell culture of predictive control, the glucose concentration was maintained within the $\pm 10\%$ against 2 g/L setpoint. We will present the additional evaluation results with other CHO cell lines and media.

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Disclosure of Interest: None declared





PO166

ON-LINE MONITORING OF DIELECTRIC CELL PROPERTIES FOR THE ANALYSIS OF VIRUS-LIKE PARTICLE PRODUCTION BY CAP CELLS

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Background and novelty: Dielectric spectroscopy sensors have been used to measure the permittivity of cell cultures, which can be related to viable cell density (VCD). Moreover, permittivity provides information about changes in the cell membrane, which correlate with cell physiological state. CAP is a novel cell line able to grow to high cell densities and to produce high titers of HIV virus-like particles (VLP). VLP assembly takes place in the membrane, changing its properties. The aim of this work was to study the events related to VLP formation reflected in permittivity measurements.

Experimental approach: A CAP stable cell line expressing a GagGFP fusion protein was cultured in a 1L bioreactor. VLPs were quantified by ELISA, the % of GagGFP-expressing cells was assessed by FACS. A capacitance sensor (Hamilton) was used to monitor the cell density.

Results and discussion: Fed-batch culture of CAP cells allowed obtaining 9×10^9 VLP/mL. Online monitoring of β -dispersion parameters (permittivity $\Delta\epsilon$, characteristic frequency f_c and α) allowed to follow VCD and characterize the process by identifying different phases throughout the culture. The first phase (0-48 h) was characterized by an accused decrease of f_c and an increase of the specific $\Delta\epsilon$, attributed to the intracellular accumulation of GagGFP. In a second phase (48-168h), VLP budding rate was higher as more VLPs were detected in the supernatant and a decrease of membrane capacitance was observed. After 168h, cell growth and VLP production reached a plateau phase and $\Delta\epsilon$ leveled off, while α profile indicated an increase of the culture heterogeneity. Finally, cell death was observed by an f_c increase and a $\Delta\epsilon$ decrease. The suitability of dielectric measures to identify key events in VLP production was demonstrated in CAP cells for the first time, proving the potential of this technology to automatize processes using online β -dispersion data acquisition.

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Disclosure of Interest: None declared





PO167

SCALE-UP AND PROCESS TRANSFER IN BIOPHARMACEUTICAL MANUFACTURING: THE PATH TO INTELLIGENT BIOREACTOR CONTROL SOFTWARE

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Background and novelty: For all biopharmaceutical manufacturing processes, scale-up and/or process transfer from one type of cultivation container to another is an intrinsic requirement. Often, the screening of appropriate cell clones takes place at a very small scale of a few mL – in a static or orbitally shaken system. Further process development and optimization is performed with L scale bioreactors, either orbitally shaken, wave-mixed or stirred. The final production scale can be up to several m³, mostly in stirred bioreactors. It is therefore clear that process transfer and scale-up are crucial and may result in limitations if they are not performed correctly.

Experimental approach: During the research project, numerous investigations were carried out in order to better understand the effects of scale-up and process transfer. Engineering characterization with classical (oxygen transfer, mixing time, power input) and modern methods (CFD for shear distribution) as well as cultivation experiments with a mammalian cell line (CHO XM111/10) were conducted in small scale TubeSpin[®] bioreactors (50mL, 600mL), shake flasks (250mL to 5L), shaken single use bag bioreactors (2L, 20L) and a wide range of scales of stirred bioreactors (700mL, 3.6L, 13L and 42L).

Results and discussion: The results from engineering characterization and cultivation experiments were evaluated using big data tools (e.g. multivariate data analysis) to identify optimal and critical process conditions for cell growth. Elements have been transferred to the new eve[®] bioprocess platform software from Infors AG and further results will continuously be integrated.

Furthermore, the introduction of user data for other cell lines allows the database to be extended. These evaluation results can then be used by the bioreactor's intelligent process control software, which recommends optimized operating conditions.

Bibliography, Acknowledgements: The project is funded by CTI (16924.1 PFLS-LS).

Disclosure of Interest: None declared





PO168

UP AND DOWN SCALE CONSIDERATIONS FOR THE CONTINUOUS PRODUCTION OF GLYCOOPTIMIZED BIOPHARMACEUTICALS

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Background and novelty: Product quality especially with respect to glycosylation is a critical product attribute which can have an immense influence on the activity of glycoproteins and should be closely monitored during process development and manufacturing. GlycoExpress® cells have been developed in this context to provide human host cell lines with robust glycosylation machineries.

This work will address challenges in two case studies for up and down scaling of production processes with focus on product quality and continuous processing.

Experimental approach: In a first section, data of a process transfer from a 200 L stainless steel to a 1000 L single-use bioreactor for a continuous cell culture application is discussed. The impact of engineering aspects for the bioreactor and the cell retention device as well as product quality considerations are addressed.

As a second part, a 10 mL down-scale system for perfusion cultivations in a microbioreactor is introduced and its application for process development is evaluated

Results and discussion: A GlycoExpress-based Phase II GMP perfusion process for mAb production at the 200 L stainless steel scale was successfully transferred to a 1000 L single-use bioreactor. Growth, productivity and stable glycosylation were maintained during scale up.

The developed perfusion down-scale system SAM shows very good comparability to larger scale ATF runs not only for USP characteristics but also for product quality. The system predicts the response to media supplementation better than batch or chemostat approaches. Design of Experiment studies can be easily performed with this system resulting in highly significant models.

Bibliography, Acknowledgements: B.Sc. and M.Sc. in Biotechnology, Associate Director for USP development at Glycotope GmbH, multiple years of experience in mammalian perfusion and fed-batch cultivation as well as scale down models for product quality prediction

Disclosure of Interest: None declared





PO169

EVALUATION OF FIXED-BED, DISPOSABLE BIOREACTOR FOR VIRAL PRODUCTION IN REPLACEMENT OF ROLLER BOTTLES

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Background and novelty: The presentation/poster will focus on the utilization of a fixed-bed bioreactor as a solution to improve manufacturing capacity & cost of goods for veterinary vaccines.

Experimental approach: The feasibility have been done at small scale with the iCellis nano by PALL Life Science. The comparison of yield productivity between roller bottles process vs iCellis nano have been done.

After the proof of concept at small scale, we will share the results obtained at large scale.

The scale-up will be done with the 66m² scale in iCellis 500 replacing 770 roller bottles of 850cm².

This system has been used for a virus currently produce in roller bottles, the fixed-bed bioreactor brings many advantages, it's a closed-system allowing controlled culture (pH, pO₂) and well monitored.

Results and discussion: Implementing this new disposable technology in manufacturing is a challenge. The first results are very encouraging and the iCellis seems to be a good alternative for the production of some vaccines on adherent cells in the future avoiding A/B class laboratories.

Bibliography, Acknowledgements: The work has been done with Pall Life Sciences

Disclosure of Interest: None declared





PO170

APPLICATION OF NEXT GENERATION ANALYTICAL TECHNIQUES TO SCALE DOWN MAMMALIAN PROCESSES

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Background and novelty: Platform approaches are widely used across the industry for biopharmaceutical process development and production. Step changes in overall productivity may be achieved using new cell lines and/or medium/feeds with further improvements coming from the increasing focus on experimental design and data interrogation coupled with new analytical techniques.

The use of mL scale bioreactors (eg ambr15) is well documented in mammalian process development. These systems facilitate small scale experimental design but because of the limited volumes can only be fully exploited with the concomitant application of high throughput small volume analytical techniques to assess process and product quality. The application of scaled-down bioreactors supported by low volume analytics offers the potential for deeper process understanding earlier in the development cycle leading to smarter process development and associated late-stage time line reductions.

Experimental approach: Over the course of an experiment the sample volumes required for conventional analytical techniques can amount to a significant proportion of the bioreactor volume in scale down processes and this is further exacerbated if purification is required for product quality analysis. In this poster we describe how we are developing and applying scaled-down analytical techniques to our upstream work packages to extend our scale down process models and thereby facilitate cell line and process decisions at an earlier stage in the development process.

Results and discussion: Data obtained from low volume based analytics including spectroscopic and microcapillary based techniques applied to process samples from ambr bioreactor experiments will be presented and will demonstrate how this approach can enhance the value of typical scale down process development experiments

Bibliography, Acknowledgements:

Disclosure of Interest: None declared





PO171

PROCESS CHARACTERIZATION OF A CHO CELL CULTURE FOR PRODUCING THERAPEUTIC PROTEINS USING A QUALIFIED SCALE-DOWN MODEL

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Background and novelty: Quality by Design concept was applied to a fed-batch process to understand impact of cell culture parameters on product qualities.

Experimental approach: Potential critical process parameters (pCPPs) were determined based on our previous development data using risk priority number scoring, as a part of Failure Modes and Effects Analysis. A 5-L scale down model (SDM) was developed to represent the 2500-L commercial manufacturing process in terms of process performance (PP) and critical quality attributes (CQAs). Cell culture harvests from bench scale bioreactors were purified by mixed-mode chromatography and then analyzed to compare PP and CQA with those from the commercial scale. One-step purification process was applied to the development of SDM and process characterization (PC) study to minimize potential impacts of downstream process on product qualities. Variability within an average \pm 2SD of each PP and CQAs from five 2500-L manufacturing batches was used as acceptable ranges for SDM qualification. PC was performed at the SDM using DoE with a central composite face-centered design to understand how the identified process parameters impact on the CQAs of the process in an individual and/or interactional manner.

Results and discussion: A statistical model was obtained for each CQA (p-value < 0.05 and $R^2_{adj} > 0.7$) and verified by comparing the empirical data with the 95% prediction interval limits from the models. As a result the design space was defined for this production process and it was confirmed that the current operating ranges for the identified CPPs in the manufacturing process were within the design space to ensure consistent product qualities.

Bibliography, Acknowledgements: *Biotechnol. Bioeng.*, Vol. 106, No. 6, August 15, 2010
Eur. J. Pharm. Biopharm., 81 (2012) 426–437

We acknowledge our colleagues of GC research center

Disclosure of Interest: None declared





PO172

COMPREHENSIVE ANALYSIS OF GLYCOSYLATION MODULATING MEDIA ADDITIVES FOR CELL LINE SELECTION

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Background and novelty: The extent and pattern of glycosylation can influence pharmacokinetics and effector functions of therapeutic monoclonal antibodies. Matching glycosylation product quality attributes within defined specification ranges when manufacturing a given antibody at different scales, different sites, or from different host cell lines poses a formidable challenge to the bio-manufacturing industry. One approach to control glycosylation attributes is to supplement cell culture media with additives known to influence glycosylation patterns.

Experimental approach: Here, we report a two-tier approach for the modulation of nine critical quality attributes in a monoclonal antibody expressed in CHO cells. In a first step, we tested ten different additives both in univariate and multivariate modes, pursuing a power-4 level design-of-experiments approach in 24-well deep well format to screen 42 experimental conditions.

Results and discussion: We found several components to influence one or more critical quality attributes, either as single agents or in combination with other additives. As a second tier, we focused on the efficacious additives and tested additional concentrations both in univariate and combinatorial experiments. We characterize the extent to which media formulation can influence critical quality attributes related to glycosylation, e.g. to match a desired target profile. This information, in turn, can be used to inform early stage cell line development in order to screen for cell lines with product quality profiles within reach of target.

Bibliography, Acknowledgements: -

Disclosure of Interest: None declared





PO173

FROM OBSERVATION TO CONTROL: USING CELL CULTURE AUTOMATION FOR ENHANCED PRODUCT QUALITY OPTIMIZATION

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Background and novelty: In this talk we will present an overview of our findings on influencing and controlling product quality attributes of different antibody derived biopharmaceuticals like standard IgGs, bispecific and glycoengineered proteins.

Experimental approach: We used the Roche in-house developed automated cell culture system which is based on shaken multi-well plates and a fully automated process workflow. More than 600 fed-batch cultivations can be handled in parallel enabling the screening of a wide range of process conditions. Combining the automation technologies in the fields of cell culture and product quality analytics enables the high throughput screening of a broad range of product quality attributes which makes this technology ideal for the process development of complex molecule formats.

Results and discussion: Several case studies from active early and late stage portfolio projects identifying levers for product quality attributes of different molecule formats will be presented in this talk. We focused on the glycosylation pattern, charge distribution, product aggregation, and trisulfide levels. The high information density provided by using high throughput cell culture enabled the identification of general levers for product quality attributes that can be used to control product quality or adjust it to a desired pattern. Furthermore the know-how could be used for the process development of new pipeline molecules. We could demonstrate that the results from the automated multi-well plates are predictive for the bioreactor and we established the system for the routine use of product quality optimization.

Bibliography, Acknowledgements: The author would like to thank the Roche project groups (K. Joeris, O. Popp, H. Wizemann), cell culture groups, analytics department and contributors from Genentech.

Disclosure of Interest: None declared





PO174

USE OF AN ANTIOXIDANT TO IMPROVE MONOCLONAL ANTIBODY PRODUCTION AND QUALITY IN CHO CELLS

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Background and novelty: Oxidative stress which is one of the major ER stress inducer is necessarily accumulated in the ER caused by mitochondrial energy metabolism and protein synthesis. Although accumulation of oxidative stress can severely disrupt the ER function, the impact on the recombinant protein production and product quality in Chinese hamster ovary (CHO) cells has not been explored.

Experimental approach: To elucidate the relationship between protein production and oxidative stress, hydrogen peroxide was added into the two different recombinant CHO cell lines that produces monoclonal antibody (high and low producer). In order to remove the oxidative stress, screening of six antioxidants (butylated hydroxyanisole, N-acetylcysteine, baicalein, berberine chloride, kaempferol, and apigenin) has been performed.

Results and discussion: It was clearly observed that the oxidative stress induced apoptotic cell death and reduced the maximum antibody concentration by 25 – 50% as well as proportion of glycosylated form of antibody. Among the six antioxidants, baicalein significantly increased culture duration by 3 - 5 days and maximum antibody concentration by 1.6 - 1.7 fold compared to the control cultures in both cell lines along with reduction of ER stress. Baicalein addition during the fed batch process showed synergistic effect on the maximum antibody concentration which was over 3.0 fold higher than control cultures. Furthermore, baicalein addition maintained the proportion of glycosylated form of antibody at the late culture period.

Taken together, the data obtained here demonstrate that baicalein is a potential culture supplements for reducing the oxidative stress and improving recombinant protein production and product quality in CHO cells.

Bibliography, Acknowledgements: DET FRIE FORSKNINGSRÅD, Danish council for independent research, Technology and Production Sciences (FTP). Novo Nordisk Foundation.

Disclosure of Interest: None declared





PO175

MODEL-BASED CELL CULTURE CONTROL – UNSTRUCTURED, UNSEGREGATED MODELS AS A KEY ELEMENT FOR ADAPTIVE SEED TRAIN AND FED-BATCH OPTIMIZATION

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Background and novelty: The production of biopharmaceuticals covers over 300 products creating approx. 200 billion US\$ sales. The corresponding cultivation of mammalian cell lines is demanding with respect to various aspects such as complex cell metabolism, variabilities in cell behavior, scale dependencies, influences of changes in cultivation conditions, medium composition etc.. Although an increasing number of measurement parameters is available, only a part of them is routinely utilized in industrial cell culture processes and their corresponding seed trains. Nevertheless, the data base grows, statistical investigation of data gains importance and process data are more easily accessible in the context of industry 4.0. Furthermore, cultivation strategies have to be adapted to new products, cell lines and clones.

Experimental approach: Cell cultivation has to take these complex requirements into account, e.g. for fed-batch control and seed train design. One approach to encounter the variabilities and to include actual process information is adaptive model-based control. One key element is the underlying process model. In order to provide an adaptive character, model parameters should be easily estimated from cultivation data available during a seed train and fed-batch without additional sophisticated measurements. Unstructured unsegregated models are one way to address this request.

Results and discussion: This contribution illustrates the usage of such a model for two applications: 1) a controller for the calculation of fed-batch feed trajectories 2) a programmed software tool for seed train design, analysis and optimization. The model implementation is shown in each case concerning the structure and the open-loop control sequence over time (“moving horizon”). An example demonstrates the adaptive character which enables feedback from the process.

Bibliography, Acknowledgements: Pörtner et al., ISBN: 978-0-444-63663-8 , pp. 463-493

Disclosure of Interest: None declared





PO176

A NOVEL SINGLE-USE DEVICE FOR EFFICIENT LARGE-SCALE MEDIA PREPARATION

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Background and novelty: As advances occur in particular bioprocessing technologies, it naturally stimulates innovation in other, related aspects. The advent of single-use equipment and flexible, functionally-closed systems has led to smaller, more efficient facility designs¹ and has also created a need for improving traditional media preparation methods².

Experimental approach: Several complete, chemically defined, dry powder media (dpm) formulations have been used to develop an in-line device capable of delivering 1,000L of ready-to-use liquid cell culture media when connected to an appropriate water source and combined with a sterilizing filter.

Results and discussion: A single-use device has been developed that shares all the advantages offered by other single-use equipment: reduced risk of cross-contamination, reduced cleaning costs, reduced capital expense, reduced time for setup or re-configuration. Because it is designed as an in-line device to be integrated into a closed system of interconnected single-use equipment, it also allows media preparation to be conducted in the same bioprocessing suite as other unit operations. The device arrives with a premeasured quantity of dpm in a fully contained vessel ready to install between an appropriate water source and a sterilizing filter in order to deliver a specific volume of liquid media directly into a sterile bioreactor or collection vessel. As bioprocesses are being intensified to deliver higher and higher volumetric productivities and facilities are being designed to be ever smaller and more efficient, liquid media preparation methods can now support these advancements without requiring special, dedicated resources.

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2. BioPlan Associates, Inc. *Powder Culture Media Packaging, Preparation and Market Trends*. (Rockville, MD 2014)

Disclosure of Interest: None declared





PO177

MODULATING GALACTOSYLATION BY CELL CULTURE FEED OPTIMIZATION

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Background and novelty: Recombinant proteins undergo of a variety of post-translational modifications, including glycosylation. In the case of mAbs, the oligosaccharide located in the CH2-domain affects protein stability and activity including ADCC and CDC. [1,2] Many strategies have been developed to modulate glycosylation by cell culture media design and by enzymatic glycoengineering. During this study, we developed a new approach consisting in the optimization of the supplement feed timing in order to tune more finely the glycosylation.

Experimental approach: mAb glycosylation was modulated by cell culture feed solutions supplemented with sugar and sugar-like components. CHO cells were cultured in fed-batch mode during 14 days in shake tubes. The Viable Cell Density (VCD) was measured by ViCell and the titer quantified using Biacore. The N-glycosylation profile of the expressed mAb was analyzed by CGE-LIF.

Results and discussion: The tested supplements induced significant changes of the glycosylation level. Early feed addition led to a greater glycosylation change in comparison to a later one. Early addition also reduced the VCD with a limited impact on titer. Moreover, we were able to obtain a 3.78-fold galactosylation decrease by adding a galactose analog during exponential growth phase and a 1.67-fold decrease when added in production phase. While the 11.5% of the molecules were galactosylated in the control, we managed to decrease the overall galactosylation level to 3% at the most. On the other hand, galactose supplemented feed entailed a 2.2- to 2.6-fold galactosylation increase.

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[2] Eon-Duval, A. *et al.* (2012). Quality Attributes of Recombinant Therapeutic Proteins: An Assessment of Impact on Safety and Efficacy as Part of a Quality by Design Development Approach. *Biotechnol. Prog.*

Disclosure of Interest: None declared





PO178

NEW APPROACH FOR CROSS FUNCTIONAL RISK MANAGEMENT FOR RAW MATERIALS USED IN A MULTISITE BIOTECH COMPANY

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Background and novelty: Periodically, issues arise during production with raw materials considered to be non-critical, but have the potential to impact cell culture performance. After several cases observed with raw materials, resulting in acute corrective actions, Roche/Genentech with its worldwide network of biotech drug substances manufacturing and process development sites is moving to a risk based approach to assess and mitigate direct material related issues from a cross-functional perspective including manufacturing, quality, procurement and regulatory aspects.

Experimental approach: Starting with case studies of raw material related issues, we established a routine process to assess and mitigate the individual risks and to apply this on manufacturing sites, considering all relevant aspects and functions. For this, a tool is used to consider the aspect of all different functions involved in raw material selection, qualification and procurement.

Results and discussion: The new established process will be demonstrated.

Bibliography, Acknowledgements: TBD

Disclosure of Interest: None declared





PO179

COMPARISON OF TWO HIGH-THROUGHPUT BIOREACTOR SYSTEMS WITH 2L GLASS BIOREACTORS FOR CLONE SELECTION

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Background and novelty: Miniaturized high-throughput bioreactor systems using disposable vessels have become increasingly important to facilitate process development and clone selection in timely manner. We compared two commercially available systems (ambr® 15 and ambr® 250; Sartorius AG, Germany) with the well-established 2 L glass bioreactor (UniVessel®, BIOSTAT® B; Sartorius AG, Germany) to perform the final clone selection (8-12 clones) for the production of complex antibody formats in CHO cells.

Experimental approach: In three clone selection experiments for three different products, all three systems were inoculated using the same pre-culture and 14 day fed-batch cultivations were carried out in parallel. At least every second day samples were analyzed. We used a combination of two bioinformatic approaches, i.e. two one-sided t-test and model-based comparisons, to evaluate which high-throughput system provides comparable results to the 2 L bioreactor.

Results and discussion: For several parameters such as viable cell concentration, product titer and glutamine concentration good comparability was found between all three cultivation systems. However, some parameters such as ammonium and lactate concentrations showed similar dynamics in the time course of the fermentation but absolute levels differed significantly. Especially the results from the ambr15 showed higher deviations to the results of the 2 L bioreactors. In addition, cell viability often decreased earlier in the ambr15. Moreover, the clone ranking based on product titer was similar between 2 L bioreactors and ambr250 but less defined in the ambr15 due to larger variations between technical duplicates. Hence, the ambr250 can be applied for the final selection and characterization of production clones while in our hands the ambr15 is more suitable for pre-screenings of cell clones to identify promising candidates.

Bibliography, Acknowledgements: n/a

Disclosure of Interest: None declared





PO180

IMPACT OF METABOLISM CHANGES ON PRODUCT QUALITY IN HIGH PERFORMING MAMMALIAN CELL CULTURES

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Background and novelty: Use of high-titer cell lines and high-quality antibody products are the primary objectives for process development for biologics production in an industrial environment. Because variations in bioprocess parameters can affect post-translational modifications of cell culture products, it is imperative to monitor product quality during cell culture process development. Metabolic changes occurring during a production process can impact product quality through waste accumulation and reactions of rich and complex media and feed components needed to sustain the cell line productivity and growth.

Experimental approach: In our study, metabolism changes were induced to assess their impact on product quality by adding specific components in a high density chinese ovary cells culture producing a recombinant monoclonal antibody. These components have been identified to play a role in targeted metabolic pathways in literature. A scale down model of the large scale fed batch process has been used for the production step in AmbrTM microbioreactors. The product quality assessment was performed on purified day 14 samples.

Results and discussion: Differences in process performances were observed during the production supplemented by the tested components in bioreactors. Indeed, the addition of some components assessed in this study improved cell growth. For example, the addition of one component enabled to reach a 20% higher maximum VCD than the control condition. However for the two selected components a 20% reduction of product titer during the production phase was observed. On the other hand, a 10% reduction of product related variants was observed. This study suggested the targeted metabolic pathways could have an impact on the recombinant protein product quality attributes.

Bibliography, Acknowledgements: Gramer, M. J., et al. (2011); Kildegaard, H. F., et al. (2016); Jefferis, R. (2016); Hossler, P., et al. (2015); Vijayasankaran, N., et al. (2013).

Disclosure of Interest: None declared





PO181

CONTROL OF ANTIBODY'S GALACTOSYLATION: APPLICATION OF A HIGH-THROUGHPUT PLATFORM

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Background and novelty: The glycan profile of a therapeutic protein is known to have a significant effect on its activity, immunogenicity and half-life. Through process optimization, to gain in productivity and facility of transfer, one can sometime assist in an unwanted drift of the monoclonal antibody (mAb) glycan profile. Many publications have documented the effect of cell culture media on this profile. To gain in glycan profile's understanding of one of our biotherapeutics, we have tested the effects of several chemicals using a high-throughput (HT) platform coupled to Design of Experiment (DOE).

Experimental approach: We have implemented an HT platform constituted of a 96-deepwell plate (96-DWP) cultivation system allowing for fed-batch cell culture, followed by purification of supernatant performed with AssayMAP cartridges and N-glycan analysis with fast-HILIC. Previous experiments allowed us to confirm the equivalency between 96-DWP and more conventional small-scale cultivation system like shake-flasks and 5L bioreactors, in terms of culture parameters (growth, viability, mAb titer) and quality attributes of the mAb produced.

Results and discussion: Using a DOE approach, we investigated the effects of three known galactosylation metabolic effectors (uridine, manganese and galactose). For this cell line and in our experimental culture conditions we found the glycans profile of our mAb to be highly dependent on manganese level. Furthermore we showed that the timing of addition of this particular component allows for a fine-tuning of the galactosylation level of the mAb produced.

These results highlight the importance of careful media design when changing culture processes to ensure equivalent bio-product quality and gain better understanding of the process tolerance to trace elements variability.

Bibliography, Acknowledgements: Gramer et al, Biotechnol. Bioeng., 108: 1591-1602 (2011)
Grainger et al., Biotech Bioeng., 110: 2970-2983 (2013)

Disclosure of Interest: None declared





PO182

QUALITY BY DESIGN APPROACH USED IN THE CHARACTERIZATION OF A COMMERCIAL CELL CULTURE MANUFACTURING PROCESS OF TWO RECOMBINANT PROTEINS: VON WILLEBRAND FACTOR AND HUMAN FACTOR VIII

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Background and novelty: The drug substances for recombinant human antihaemophilic Factor VIII (rFVIII) and recombinant von Willebrand factor (rvWF) are produced by the same genetically engineered Chinese Hamster Ovary (CHO) cell line. rvWF stabilizes rFVIII during cell culture and is separated from the drug substance during the purification process of rFVIII.

The principles of quality by design (QbD) were applied in process characterization of rFVIII and rvWF proteins, however this was done sequentially for both molecules. When implementing the QbD approach, the rFVIII was already commercially available whereas rvWF was in clinical development phase.

Experimental approach: We share our approach in characterizing the cell culture process using QbD principles for establishing a process control strategy at manufacturing scale. Process characterization started with critical quality attributes (CQAs) identification and evaluation of potential impact of these CQAs on the patient. Then, a process risk assessment was performed to identify Critical Process Parameters (CPPs) by evaluating their impact on CQAs and Process Consistency Indicators (PCIs).

CQAs, PCIs and CPPs were monitored and controlled through the process to ensure a consistent process and product delivering the expected quality. Finally, this preliminary control strategy was validated and allowed the development of a Continued Process Verification (CPV) plan.

Results and discussion: The QbD implementation was challenging given a concomitant production of two complex molecules within one single cell culture process. The key elements of QbD including quality target product profile, CQAs, PCIs, risk assessments through process Failure Mode and Effects Analysis (pFMEA), control strategy and product lifecycle management are discussed to understand the impact of process on CQAs.

Bibliography, Acknowledgements: -

Disclosure of Interest: None declared





PO183

CREATING A SUITABLE MICROENVIRONMENT FOR GROWING HUMAN PRIMARY T CELLS TO HIGH CELL DENSITIES

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Background and novelty: Ex vivo expansion of human primary T cells is of considerable scientific and medical interest, e.g. as for T cell therapy. Currently, this requires the addition of massive amounts of stimuli. Here, we present the possibility to cultivate such cells, freshly isolated from blood, to high densities in semipermeable polyelectrolyte microcapsules within less than 10 days. Significant advantages, such as great mechanical stability, good biocompatibility and good mass transfer properties characterized these capsules.

Experimental approach: Cell growth, cytokines production and phenotype were analyzed in non-encapsulated and encapsulated cells grown under standard culture conditions. Moreover, we analyzed the interplay between the secreted cytokines and the cellulose sulfate (SCS) within the capsules and its putative influence on cell growth.

Results and discussion: Cells mixed in the cellulose sulfate solution under physiological conditions can be safely trapped within a liquid core during capsule formation. One major advantage of these polyelectrolyte capsules is the low MWCO (< 10 kDa). This restricted permeability allows for a conditioning of the capsule core by autocrine factors, which in turn permits the use of basal cell culture medium instead of expensive T cell specialized media, does not necessitate high amounts of rhIL-2 and thus reduce the cultivation costs. Gentle digestion with endocellulase allows an easy release of the cells out of the capsules. Our results suggests that the SCS, we used for encapsulation has biomimetic properties, creating an artificial extracellular matrix mimicking heparan sulfate which in turn positively affect T cell proliferation *via* trans-presentation of IL-2. We consider that the described method for T cell expansion may be an appropriate alternative to expand T cells while creating a local microenvironment mimicking *in vivo* conditions.

Bibliography, Acknowledgements: This research was supported by the DFG.

Disclosure of Interest: None declared





PO184

NOVEL BIFUNCTIONAL PEPTIDE TAG FOR THE PRODUCTION OF O-GLYCOSYLATED HUMAN BIOTHERAPEUTICS

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Background and novelty: Currently, clinical use of human recombinant therapeutic proteins entails certain limitations due to their low stability and short half-life. To overcome this, our laboratory developed a novel bifunctional peptide (GMOPm), derived from the N-terminal end of human granulocyte and macrophage-colony stimulating factor (hGM-CSF). This 15-amino acid tag has 6 potential O-glycosylation sites and includes a linear epitope recognized by a monoclonal antibody (mAb CC1H7). Our goal was to study the capacity of GMOPm to both improve the properties of chimeric therapeutic proteins and to provide an operational advantage through specific immunochemical quantification and purification techniques.

Experimental approach: Five variants were designed by adding GMOPm to the N- and/or C-terminal ends of human interferon- α 2b (hIFN- α 2b) in different proportions. CHO-K1-producing cell lines were generated, molecules were produced and analyzed carrying out immunochemical techniques based on the mAb CC1H7-GMOPm interaction.

Results and discussion: GMOPm/IFN chimeras presented higher molecular masses (31-60 kDa) than the CHO-K1-derived wild type hIFN- α 2b (21,5 kDa), and retained both in vitro antiviral (20-100%) and antiproliferative (2-47%) bioactivity, relative to the unmodified hIFN- α 2b. An experimental design was performed to study the effect of salts and pH on the mAb CC1H7-GMOPm interaction. These results were applied to develop a competitive ELISA with an adequate sensitivity (492 ml/ng) and limit of detection (20 ng/ml) to quantify GMOPm tagged proteins. Also, optimal binding conditions (NaCl 4,5 M-pH 7) that increased 70 times the apparent binding affinity in relation to the control condition (no salt, pH 7) were identified. These findings constitute an important basis for the future optimization of purification processes of GMOPm chimeric proteins.

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Disclosure of Interest: None declared





PO185

POLY-PATHWAY MODELLING APPROACH SIMULATING MULTIPLE METABOLIC STATES BY LARGE KINETICS MODEL

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Background and novelty: Mammalian cells have a complex and flexible metabolism, with varied metabolic behaviors depending on the culture conditions. The kinetics model of process simulating and predicting these variations could be used as optimization tool for the development of media, feeds or processes. We have developed a novel approach to provide such kinetics model without limitation of the number of biochemical reactions and rendering multiple metabolic states.

Experimental approach: We have introduced the poly-pathway model approach to capture multiple metabolic states in one single model. The pathways are represented by macro-reactions, obtained via elementary flux mode (EFM) analysis of a metabolic reaction network. The model is identified using rich information obtained in varied cell culture experiments.

Results and discussion: The EFMs identification has been traditionally carried out by systematic enumeration. However this enumeration becomes prohibitive with the network complexity, limiting the number of reactions in the network. We have developed an novel approach by a new algorithm, called column generation, to identify reduced sets of macro-reactions, which are relevant to the experimental data [1]. We present here a network of ≥ 120 reactions with kinetics including saturation and inhibition effects. Using this approach, an excellent fit between simulated and experimental data is obtained, rendering the different metabolic states triggered by various stimuli. Furthermore, we applied the column generation algorithm to determine the EFMs relevant to the data in genome-scale model and provide a tool to determine the main fluxes relevant for the experimental data occurring in a biological system.

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Acknowledgements: We thank KTH for funding of Erika Hagrot and VR for funding of Hildur Æsa Oddsdóttir.

Disclosure of Interest: None declared





PO186

HIGH CAPACITY PROTEIN A- MAGNETIC BEAD FOR CELL CLARIFICATION AND ANTIBODY CAPTURE IN ONE STEP FOR MANUFACTURING PROCESS

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Background and novelty: The batch cultivation processes of antibody production are typically followed by the steps of cell clarification and capture of the product of interest by protein A chromatography in packed columns. These steps are expensive and time consuming. Magnetic bead-based separation has been used since decades but their capacity and magnetic properties have been too low for an application in biopharmaceutical processes. In this study an alternative novel high capacity magnetic bead, LOABeads, has been investigated for its potential of performing the cell clarification and antibody capture in one step. The beads have also been studied for their application in perfusion process for continuous capture operation.

Experimental approach: LOABeads were first studied for their binding properties of two model antibodies. Processes of antibody capture were then developed for a fed-batch process of antibody producing Chinese Hamster Ovary cell and for a perfusion process of these cells.

Results and discussion: We performed kinetics analysis of the antibody uptake to study the binding rate and preliminary determine the operation conditions to be applied to culture harvest. A magnetic bead based capture process of antibody in the cell broth was then developed, showing promising results at reduced cost of equipment, resource and time. The technology could also be applied to continuous harvesting of perfusion culture.

Bibliography, Acknowledgements: This work has been carried out with the financial support of VINNOVA, Sweden's innovation agency.

Disclosure of Interest: None declared





PO187

USING AMBR AS SCALE DOWN MODELS IN CELL CULTURE

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Background and novelty: Small scale systems, such as microtiter plates and miniature bioreactors (MBR) have found their way in numerous applications in the biotechnology industry. Activities cover several steps in a lifecycle of a drug. High Throughput (HT) technology is not only used for a large number of individual processes, i.e. cell line development, medium/feed/process parameter screening, but also for increasingly formal process characterization studies in a scale down model (SDM) context. Here we summarized which SDM heuristics in ambr were found useful, and which level of process characterization is required before crossing the border of screening studies and entering the realm of qualified SDMs.

Experimental approach: Literature on publicly available studies in ambr15 and ambr250 was collected and summarized for CHO cell cultures. Challenges in the selection of classical SDM criteria in ambr were identified, and new SDM criteria as well as novel tools to characterise both scales were discussed. Formal requirements and expectations for qualified SDM were studied and the state of the art including data handling was summarized.

Results and discussion: Novel SDM heuristics of computational nature (i.e. CFD, kLa, gradients and stress), together with wet-lab characterisation studies (i.e. pCO₂, kLa and stress) may be more meaningful in MBRs. Once HT data sets are available, uni- and multivariate data analysis is required to support certain claims that were made in the SDM. In conclusion, establishing MBRs as SDM required much more attention and additional work than simply using them as screening tools. Classical SDM criteria did not always work well for such systems, but recent efforts showed promising results to qualify ambr as SDM in the near future.

Bibliography, Acknowledgements: This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Curie Skłodowska-Curie grant agreement No 643056.

Disclosure of Interest: None declared





PO188

INVESTIGATION OF PROTEIN RETENTION IN FILTER BASED HIGH DENSITY PERFUSION PROCESS

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Background and novelty: Membrane based perfusion devices are the most widely used technology for performing high cell density (HD) perfusion processes with efficient cell retention. However, one potential complication encountered with filter technology is protein retention. It has been reported that XCell™ Alternating Tangential Flow (ATF) demonstrates less protein retention compared to TFF-based perfusion system, due to XCell ATF's uninterrupted pressure and exhaust cycles creating a continuous self-cleaning (backflush) through filter pores (Karst, *et al* 2016; Clincke, *et al* 2013). The continuous backflush in XCell ATF is assumed to mitigate rate of filter fouling followed by protein retention.

Experimental approach: Several parameters, such as antifoam, cell debris, media components and protein related issues, alone or in combination, could lead to protein retention in HD perfusion culture. Despite protein retention is frequently observed in most perfusion processes, the potential root cause might be different from one process to another. We investigated the root cause of protein retention and provide troubleshooting guidance. A number of CHO perfusion bioreactor and shake flask experiments were performed to determine the impacts of antifoam, cell lysis and cell debris on protein retention. Particle size analysis was also conducted on perfusion culture samples to quantify the concentration and size distribution of debris.

Results and discussion: The preliminary results suggest that cell debris might be a potential root cause for protein retention, while Antifoam C had no major impact. Further, reducing cell debris in perfusion culture also decreased protein retention. To gather further evidence, several CHO perfusion cultures are being investigated using various filter pore sizes and particle size distribution, and results will be discussed.

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Disclosure of Interest: None declared





PO189

ACCELERATING TIMELINE TO IND BY USING POOL FOR TOX STRATEGY

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Background and novelty: Speed to IND submission is essential in establishing a competitive advantage for a therapeutic product. The IND-enabling Toxicology (Tox) studies and the associated CMC activities to provide material are often the rate limiting steps to IND filing. We have demonstrated that a “Pool for Tox” strategy, where a pool of clones is used instead of a single clone to generate material for Tox studies, can accelerate timelines to IND submission by months.

Experimental approach: The Pool for Tox strategy pools clones during cell line development prior to final clone selection to enable earlier generation of Tox material. The number of clones to pool can range from many (e.g. >60) to a few (e.g. 4) depending on the speed of the program. The success of the strategy relies on the ability to generate Phase I material using a single clone with comparable product quality to the Tox material. The importance of individual product quality attributes may be project-specific. For instance, high comparability of afucosylation levels would be critical for an IgG1 with antibody dependent cellular cytotoxicity as the primary mechanism of action. To maintain the acceleration through IND submission it is essential to reduce the development timeline to Phase I with cross-functional streamlining and/or accepting the risk of delaying certain activities.

Results and discussion: We have successfully implemented the Pool for Tox strategy for multiple projects and achieved the targeted acceleration to IND submission. Each project had its own challenges, such as balancing titer advantage with product quality advantage in selecting the lead clone, or taking risks in locking processes with preliminary data for timeline gain. In this presentation, we will describe the application of the Pool for Tox strategy, considerations for clone selection, approaches to shortening the Phase I development timeline, and risks associated with the strategy.

Bibliography, Acknowledgements: Amy Shen, Mike Laird, Masaru Shiratori.

Disclosure of Interest: None declared





PO190

THE NEW AGE OF DIGITAL BIOMANUFACTURING

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Background and novelty: Digital manufacturing heightens the productivity and robustness of existing processes while enabling the development of previously unmanageable products or processes. More than distributed control systems and supervisory control and data acquisition, it is the interconnection of divergent sources of information with deep real-time analysis and comprehensive application.

Experimental approach: In Digital Biomanufacturing (DB), living components add new dimensions to the systems employed. DB addresses the complexity of biological systems, distributed heterogeneous data, and limited at-line or on-line data sources. The costs of omics data generation are decreasing rapidly, while process monitoring and analytical technologies, computational power, predictive modeling and data management infrastructures are greatly improving. These are removing roadblocks that used to limit transformation of the bioeconomy into an industry based on digital knowledge and connectivity. The application of predictive models for bioprocess optimization greatly improves established platforms and ultimately leads to a massively increased mechanistic process understanding.

Results and discussion: Five essential benefits result from the increased bioprocess understanding, development and control of DB: (i) personnel are relieved of many repetitive tasks (ii) strategic planning and operational efficiency are improved, (iii) real-time optimization of end-to-end manufacturing can be based on such high-value criteria as projected product quality and profitability, (iv) previously unmanageable operations are enabled and innovative solutions created, and (v) comprehensive analysis can now extend beyond operations performance data from production to data driving such activities as raw materials security of supply and business continuity management systems.

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Disclosure of Interest: None declared





PO191

SOFT SENSORS: NEW INNOVATIVE APPROACH FOR PROCESS MONITORING OF CELL GROWTH IN SMALL SCALE FERMENTATION SYSTEMS

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Background and novelty: Process development and in particular the use of high throughput systems required sampling for controlling. One of the most important parameter is the cell growth, but sampling, sample dilution and analyzing is time consuming and generates high efforts in the case of high throughput fermentation systems. Sampling allows also only a look in the culture status at a certain time point, the information between two sample points is missing. Therefore we develop a new softsensor, which takes online signals of the bioreactor, which are correlated to cell growth to estimate the cell growth.

Experimental approach: The new approach based on multiple linear regression and on artificial neural network processed the common online signals of the bioreactors to estimate the cell growth as online signal during cultivation time.

Results and discussion: The cell growth estimated by soft sensor was successful implemented in the multiple small scale bioreactor system and resulted estimated values with high confidence and low root mean squared error below 15 %. The new approach can be integrated in process modelling.

Bibliography, Acknowledgements: Special thanks Bernhard Keil and Daniel Christofori for working on the soft sensor development.

Disclosure of Interest: None declared





PO192

MONITORING BETWEEN-BATCH BEHAVIOR OF REAL-TIME ADJUSTED CELL-CULTURE PARAMETERS

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Background and novelty: Some cell-culture parameters (CCP), are continuously measured online and may be subject to automated real-time adjustment. This is an efficient method to maintain the parameter within limits. However, it only offers a within-batch control, and does not account for between-batch variability. We propose a strategy to monitor the between-batch culture behavior, providing process knowledge and understanding. The strategy offers the possibility of termination of a batch in case of unexpected behavior.

Experimental approach: Typically, online measurements provide time-dependent curves presenting two types of transitions:

From a state in which the real-time adjustment is needed to one in which it is not, and the other way around.

In this work, the timepoints at which those transitions take place are called changepoints. The positions of those reflect aspects of the cell-culture process. They should be submitted to statistical control.

The proposed methodology is basically a control-chart-like method to monitor the position of the changepoints. This method requires the identification of the changepoints and the definition of control limits.

Results and discussion: Identification of the changepoints can be model-based or based on some predefined rules. The model based approach is more objective, but relies on one's ability to fit the model and requires the complete curve. The rule-based identification is easier to use and does not require the complete curve.

A multi-response model, is adjusted on the identified changepoints. From this model, 95% and 99% prediction regions are obtained and should be used as warning and control limits.

This control methodology is simple and offers the opportunity to terminate a culture in case of unexpectedly early or late first changepoint.

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Disclosure of Interest: None declared





PO192-a

OPTIMIZING THE AMINO ACID METABOLISM OF CHINESE HAMSTER OVARY CELLS TOWARDS ENHANCED FED-BATCH PROCESS PERFORMANCE

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Background: Chinese hamster ovary (CHO) cells are the current 'work-horses' for recombinant therapeutic protein production. In fed-batch cultures, CHO cells consume large amounts of nutrients and divert significant fraction of them towards inhibitory byproduct formation such as lactate and ammonia. Lactate accumulation can be controlled by limiting the supply of glucose using the HIPDOG (Hi-end pH controlled delivery of glucose) strategy, and ammonia accumulation can be controlled through use of the glutamine-synthetase expression system. Even under such optimized conditions of reduced lactate and ammonia accumulation while in the presence of sufficient nutrients, cell growth slows and ceases, the reasons for which are currently unknown.

Experimental Approach: Omics methodologies were employed to the above mentioned optimized conditions which led to the identification of a number of compounds that accumulated in cell culture. Using add-back experiments, the compounds (or putative inhibitors) that had negative effect on growth were identified. The carbon sources for these novel inhibitors were subsequently ascertained. Cell culture processes that limit the supply these carbon sources, in addition to employing HIPDOG strategy, were developed to limit the production of these inhibitors.

Results and Discussion: A sizable fraction of these growth inhibitory compounds were either byproducts or intermediates of amino acid catabolism pathways. By controlling levels of amino acids in a low concentration range, the production of these inhibitors was significantly reduced. The reduction in inhibitor production led to higher peak cell densities and titers. Details of the novel inhibitors, their nutrient source and their effect on cell physiology, along with other strategies employed to control inhibitor levels will be presented.





PO193

TOWARDS A PREDICTIVE MANUFACTURABILITY ASSESSMENT PLATFORM FOR THERAPEUTIC KL BODIES

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Background and novelty: Bispecific antibodies (BsAb) represent a powerful approach for dual targeting strategies. NovImmune's solution for generating a fully human bispecific IgG is the kappa-lambda ($\kappa\lambda$) body(1). Having an ability to predict manufacturability of $\kappa\lambda$ bodies early in bioprocess development is an important component of lead candidate selection.

Experimental approach: In order to effectively evaluate robust growth and high productivity properties of the multiple $\kappa\lambda$ body expressing CHO cell pools, a small scale model for fed-batch evaluation of BsAb candidates as well as a rapid $\kappa\lambda$ body purification method have been established. A new cell sorting based strategy for generating rapidly good $\kappa\lambda$ body producer pools was also investigated.

Results and discussion: A small scale approach for fed-batch evaluation of BsAb candidates was assessed to manage multiple pools per candidate. We identified the appropriate system that would be predictive of larger scale manufacturing. We also developed a rapid $\kappa\lambda$ body purification method allowing us to quickly generate sufficient amounts of $\kappa\lambda$ bodies from a large panel (e.g., 40) of candidates to identify those with good quality attributes for biophysical characterization and testing in biological activity assays. The outcome was the development of a new cell sorting based strategy that provided good quality material in order to rapidly conduct experiments and identify the candidate(s) to progress. This method allows the efficient, cost effective and timely production of multiple $\kappa\lambda$ body candidates (in 8-10 weeks versus 12 weeks for the classical method).

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Disclosure of Interest: None declared





PO193-a

ENHANCING AUTOMATED SAMPLING, PROCESS MONITORING, AND NUTRIENT FEEDBACK CONTROL FOR A SYSTEM OF 3-L BIOREACTORS

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Background and novelty: Key to effective upstream development is the presence of resource-efficient systems for examining complex process options. In this presentation, we report on the progress of a completely automated sampling and feed control system, capable of simultaneously evaluating different metabolic-based feed strategies for bench scale cell culture processes. Though there are automated feed systems available for micro-scale vessels, to date there is not a complete 3-L scale sample and feed automation solution as described here, with capability to transfer samples from multiple bioreactors (Single-Use or Glass) to a suite of independent analyzers with concurrent feedback control of nutrient feed pumps. There are multiple drivers for developing automated systems for benchtop bioreactors, ranging from increased operational efficiency to enhancing the toolbox for process development. Deeper process understanding, obtained through examining metabolic-tailored feeding strategies, is essential to the development of state-of-the art upstream processes with emphasis on product quality attributes.

Experimental approach: The automated on-line sampling and feed system is capable of feedback control for a variety complex feed strategies, that incorporate data inputs from integrated third party analyzers. Feed control inputs can be cell density and/or up to four measured residual metabolites. The completely automated system has supported experiments from two to eight 3-L bioreactors, with sampling frequencies ranging from 4 to 24 hours per tank, depending upon experimental needs.

Results and discussion: In a recent typical experiment, eight tanks were sampled three times per day with feedback control for multiple separate nutrient feed solutions per tank, based upon independent metabolite triggers. Over the course of the run, each vessel was triggered feeds 35-40 times with feed volumes ranging from 2-40 mL, for over ~300 automated feeds across the eight vessels. Refined data extraction templates streamlined data imports into the electronic notebook system and PI integration can enable remote monitoring. The enhanced system capabilities are being increasingly leveraged to support ongoing process development efforts.





PO194

DEVELOPMENT OF SUSPENSION MDCK CELLS CULTURED IN CHEMICALLY-DEFINED MEDIUM FOR INFLUENZA VIRUS PRODUCTION

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Background and novelty: In response to rapid antigenic drift in influenza viruses, vaccination is considered as the most effective intervention. Traditionally these vaccines are produced in embryonated chicken eggs. However, in the case of a pandemic outbreak, this egg-based production system may not be quickly enough to meet the surging demand. MDCK cells are widely considered as an alternative host to embryonated eggs for influenza virus propagation.

Experimental approach: In this study, a previously characterized adherent MDCK (aMDCK) cell line (Bioreliance, U.K.), were cultured and adapted in a chemically-defined (CD) media prototype through two-step processes. Experiments were conducted to evaluate the adapted cell-growth kinetics and its productivity of culturing avian influenza viruses.

Results and discussion: The cell concentration of the suspension MDCK (sMDCK) cells up to 2×10^6 cells/ml after 96 hrs was obtained, and the doubling time of 30-35 hrs was found very similar to the aMDCK cells cultivated on microcarriers (5g/L). The H7N9 candidate vaccine virus (NIBRG-268 which was derived from A/Anhui/1/2013) was used and infected both sMDCK and aMDCK cells with a low multiplicity of infection. The harvest of viruses was collected on day three post infection. The HA titers in sMDCK and aMDCK cells were reached to 996 and 574 HA units/₅₀ul, respectively. The HA titer of sMDCK cultured in the serum-free medium increased nearly 74% compared to the aMDCK cell culture.

In summary, a new adapted sMDCK cell line was developed. This new combined technology of sMDCK with specifically-optimized CD medium provides a new solution to the bottlenecks for establishing a large-scale cell culture using adherent MDCK cells.

Bibliography, Acknowledgements: The authors would like to thank Dr. Jessie T-H Ni and Ms Jenny Bang for technical consultation. The authors also thank the funding support from Irvine Scientific (02D4-1IV10) and NHRI (IV-105-PP-25).

Disclosure of Interest: None declared





PO195

PROCESS INTENSIFICATION OF YELLOW FEVER VIRUS AND ZIKA VIRUS PRODUCTION IN PERFUSION BIOREACTORS

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Background and novelty: Increased cases of yellow fever virus (YFV) and Brazilian Zika virus (ZIKV) infections in new habitats left no doubt about emergence and spread of mosquito-borne flaviviruses. The most effective countermeasure against this global threat is controlled vaccination. However, as recently faced by depleted YFV vaccine stockpiles in Angola, current egg-based production processes are rarely capable to promptly meet increasing demands. Hence we present a cell culture-based YFV-17D vaccine and Brazilian ZIKV production process using suspension BHK-21_{SUS} cells.

Experimental approach: BHK-21 cells were adapted to suspension and SF or CD media. Infection studies with YFV-17D and four different Brazilian ZIKV isolates were conducted in static T-flasks and shaken flasks. Process knowledge was transferred towards higher cell density cultivations in bioreactors with cell retention devices.

Results and discussion: Scouting experiments of BHK-21_{SUS} revealed cell-specific YFV yields in the range of 20 PFU/cell, similar to adherent Vero cells (WHO lineage). To increase overall virus yields, a tangential flow filtration (TFF) perfusion system was established which allowed to achieve cell concentrations of 2.8×10^7 cells/mL and YFV titers up to 1.6×10^8 PFU/mL. This corresponds to about 2 Mio YFV vaccine doses from a 0.7 L working volume within 10 days. In addition, the use of BHK-21_{SUS} cells was evaluated for Brazilian ZIKV production. RT-qPCR studies revealed high viral RNA copy numbers within the cell, but poor virus release. Cultivations in a single-use bioreactor with alternating tangential flow (ATF) perfusion, enabled to achieve reasonable ZIKV^{PE} yields of up to 3.6×10^7 PFU/mL. While a better understanding of intracellular virus replication and virion release is crucial for further process optimization, this study clearly demonstrated that cell culture-derived production of inactivated or live-attenuated ZIKV vaccines is feasible.

Bibliography, Acknowledgements: -

Disclosure of Interest: None declared





PO196

“THE ALPHAVIRUS STRUCTURAL PROTEIN EXPRESSING USING PICHIA PASTORIS AND BACULOVIRUS”

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Background and novelty: The *Mayaro virus* (MAYV) is an *Alphavirus*, causing a disease characterized by fever, headache, vomiting and diarrhea. The mosquito of genus *Haemagogus* is responsible for the transmission and it is a characteristic mosquito of isolated regions. Recent studies have shown that *Aedes aegypti* is also a competent vector of this virus, enabling the urbanization of MAYV fever. This work aims to expression of VLP (*Virus Like Particles*) of *Alphavirus*, using two different expression systems, one based on the yeast *Pichia pastoris*, and another in baculovirus (BEVS). The VLPs will be characterized and evaluated for the immunogenic potential.

Experimental approach: Construction of a recombinant baculovirus containing the corresponding genes for structural proteins of the virus Mayaro, through the *Bac-to-Bac* system (ThermoFisher). The fragment containing the structural genes *Mayaro virus* (E-MAYV) was synthesized and cloned into a commercial vector (*pFastBac*). After confirmation of the construction, the vector was used for transformation into DH10Bac bacteria (ThermoFisher), contained in the Bac-to-Bac kit in order to obtain a recombinant bacmid, capable of producing recombinant baculovirus when transfected into insect cells. The recombinant baculovirus obtained was then used to infect Sf-9 and Sf-21 cells and the production of VLP was analyzed. The recombinant *Pichia pastoris* was obtained by electroporation with pPIC9K plasmid, contained the structural MAYV genes. These yeast were grown in shaker at 30° C and induced with methanol, the expression of VLPs analyzed by *Western blot*.

Results and discussion: *Western blot* results indicated expression of virus structural proteins by both systems, where it was possible to view the E2 and C protein on their expected size.

Bibliography, Acknowledgements: Supported by FAPESP and CNPQ.

Disclosure of Interest: None declared





PO197

PROCESS DEVELOPMENT OF INFLUENZA GAG VIRUS-LIKE PARTICLES PRODUCTION IN HEK-293 SUSPENSION CULTURE

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Background and novelty: The immunogenic efficiency of virus-like particles (VLP) as an influenza vaccine candidate has been demonstrated in several studies [1]. Different expression systems have been explored for VLP production including: insect, plant, and mammalian cells. Mammalian cell platform possess the capability to perform superior post-translational modifications and the high cell densities achieved in bioreactor. However, the upstream and downstream process of mammalian cells produced VLP has been poorly addressed in the literature. Greater understanding of the production process could increase the productivity and make large-scale VLP vaccine manufacturing feasible.

Experimental approach: In this study, a suspension culture adapted human embryonic kidney HEK-293 cell line stably expressing three proteins, hemagglutinin, neuraminidase and HIV-1 Gag protein, was generated. The Gag protein was fused to the green fluorescent (f) protein to monitor the production by flow cytometry. The cell line was process optimized, in order to reach higher volumetric productivity, by increasing cell density at the time of induction.

Results and discussion: A fed-batch strategy in SFM4-TransFx medium and a batch culture in HyCell-TransFx-H increased by 5-fold the VLP production. Furthermore, a 3-L bioreactor in perfusion mode mirrored the performance of the cells under media replacement at small scale increasing the volumetric productivity 60-fold higher (1.54×10^{10} f.events/ml) than the control (2.58×10^8 f.events/ml). The 9.5L harvest from the perfusion bioreactor was semi-purified by tangential flow filtration at low shear forces. The electron micrographs of the final retentate revealed the presence of VLPs of 100-150nm with the characteristic dense core of HIV-1 particles. This work contributes much needed technical knowledge to the bioprocess development of VLPs produced from mammalian cells.

Bibliography, Acknowledgements: 1-Venereo-Sanchez et al. Vaccine. 2016;34:3371-80

Disclosure of Interest: None declared





PO198

VIRUS PRODUCTION IN SINGLE-USE BIOREACTOR SYSTEMS USING PRE-STERILIZED MICROCARRIERS

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Background and novelty: Since the 80's, microcarriers have been used to provide growth support for adherent cells in vaccine biomanufacturing processes at scales up to 6000 L. Today, as yields are increasing, most newly developed vaccines are manufactured at smaller scales, making single-use technologies suitable. Because many anchorage-dependent cell lines grown on microcarriers are sensitive to shear stress, some of the remaining challenges are the need for homogenous well-suspended microcarriers and efficient oxygenation of the medium. These prerequisites can limit operating conditions and volumes of the bioreactor process, by operating parameters are selected carefully, good cell growth and virus yields can be achieved in a single-use bioreactor system.

Experimental approach: In this study, we describe different approaches for the serum-free cultivation of Vero cells to high cell densities using pre-sterilized microcarriers. Vero cells were grown in single-use bioreactor system, and then infected with influenza virus during exponential growth phase. The concentration of infectious virus was determined by 50% tissue culture infective dose measurement (TCID₅₀).

Results and discussion: Viral titers of 10⁷ or higher were observed in all cultures. Comparable results in cell growth and cell concentrations were observed when using microcarriers in a single-use bioreactor system [ReadyToProcess WAVE 25 or Xcellerex XDR-10 single-use bioreactor system.] We show that by carefully selecting operating parameters, good cell growth and virus yields can be achieved in a single-use bioreactor system. The time-consuming process of preparation and sterilization of the microcarriers is reduced significantly by using pre-sterilized microcarriers. Our results offer valuable information to facilitate novel process development of single-use bioreactor systems and pre-sterilized microcarriers [Cytodex Gamma], enabling simplified vaccine productions at smaller scales.

Bibliography, Acknowledgements: NA

Disclosure of Interest: None declared





PO199

EFFECT OF INSULIN ON INFLUENZA PRODUCTION IN HEK293 CELLS

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Background and novelty: Mammalian cells are considered an alternative to eggs for the production of influenza vaccines. HEK293SF-3F6 is a suspension GMP cell line that grows in serum-free media, and influenza production has been achieved in flasks and large bioreactors. In order to boost influenza production, insulin was added to the cultures. This growth factor was chosen because it has cell survival effects, it acts on cellular signaling pathways that are exploited by the influenza virus and it is approved by regulatory agencies.

Experimental approach: HEK293SF-3F6 cells were infected with H1N1/A/Puerto Rico/08/34 or H3N2/A/Aichi/8/68 in a 24-well microbioreactor cassette. Next, 5 to 100mg/L insulin was added. After 48hrs, supernatants were collected and hemagglutinin (HA) was quantified. HA is highly expressed at the surface of the virus, and it was quantified by dot blot using a pan-HA antibody developed in-house.

Results and discussion: The HA concentration was increased by almost 2-fold with insulin: Depending on the media, the effective insulin concentration varied; with H1N1/A/Puerto Rico/08/34, 25 to 100mg/L insulin increased the yield in CD293 media, whereas concentrations between 5 and 25mg/L resulted in a similar increase in an in-house media (IHM-03). H3N2/A/Aichi/8/68 virus yield was increased with 25 mg/L insulin in CD293 media. Overall, a concentration of 25mg/L insulin provided an increase in influenza yield regardless of the media or viral strain used in HEK293SF-3F6 cells. A concomitant activation of signaling pathways associated with cell survival (PI3K-Akt pathway) was observed. In conclusion, insulin is known to stimulate cell proliferation. Here we show that adding 25mg/L insulin is also an effective way of increasing influenza production and can easily be implemented in a vaccine bioprocess. Evaluation of the effect of insulin on the production of other viruses and viral vectors is in progress.

Bibliography, Acknowledgements: .

Disclosure of Interest: None declared





PO200

DEVELOPMENT OF VIRTUAL EXPERIMENTATION OF MAMMALIAN CELLS PROCESS IN BIOREACTORS

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Background and novelty: The scope of this work is the simulation and optimization of biological processes through modeling tools and simulation of integrated and complex systems.

Experimental approach: The development of a modeling and simulation software allows to:

- Reproduce experimental conditions and run virtual tests of mammalian cells process in bioreactors;
- Limit time-consuming and expensive experiments and secure the design of products and processes;
- Increase the level of knowledge of our products and processes;
- Provide the teams with the tools and methods to extract all the knowledge from the data gathered from experimental data and bibliography.

Results and discussion: The interest of modeling tools for up-stream process development has been demonstrated. This work is a collaborative project between R&D and engineering process teams, connecting 3 fields: mathematics, data processing, and biology. The first simulations obtained were successful and allowed to reproduce the observations made through laboratory experimentations such as cell growth, metabolic consumptions and virus production. This project has allowed better product knowledge and can be used for process development enhancement.

Bibliography, Acknowledgements: Within follow up work, software will be applied to new cell lines and processes, supplemented with additional libraries. At middle or long-term perspective, tool will be deployed consistently in development work plan.

Source of funding: Sanofi Pasteur

Disclosure of Interest: None declared





PO201

EVALUATION OF THE XPANSION BIOREACTOR SYSTEM FOR CELL & VIRAL CULTURE

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Background and novelty: Merial is since January 1st, 2017 a Boehringer Ingelheim, with this merger we are the 2nd animal health company in the world. It endeavors to answer the market needs while reducing its Costs of Goods to stay competitive.

Experimental approach: To answer these challenges, Merial is taking a strong interest in new single use technologies. One of them is Xpansion® technology that could represent a good alternative for roller bottles & Cell factory for the production of cell banking and vaccine.

Xpansion® have many advantages:

- Closed systems = less manipulations than roller bottles, no open phases, fewer needs in area classification, possibilities of multiple production in the same lab, reduce risks of contaminations
- Culture parameters monitoring = better control of the process parameters (pH, D.O., temperature...) during process screening & optimization.
- Increase of the capacities = produce the equivalent of thousands of roller bottles in less space and time (1XP-200 equal 144RB 850cm² or 21 CF-10).
- Xpansion® allow better ergonomoy for operators.

We evaluated the Xpansion® by PALL Life Science for :

- cell growth using primary cell chicken embryo fibroblast and canine cell line.
- viral production

Results and discussion: Different tests have been performed at laboratory scale with various modalities to assess the possibility to adapt :

- one of our viral production on canine cell line, we achieved comparable performance for both technologies (Xpansion® & roller bottle) without any optimization.
- one of our current viral production on CEF production process to this new technology and to identify the key parameters for an industrial application. We faced some challenges with cell trypsinization & harvest.

Bibliography, Acknowledgements: PALL/MERIAL

Disclosure of Interest: None declared





PO202

DEVELOPMENT OF HIGH CELL DENSITY HEK293 FED-BATCH PROCESS FOR HIGH YIELD PRODUCTION OF ADENOVIRUSES

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Background and novelty: Fed-batch culture represents the most attractive approach to increase process productivity and reduce the manufacturing cost of cell culture-based viral vaccines. Presently, max volumetric virus productivity is generally achieved in cultures infected at cell densities not higher than 2×10^6 cells/mL. Reductions in virus productivity have been reported frequently in high cell density cultivations due to decreased cell-specific virus yields. Therefore, improving virus productivity through infecting at high cell densities still remains a challenging task.

Experimental approach: Three commercial serum-free media and one in-house developed medium (IHM03) were evaluated for supporting the growth of HEK293SF-3F6 cells and production of adenovirus. Commercial SFM4Transfx-293 and IHM03 were selected for the development of high cell density fed-batch processes for high yield production of adenovirus. The processes were thereafter evaluated in 3L bioreactor to assess scalability of cell growth and adenovirus production.

Results and discussion: The max cell density of batch cultures using the four media varied from 3.0 to 5.8×10^6 cells/mL. The virus productivity ranged from 4×10^8 to 5.5×10^9 total viral particles per mL (TVP/mL) when the cultures were infected at a cell density of 1×10^6 cells/mL.

A max cell density of 18×10^6 cells/mL was achieved in both fed-batch cultures using commercial SFM4Transfx-293/feed and in-house prepared medium/feed, respectively. The virus productivity was dramatically improved with increasing cell density at infection using IHM03/feed, reaching a titer of 40×10^9 TVP/mL in cultures infected at 4.5×10^6 cells/mL.

Significant differences in cell growth and nutrient consumption were observed between shake flask and bioreactor cultures. Interestingly, the max cell density and virus productivity were much lower in fed-batch bioreactor cultures. More research is underway to explain these differences.

Bibliography, Acknowledgements: None

Disclosure of Interest: None declared





PO203

A MODULAR STRATEGY FOR MULTI-HA INFLUENZA VLPs PRODUCTION: COMBINING STABLE AND BACULOVIRUS-MEDIATED EXPRESSION IN INSECT CELLS

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Background and novelty: Safer and broadly protective vaccines are needed to cope with the continuous evolution of Influenza virus and promising strategies based on the expression of multiple hemagglutinins (HA) in a virus-like particle (VLP) have been proposed. However, expression of multiple genes in the same vector can lead to its instability due to tandem repetition of similar sequences. By combining stable with transient expression systems we can rationally distribute the number of genes *per* platform reducing such risk. In this work, we designed a modular system comprising stable and baculovirus-mediated expression in insect cells for production of multi-HA influenza VLPs.

Experimental approach: A stable High Five cell pool expressing two different HA proteins from H3 subtype was established. Pentavalent VLPs were expressed by infecting this cell pool with a baculovirus vector encoding M1 plus three other H3 HA proteins. Rational bioprocess optimization included the design of refeed strategies and manipulation of cell concentration at infection (CCI).

Results and discussion: Differential HA expression in non-infected and infected cultures was assessed by Western blot and hemagglutination assay, and VLPs formation demonstrated by electron microscopy. Aiming at increasing productivity, the stable cell population was infected at increasingly higher CCI though it was observed that HA titers *per* cell decreased with increasing CCI. Therefore, a tailor-made refeed strategy was designed based on the exhaustion of key nutrients during cell growth leading to an increase of up to 4-fold in volumetric productivity. Scalability was successfully demonstrated in 2L stirred tank bioreactors. This work validates the suitability of the modular strategy herein proposed to overcome specific downsides of the baculovirus-insect cell system.

Bibliography, Acknowledgements: This work was funded by EU FP7/2007-2013/ under REA grant agreement n° [602640].

Disclosure of Interest: None declared





PO204

OPTIMIZATION OF THE PRODUCTION PROCESS FOR A VLP-BASED RABIES VACCINE

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Background and novelty: Our group had previously developed an effective VLP-based rabies vaccine candidate produced in high density HEK293 cells with serum free medium (SFM)^{1,2}. One of the aims in vaccine production process is the achievement of a good productivity with a low cost per dose, mainly in the case of vaccines for animal use in which case the SFM is one of the principal expenses. In this work, we showed the adaptation of our producer clone to a home-made culture medium, as well as the evaluation of different adjuvants in the formulation of the RV-VLPs, in order to increase the productivity and reduce the global cost of the process.

Experimental approach: First, we compared a direct and a sequential adaptation protocol from 100% of the commercial SFM to a new formulation with only 50% of the SFM and a minimum essential medium. The specific productivity of RV-VLPs was measured by ELISA. On the other hand, in mice experiments we evaluated three different formulations of saponine-based adjuvants that were compared with alum and with RV-VLPs without adjuvant. The triggered immune response was evaluated measuring the total and neutralizing antibody titers.

Results and discussion: After the adaptation process, we obtained suspension cultures without aggregates or clumps and with the same specific growth rate, but reaching a lower maximum cell density. The specific RV-VLPs productivity per cell was maintained in the new medium formulation, either for sequential or direct adaptation.

Further, the antibody titers obtained with the saponine-based adjuvants were significantly higher than the obtained without adjuvant and appreciably superior than the one achieved with alum. Thus, the results obtained represent an interesting advance in the optimization of this vaccine production process.

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Disclosure of Interest: None declared





PO205

NOVEL AVIAN DUCKCELT-T17 CELL LINE FOR PRODUCTION OF VIRAL VACCINES : APPLICATION TO INFLUENZA VIRUSES PRODUCTION

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Background and novelty: Influenza virus causing severe epidemics in human and animals is an important target for the vaccine manufacturing sector. Manufacturers are still looking for production processes to replace/supplement embryonated egg-based process and to provide efficient response to such threats. Cell-based production, with a focus on avian cell lines, is one of the promising solutions. Three avian cell lines (EB66@cells , AGE.CR@ cells & QOR/2E11 cells), are now competing with traditional mammalian cell lines used for influenza vaccine productions (Vero and MDCK cells).

Experimental approach: The DuckCelt™-T17 (T17) cells presented here are a novel avian cell line developed by Transgene SA [1]. The Transgene proprietary immortalization technology consisting in constitutively expressing the duck telomerase reverse transcriptase (dTERT) in primary embryo duck cells from SPF eggs was used to generate the cell line. T17 cells were able to grow in suspension at high cell density (7×10^6 cell/ml) in serum-free conditions.

Results and discussion: Various influenza strains from different origins-human, avian and porcine- were produced on T17 cell line with titres higher than 5.8 log TCID₅₀/ml. H1N1 human strains and H5N2 and H7N1 avian strains were the most efficiently produced with titres up to 8 log TCID₅₀/ml. Porcine strains were also greatly rescued with titres of 4 to 7 log TCID₅₀/ml depending of the subtypes. Interestingly, maximal titres were obtained at 24h post-infection, allowing early harvest time. Process optimization on H1N1 2009 Human Pandemic strain allowed to identify best operating conditions for production (MOI, trypsin concentration, medium and density at infection) allowing to improve the production level by 2 log.

Bibliography, Acknowledgements: 1. Balloul Jean-Marc, Duck cell line dedicated to the production of virus-based vaccines and therapeutic products BioProduction Optimization Workshop, Sept 22 & 23-2010 Frankfurt Germany

Disclosure of Interest: None declared





PO206

PRODUCTION OF INFLUENZA VIRUS-LIKE PARTICLES USING MAMMALIAN (HEK293) AND INSECT (SF9) CELL PLATFORMS

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Background and novelty: Several Virus Like Particle (VLP)-based vaccines are now commercialized and few influenza-VLP are presently under clinical trials as vaccine candidate. But, production and purification process optimization is still needed to bring influenza-VLP up to a valid alternative to egg-based processes and development of specific VLP characterization and quantification methods is still strongly needed.

Experimental approach: In the present study, two cell platforms were compared, Sf-9 insect and HEK293 mammalian cells. The influenza proteins chosen for VLP structure were the two antigens HA and NA and the matrix protein M. Influenza proteins were expressed in Sf9 cells thank to infection with recombinant baculovirus carrying the influenza genes. In mammalian cells, two gene transfer protocols were tested; plasmids transfection or baculovirus BacMam transduction. Production protocols were optimized according to the specific parameters for each cell line and processes (influenza proteins, DNA:cell and DNA:transfectant ratios, MOIs, harvest time, cell density). In parallel, VLP characterization protocols were developed in order to rapidly screen our various production and purification conditions.

Results and discussion: For each infection/transfection/transduction conditions tested, HA and NA protein expression was validated on cell pellets, and influenza-like particles of about 100nm were detected by EM. Our newly developed HPLC detection method and TRPS analysis also allowed to rapid screen of the different VLP production lot and to select best production and purification conditions for each cell platforms allowing to compare based on VLP productivity and contaminant presence levels.

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Disclosure of Interest: None declared





PO207

GENERATION OF INFLUENZA VIRUS SEED STOCKS IN HEK-293 SUSPENSION CELL CULTURES BY REVERSE GENETICS

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Background and novelty: The influenza virus continuously undergoes antigenic evolution requiring the release of new seasonal vaccine lots. Although current egg-based production processes are well established for manufacturing seasonal vaccines, significant limitations have been underlined in the case of pandemic outbreaks. In this study, two influenza A strains (H1N1 and H3N2) were generated using Reverse Genetics (1) in suspension HEK-293 cells in serum free medium as an alternative platform to streamline the production of Candidate Viral Vaccine (CVV) for influenza vaccine manufacturing.

Experimental approach: Influenza A H3N2 was generated after transfection of 1×10^6 cells/mL suspension HEK-293 cells with 8 and 4 μg of total plasmid. The virus in the supernatant was harvested at 48 hpt and amplified in MDCK cells. Influenza A H1N1 was generated by transfecting 1×10^6 cells/mL suspension HEK-293 cells using 8 μg of total plasmid. The supernatant was harvested after 48 hpt and used to infect suspension HEK-293 cells for virus amplification. Quantification by TCID₅₀ was performed for virus quantification.

Results and discussion: Small-scale transfection for influenza A H3N2 generated 2.5×10^9 and 1.3×10^{10} IVP/mL respectively after amplification in MDCK cells when 8 and 4 μg total plasmid were used. The infectious titer of influenza A H1N1 after amplification in MDCK was 2.2×10^7 IVP/mL. To favor the scalability, suspension HEK-293 cells were used for influenza A virus amplification. The values of influenza A H1N1 ranged from 6.2×10^6 IVP/mL to 2.4×10^6 IVP/mL after one and two amplifications respectively. These results, demonstrate the efficient generation by Reverse Genetics of both influenza A H1N1 and H3N2 strains in suspension HEK-293 cells. This approach could deliver a CVV for influenza vaccine manufacturing within two-weeks.

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Disclosure of Interest: None declared





PO208

HIGH-THROUGHPUT PROCESS VACCINE DEVELOPMENT USING FULLY INTEGRATED ROBOTIC PLATFORM (AMBR & NOVAFLEX)

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Background and novelty: Boehringer Ingelheim is a world leader in animal healthcare. The world is evolving each and every day, and changing global trends affect the overall health of our animals with new emerging infectious diseases new pandemics. The challenge of the process developer is to shorten development time, and increase process knowledge to design a robust, scalable process. Merial is evaluating the opportunity to implement high throughput process with online in process control integrated for vaccine production.

Experimental approach: The new automation platform is based on the Ambr 250® (TAP Biosystem Sartorius) robot link to the Novaflex 2® (Novabiomedical). - Thanks to numerous on line and in line analysis (cell count, metabolites,...), it's a game to control a design of experiment (DoE). These results are integrated in real time and are used to manage automatically the upstream step. The process knowledge is wide to allow the scale up. The system is versatile, it is possible to run cell culture, viral culture or microbial fermentation.

Results and discussion: The challenge is the huge amount of data generated by the high throughput upstream process and the analysis. A data management and the data meaning tool are required!

Bibliography, Acknowledgements: Acknowledgements: Didier ROZE ; Chloé DAMIANY ; Laurent GIROUD ; Raphaël GONTARD ; Sylvain LAGRESLE; Christine COUPIER ; Noel DETRAZ ; Zahia HANNAS

Disclosure of Interest: None declared





PO209

ADENOVIRUS PRODUCTION IN A SINGLE USE STIRRED-TANK BIOREACTOR SYSTEM

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Background and novelty: Adenovirus vectors are attractive delivery systems for vaccines and cancer treatment. Scalable and cost-efficient production technologies are needed to enable manufacturing of safe and efficacious clinical-grade virus. In early-stage studies, anchorage-dependent cells cultured in roller bottles or cell factories are commonly used for production. However, scale-up using these techniques is complicated and limited by the surface available for cell growth. One alternative is to scale up the production on microcarriers. Another solution is to use suspension-adapted cells, which might facilitate scale-up but also could lead to lower productivity per cell as compared with adherent cells. We have established an efficient, scalable process for adenovirus production using suspension HEK 293 cells cultivated in serum-free cell culture medium in a single use stirred-tank bioreactor.

Experimental approach: Human adenovirus 5 expressing green fluorescent protein was used as a model system. HEK 293 cells adapted to different cell culture media were evaluated for cell growth and virus productivity. Based on this data a chemically defined serum-free medium was selected for further process development at bioreactor scale. HEK 293 cells were inoculated at a cell density of 0.3×10^6 cells/mL in 5–10 L scale in a single-use stirred-tank (Xcellerex™ XDR-10) bioreactor system. The cells were infected using a multiplicity of infection of 10–20 at a cell density of $0.5\text{--}1 \times 10^6$ cells/mL. Cell lysis and concentration was performed 42 h post infection. Infectious viral titer was determined by standard TCID₅₀ and a time-saving fluorescence-based technique.

Results and discussion: Our results demonstrate an efficient process for adenovirus production in a single-use stirred-tank bioreactor. Furthermore, the tools and techniques used in this study can be applied to other suspension cell lines and viral vectors that are currently being evaluated for clinical use.

Bibliography, Acknowledgements: N/A

Disclosure of Interest: None declared





PO210

INTRANASAL VACCINATION OF MICE AGAINST HEPATITIS E VIRUS USING ADENO-ASSOCIATED VECTORED VACCINE

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Background and novelty: Hepatitis E virus (HEV) is an emerging enteric viral hepatitis, mainly in developing countries. ORF2 encoding the viral capsid protein, is highly immunogenic, producing long lasting neutralizing antibodies. This antigen has been used in all vaccine studies so far. In this work, we suggest to develop a novel vaccine against hepatitis E infection using adeno associated virus (AAV) as a vector containing the gene of a truncated form of HEV capsid protein (112aa-660 aa). rAAV will be delivered via the nasal route.

Experimental approach: rAAV5 and rAAV6 production in Sf9 insect cells were optimized in Erlenmeyer flasks according to the experimental design approach. The effects of initial cell density, time of infection, temperature and Multiplicity of infection (MOI) were studied. Different chromatographic methods were then assessed for the purification of rAAVs. Collected fractions were analyzed by qPCR to determine rAAV titer, and by SDS-PAGE. To evaluate the immunogenicity of the vaccine, groups of Balb/c mice were immunized via the nasal route according to different protocols. The effect of rAAV dose, rAAV serotype, the use of an adjuvant (MPLA) and adjuvant concentration, were studied.

Results and discussion: Optimal production conditions were determined for both serotypes (rAAV5 and 6). We also found that affinity chromatography using AVB Sepharose column had resulted in the best overall yield, which was around 100% for both serotypes. Immunization of mice showed that the rAAV5 gave better immune response compared to rAAV6. After intranasal administration of rAAV, both non-adjuvanted and MPLA adjuvanted vaccines induced high anti-HEVORF2 IgG titers. The MPLA adjuvanted rAAV induces a better immune response than rAAV alone. The study of the distribution of rAAV5 showed its presence in the target organs (heart, muscles, lungs, central nervous system) and the highest amount of rAAV5 was observed in lungs.

Bibliography, Acknowledgements: -

Disclosure of Interest: None declared





PO211

APPLICATION OF NANOTECHNOLOGY TECHNIQUES TO THE CHARACTERIZATION OF BIOPROCESSES: OBTENTION OF HIV-1 BASED VLPs BY PEI-MEDIATED TRANSIENT TRANSFECTION IN THE HEK 293 CELL LINE

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Background and novelty: The application of novel nanotechnology techniques in the field of biomedicine has increased interest. In this study, those techniques are applied to the characterization of PEI-mediated transient transfection of mammalian cells for the production of HIV-1 based VLPs as vaccine platform.

Experimental approach: The production of HIV-1 Gag-GFP VLPs was performed with a protocol previously developed in the HEK 293 cell line. The characterization of complexes was performed through particle tracking and electron microscopy techniques. The transient transfection process was followed up by flow cytometry, confocal microscopy and quantified by fluorometry. The HIV-1 based VLP production was characterized by electron microscopy and advanced multifrequency (MF) atomic force microscopy (AFM).

Results and discussion: The main outcomes of this study showed that DNA/PEI complexes are compacted structures of 250 nm with a concentration of 1.08^{10} particles/mL, that undergo an aggregation process when are incubated in culture medium, representing more than 250 complexes per cell at the time of transfection. Two complex populations are clearly visualized by electron microscopy coupled with X-ray spectroscopy.

DNA/PEI complexes enter throughout every endocytosis pathway and DNA/PEI aggregates represent the major specimen contributing to transient transfection efficiency. The kinetics of DNA/PEI disassembly and its arrival to the cell nucleus was observed. Finally, the discrimination between VLPs and other vesicular bodies and more remarkably the nanomechanical qualities of the VLPs were characterized applying advanced MF AFM.

The application of novel nanotechnologies unveils the change to better understand the physicochemical properties of bioprocesses and guarantee the product quality.

Bibliography, Acknowledgements:

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González-Domínguez 2016

González-Domínguez 2017 (*Submitted*)

Disclosure of Interest: None declared





PO212

DEVELOPMENT OF A CHEMICALLY DEFINED MEDIUM FOR VIRUS VACCINE PRODUCTION IN A DUCK SUSPENSION CELL LINE

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Background and novelty: A project was initiated to improve upon the production process of viral vaccines through the use of the EB66® cell line, which is derived from duck embryonic stem cells. The primary goal of this project was to develop a single chemically defined medium that could be used instead of the multiple media process previously recommended. As with many viral cell culture-based production processes, virus production in EB66 cells required separate media for the cell proliferation phase and for the virus production phase, and neither of these processes was considered chemically defined.

Experimental approach: An initial growth-only screen of 20 different media formulations, mixtures, and/or media fortifications was performed to identify the most promising formulations to support good cell growth, viability, and consistent morphology. Screened formulations were assessed for viral production with representatives of secreted and nonsecreted viruses, namely measles (var. Schwarz) and modified vaccinia Ankara (MVA) virus. Viral titer was determined via standard TCID₅₀ methods. Scale-up to bioreactor level was also evaluated, prior to selection of a final formulation.

Results and discussion: Using nonoptimized production processes, viral titer in the new formulation has been comparable to the non-chemically defined processes (TCID₅₀ of 10^{7.5}/mL for MVA and 10⁷/mL for measles). This new formulation, HyClone™ CDM4Avian medium, and this single-medium approach are currently being investigated with several other viruses, and results are encouraging. Overall, CDM4Avian medium is the first chemically defined medium for EB66 cells that fulfills all critical requirements needed to grow viruses in a modern cell culture system.

Bibliography, Acknowledgements: The assistance of the USU Institute of Antiviral Research is gratefully acknowledged.

Disclosure of Interest: None declared





PO213

DISRUPTIVE VACCINE MANUFACTURING PLATFORM AIMING TO REACH EXTRA LOW PRODUCTION COST

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Background and novelty: Vaccines are one of the most powerful and effective health interventions ever developed, providing tremendous economic and societal value. Yet a number of factors interact to limit complete global immunization coverage, as is the capacity to develop fast and effective vaccines and cost-effective vaccine production. Innovations are needed to develop manufacturing platforms able to transform production economics.

Experimental approach: Univercells is offering a disruptive vaccine manufacturing platform being developed under a grant from the Bill&Melinda Gates foundation, aimed at increasing availability and affordability of biologics. In this disruptive setup, improvements are introduced at each step of the manufacturing process. Densification of the cell culture and purification are achieved with a new type of single-use, high-density fixed-bed bioreactors operated in perfusion, and cutting-edge high-performance chromatography membranes integrated with the cell culture through a sequential-continuous purification process. This innovative process is automated, and can do without intermediate storage tanks, therefore significantly reducing the footprint of the equipment. This intensified and integrated manufacturing process is encapsulated into an isolator, making it a self-contained vaccine manufacturing facility (2m x 3m), increasing safety for the operators and having a tremendous impact on the factory design.

Results and discussion: This new facility is expected to be able to manufacture any type of viral vaccine at a very low cost (0,15\$/dose for a trivalent sIPV) and could be deployed at the site of the manufacturer in emerging countries, killing the two birds of cost of manufacturing and distribution with one stone. The presentation will feature the description of the engineering development, but also the preliminary results of cell growth, infections and product quality, as well as a description of the COGS calculation.

Bibliography, Acknowledgements: N/A

Disclosure of Interest: None declared





PO214

AVIAN AGE1.CR CELL LINES FOR THE PRODUCTION OF AN ATTENUATED STRAIN OF RABIES VIRUS

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Background and novelty: Rabies is a viral zoonosis caused by negative-stranded RNA viruses of the *Lyssavirus* genus. The vast majority of human cases (>90%) result from the bites of rabid domestic or feral dogs making prevention of canine rabies essential in the fight against human rabies. Inactivated rabies vaccine has been shown to be effective for rabies control in dogs. In this work, we evaluated different cell lines of avian origin for the replication of rabies virus. By using these cell lines, we aim to achieve superior titers of reactogenic rabies vaccine antigen compared to conventional production processes.

Experimental approach: AGE1.CR, AGE1.CR.pIX, AGE1.CR^o and AGE1.CR.pIX^o cultures were carried out in suspension, in shake flasks at 36.5°C, 8% CO₂ and 150 rpm. CR and CR.pIX were grown in the chemically defined medium CD-U5 supplemented with 10 ng/ml of LONG-R3IGF whereas CR^o and CR.pIX^o were cultivated in the same medium without LONG-R3IGF. Upon cell infection, temperature was decreased to 34 °C and CO₂ to 4.4%. Cells were infected at 2x10⁶ cells/ml, with PV/BHK-21 rabies virus strain at different MOIs. Viral titer was determined by a fluorescent method.

Results and discussion: All cultures were initially infected at an MOI of 0.01. The different cell lines exhibited different growth profiles after cell infection. Both CR.pIX lines resulted in a high virus titer in the range of 2x10⁸ FFU/ml. CR^o cells yielded the lowest titer (around 10-fold lower). Virus replication optimization was pursued and different MOIs (0.05, 0.01, and 0.001) were tested. An MOI of 0.001 was optimal, and gave a titer of 6.5x10⁸ FFU/ml for CR.pIX^o cells and 4.3x10⁸ FFU/ml for CR.pIX cells. Thus the LONG-R3IGF-independent CR.pIX^o cell line appears to be the best producer cell line. Currently we are investigating the effect of other operating parameters to further optimize rabies virus replication in the selected cell line.

Bibliography, Acknowledgements: -

Disclosure of Interest: None declared





PO215

PROCESS ECONOMICAL EFFECTS OF IMPLEMENTATION OF READY-TO-USE MICROCARRIERS IN CELL-BASED VIRUS VACCINE PRODUCTION

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Background and novelty: Microcarriers are used as support for the growth of adherent cells. By providing a large cultivation surface in bioreactor cultures, microcarriers have to a great extent replaced cultivation in Cell Factory™ systems or roller bottles over the last decades.

At Sanofi Pasteur, one of the world leaders in human vaccines, Cytodex™ 1 microcarriers have been used for decades in the production of viral vaccines in Vero cells. In accordance with the supplier's recommendation, the microcarriers that are delivered dry are swollen in buffer, washed, and heat-sterilized before use. From October 2016, the supplier proposes a ready-to-use Cytodex™ 1 alternative delivered presterilized by gamma irradiation.

Experimental approach: First, the presterilized alternative was evaluated with regards to reduced preparation time and cost. With a two-year shelf life, the presterilized alternative reduces utility cost and adds flexibility to operations by decreasing the need for vapor and stainless steel materials in viral production facilities tooled up with single-use bioreactors.

Confirming the benefits of using presterilized microcarriers, the second step was to evaluate cell growth and viral productivity using the ready-to-use alternative in comparison with using the reference product already in place. Both cell growth and viral productivity were comparable, which enabled pursuing the documentation for implementing the presterilized microcarriers in GMP manufacturing for new R&D vaccine projects.

Results and discussion: While the presterilized Cytodex™ 1 microcarriers are implemented in process development for new vaccines and are qualified for manufacturing of clinical batches of new vaccine products, the next step will be to evaluate the benefits and impacts of replacing the microcarrier reference product with the gamma sterilized alternative on industrial products.

Bibliography, Acknowledgements: I acknowledge Cell & viral team and GE for their support.

Disclosure of Interest: None declared





PO216

NOVEL AND SCALABLE PRODUCTION PROCESS FOR A PESTE DES PETITES RUMINANTS VIRUS VACCINE

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Background and novelty: Peste des Petites Ruminants Virus (PPRV) is a highly contagious disease affecting small ruminants in Africa and Asian. Aiming to eradicate the disease, a novel/scalable PPRV vaccine production process is needed. Built upon work previously done at iBET, a new production process is herein proposed using Vero cells growing on microcarriers (MCs), serum-free medium (SFM) and stirred-tank bioreactors (STB). This includes a new method for cells detachment from MCs and perfusion culture.

Experimental approach: The PPRV vaccine production process was developed in 2L STB (BIOSTAT B-DCU3, Sartorius) using Nigeria 75/1 strain. Engineering correlations were used to optimize culture conditions. Vero cells were adapted to grow in ProVero™-1 SFM (Sartorius). New enzymatic/mechanical method for *in situ* cell detachment from MCs was designed. Perfusion culture was evaluated in 2L STB (spin-filter). PPRV was clarified using depth filtration (Sartopure PP2, Sartorius).

Results and discussion: Vero cells were adapted to SFM reaching growth rates of 0.03h⁻¹ (similar to serum-containing cultures). The new *in situ* cell detachment method was successfully implemented; yields above 80%, no impact on cell re-attachment or virus productivity. A 2-fold increase in maximum cell concentration was obtained using perfusion when compared to batch culture. Combining the new *in situ* cell detachment method with perfusion culture will enable the scale-up directly from 2L to 20L STBs, reducing seed-train time. The potential of depth filtration for PPRV clarification could be confirmed (yields up to 90%). Process scalability will be validated at the 20L scale in BIOSTAT C-Plus (Sartorius) comparing cells growth, metabolic and PPRV production profiles to those achieved in 2L STB.

The novel/scalable vaccine production process herein proposed has potential to assist the upcoming vaccination program for eradication of PPRV disease.

Bibliography, Acknowledgements: Support of Carina Silva and Margarida Serra.

Disclosure of Interest: None declared





PO217

PROCESS ECONOMY MODELING FOR VIRAL VECTOR PRODUCTION

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Background and novelty: Viral vectors such as adenovirus, adeno-associated virus, and lentivirus can be used in cancer therapeutics and in vaccines for chronic diseases, and are thus expected to become high-value products. Cost-efficient production is therefore important and should be considered early in development. Obtaining a safe and efficacious product that can be produced at a competitive cost is a balance between required purity, yield, throughput, and productivity. Traditionally, process development starts with evaluation of different unit operations in small scale, with more focus on performance than on cost efficiency. An alternative strategy is to start early with process economy calculations for larger scales, using the outcome as a guide to identifying economically feasible unit operations to evaluate experimentally.

Experimental approach: Here, experimental work and process economy calculations are combined, from adenovirus production in cell culture to purified bulk product. BioSolve™ software was used for the modelling part. Data from upstream production was used to investigate the effects of various cell culture medium formulations and bioreactor formats on process economy. For the downstream process, a number of unit operations were investigated. Different formats for anion exchange chromatography, such as bead-based resins and membrane adsorbers, were compared. The use of other separation techniques, such as core beads and size exclusion chromatography, was also evaluated.

Results and discussion: Modeled scale-up scenarios were created for several process options with regards to process economy. Advantages and disadvantages for the use of different purification techniques and formats of equipment, such as membrane adsorbers and bead-based resins in stainless steel or single use columns for early stage clinical trial material production scale and manufacturing at commercial scale will be discussed.

Bibliography, Acknowledgements: N/A

Disclosure of Interest: None declared





PO218

CELL CULTURE-BASED PRODUCTION OF SPECIFIC DEFECTIVE INTERFERING PARTICLES FOR INFLUENZA ANTIVIRAL THERAPY

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Background and novelty: Influenza A virus defective interfering particles (DIPs) are a sub-population of non-infectious viruses with an internal deletion in one or more genome segments. Therefore, DIPs require standard virus (STV) genome for replication. In addition, DIPs interfere with STV replication in co-infected cells. Due to this property, DIPs have potential to be used as antivirals. One specific DIP, the DI244 that was produced in chicken eggs, has been previously described to be a good candidate for antiviral therapy [1]. We now propose cell culture-based production of DI244, allowing more defined replication conditions.

Experimental approach: We have identified optimal cultivation parameters for enrichment of DI244 and parallel depletion of unspecific DIPs in the avian suspension cell line AGE1.CR.pIX using a design of experiments approach. Since DI244 is indistinguishable from STV by virus titration methods, the DI244 yield was monitored by real-time RT-qPCR with primers which bind to the junction region of the defective segment.

Results and discussion: We observed that conditions which deplete unspecific DIPs (multiplicity of infection (MOI): 10^{-6} and 10^{-7} infectious virions/cell), decrease the DI244 yield by 1.2 orders of magnitude. At higher MOIs (10^{-5} - 10^{-2} infectious virions/cell) the DI244 yield increases proportionally to the MOI whereas STV decreases linearly to DI244. However, unspecific DIPs were also detected. These unspecific DIPs accumulate randomly and cannot be avoided at conditions which increase DI244 yields. However, they could be useful as adjuvants. A seed virus without unspecific DIPs and a proportion of 5.5% DI244 and 94.5% STV was produced. Additionally, a seed virus with highly enriched DI244, 55.8%, but also unspecific DIPs was obtained. Overall, we increased the DI244 yield by cell culture based production by 17% in comparison to the egg-based production.

Bibliography, Acknowledgements: [1] Dimmock et al. (2012) PLoS ONE 7(12)

Disclosure of Interest: None declared





PO219

ADAPTIVE EVOLUTIONARY ENGINEERING OF INSECT CELLS FOR IMPROVED INFLUENZA HA VLPS PRODUCTION

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Background and novelty: Cell culture-based hemagglutinin (HA)-displaying virus-like particles (VLPs) have been shown to be vaccine candidates against Influenza. With the increasing need for larger quantities of vaccines and the unpredictability of novel pandemic outbreaks, bioprocess engineering strategies capable of improving production yields are vital. In this work, a high-performance insect cell line was generated through adaptive evolutionary engineering (AEE) and evaluated for Influenza HA VLPs production in the baculovirus-insect cell system.

Experimental approach: Insect High Five cells were adapted to high-pH medium by sequential sub-culturing over 2-3 months. Different cell concentrations at infection ($CCI=1-3 \cdot 10^6$ cell/ml) and multiplicities of infection ($MOI=0.1-10$ pfu/cell) were evaluated to identify optimal infection conditions. To discriminate the impact of high-pH medium and the adaption process on HA expression, adapted and parental cells were cultured in their respective medium under optimal infection conditions ($CCI=2 \cdot 10^6$ cell/ml; $MOI=1$ pfu/cell). HA expression was assessed by Western blot and hemagglutination assay, and VLPs formation demonstrated by electron microscopy.

Results and discussion: High-pH adapted cells grow similarly to parental cells ($0.04h^{-1}$) and, in most infection conditions tested, show higher specific HA productivities (up to 2-fold) when compared to parental cells. Noteworthy, the adaptation process seems to be the critical factor for maximizing titers as cells cultured directly in high-pH medium without adaptation induced expression levels similar to those of cells cultured in standard medium. Proof-of-concept in 0.5L stirred tank bioreactor is currently being evaluated. This work demonstrates the potential of AEE for process optimization of Influenza HA VLPs.

Bibliography, Acknowledgements: This work was supported by EU FP7/2007-2013/ under REA grant agreement n° [602640] and Portuguese FCT (IF/01704/2014 and IF/01704/2014/CP1229/CT0001).

Disclosure of Interest: None declared





PO220

CYPHER ONE: AUTOMATED INTERPRETATION OF HEMAGGLUTINATION INHIBITION (HAI) ASSAYS

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Background and novelty: Hemagglutination (HA) and hemagglutination inhibition (HAI) assays have been utilized for 70+ years and play a critical role in flu vaccine development. In particular, HAI is critical in antigenic characterization of flu viruses and in evaluating immunogenicity of cell-based and traditional egg-based vaccines. HAI assays are prone to poor lab-to-lab consistency due to subjectivity in interpretation between human “readers” where a difference of ± 1 dilution is often considered an ‘equivalent’ result. In addition, the presence of non-specific inhibition (NSI) can further complicate analysis. The Cypher One system both images plates for HA/HAI assays and automates the analysis in an effort to standardize interpretation and create a permanent record of results generated. Here we address whether or not the common practice of “tilting” HAI plates is necessary to obtain accurate results.

Experimental approach: We compared performance of Cypher One automated interpretation to visual interpretation of a human expert reader for a HAI dataset of 1,238 samples analyzed during serology testing by a US government agency. Cypher One titer was obtained without tilting and compared to that obtained by the human expert reader employing plate tilting for interpretation.

Results and discussion: Percent agreement between manual interpretation with tilt and Cypher One automated interpretation without tilt was $94.3\% \pm 1$ dilution. Importantly, $\sim 25\%$ of clinical samples screened exhibited NSI, which is known to complicate manual interpretation and often cited as a cause for the need to tilt the plate for accurate interpretation. Even in the presence of NSI Cypher One showed high agreement for HAI without the need to tilt the plate. The Cypher One system thus achieved high accuracy and consistency while providing a digital record of plate images and associated results to meet data integrity requirements.

Bibliography, Acknowledgements: Funding provided by NIH/NIAID Grant R43AI106054

Disclosure of Interest: None declared





PO221

VAXARRAY SEASONAL INFLUENZA ASSESSMENT OF CELL-DERIVED INFLUENZA VACCINE POTENCY

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Background and novelty: WHO guidelines dictate flu vaccine producers determine potency & stability prior to & as a function of time after release. The current accepted potency determination assay is single radial immunodiffusion (SRID) to measure hemagglutinin (HA) concentration. SRID is labor and reagent intensive, established for egg-based vaccines & is not always optimal, or even possible, for novel vaccine produced outside of eggs, such as VLPs. The VaxArray seasonal HA assay (VXI sHA) is a new alternative potency assay compatible with cell-based vaccines during all manufacturing stages, ranging from crude samples in tissue culture media to monobulk drug substances & multivalent formulations. VXI sHA is based on a panel of subtype-specific yet broadly reactive monoclonal antibodies. Multiple antibodies against seasonal subtypes are printed in an array on a glass substrate, & a multiplexed immunoassay is performed with signal readout based on fluorescence from a conjugated “universal” antibody label.

Experimental approach: To demonstrate utility of VXI sHA in characterizing cell-based flu vaccines, cell-derived vaccines provided by de-identified manufacturers tested using VXI sHA & analyzed for accuracy, precision, & linear range. To assess stability indication capabilities, a 6 month stability experiment was performed with H1, H3, B/V & B/Y monovalent VLP vaccines, tested monthly.

Results and discussion: VXI sHA can quantify potency of novel cell-based vaccines & traditional cell-based subunit vaccines with a LOD of approximately 0.01 µg/mL & linear range that spans two orders of magnitude. VXI sHA generated paBCA equivalent measurements with high accuracy ($\pm 20\%$) & precision ($\pm 15\%$) for all subtypes. Over the course of the 6 month stability study, VXI sHA signal decreased linearly to 84%, 71%, 75%, & 63% for H1, H3, B/V, & B/Y vaccines, respectively, illustrating ability to track degradation.

Bibliography, Acknowledgements: We acknowledge funding from the NIAID (NIH/NIAID AI102318).

Disclosure of Interest: None declared





PO222

FOOT AND MOUTH DISEASE VACCINE PRODUCTION IN CELL CULTURE

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Background and novelty: Foot and mouth disease virus (FMDV) is a highly contagious and acute viral affliction of cloven footed animals [1]. The seven distinct virus serotypes of FMD including A, O, C, Asia-1, and the Southern African Territories types 1 to 3 as well as multiple subtypes are endemic globally. Although South Korea had remained FMD-free for 66 years, however since 2000, six FMD epidemics have been recorded [2]. Since then, the Korean government has adopted vaccine strategy for FMD. We have developed a new technology for FMDV vaccine production by utilizing serum-free cell culture platform, which can promote the rapid preparation of vaccine against new viral strains.

Experimental approach: Baby Hamster Kidney (BHK-21, clone 13) cell line which act as a host, was developed to be a suspension-adapted cell line in serum-free medium (BHK-S). Twenty media were screened for maximum viable cell concentration using BHK-S, and then, feeding strategy was developed using selected basal media.

Results and discussion: BHK-S has a high growth rate and is highly sensitive to domestic FMDV strain. Compared to the adherent BHK cells, most of the attachment proteins on the extracellular membrane showed decreased expression level in BHK-S. The titer of FMDV increased with the viable cell concentration. Inoculating 0.01 MOI of FMDV to 10^5 , 10^6 , and 10^7 cells/ml BHK-S resulted in 4.84 ± 0.1 , 6.31 ± 0.5 , and 6.75 ± 0.6 TCID₅₀, respectively. The animal component free basal media and additive feeding media were screened to obtain higher viable cell concentration and better FMD viral production. Additionally, the feeding strategy was developed to augment maximum viable cell concentration up to 2.5×10^7 cells/ml within six days. This cell culture based-FMDV production technology can attribute to develop an effective viral vaccine in case of FMD outbreak.

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Disclosure of Interest: None declared





PO223

DYNAMICS OF INTRACELLULAR METABOLITE POOLS DURING MDCK SUSPENSION GROWTH AND INFLUENZA VIRUS REPLICATION

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Background and novelty:

MDCK suspension cell lines are used both in industry and academia for the production of cell culture-based influenza vaccines. So far, only limited information is available for the dynamics of intracellular metabolite pools during cell growth. In addition, the impact of influenza virus infection on the metabolism of MDCK_{SUS} cells has not been studied. To support media design and process optimization, however, a deeper insight in critical metabolic pathways is essential.

Experimental approach:

MDCK_{SUS} cells were cultivated in shaker flasks in a chemically defined medium over seven (mock) or four days (infection) respectively. MDCK cells were infected after 48 h (influenza A/PR/8/34 (H1N1)) with a high MOI (10) to achieve a synchronous infection of the whole cell population. Extracellular samples were taken for metabolite and virus analysis. Additionally, 2 mL of cell suspension were taken as described by Sellick *et al.* ^[1], metabolites were extracted and quantified as described previously ^[2].

Results and discussion:

Besides the characterization of growth dynamics, the main focus of the analysis was put on early events in virus replication, before cell count, viability and size were affected (12 hours post infection, hpi). Here, early glycolytic pools (G-6-P, F-6-P, F 1-6-bp) decreased already 2-4 hpi, whereas metabolic pools of the citrate cycle either remained constant or showed an increase in the later phase of the infection (ketoglutarate). Energy metabolites (ATP, ADP, AMP) and energy charge did not show major alterations confirming that influenza virus replication poses only a low energetic burden on cells. Overall, early influenza virus infection seems to alter central carbon metabolism by reducing glycolytic activity, but leaving the activity of the TCA primarily unaffected.

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Disclosure of Interest: None declared





PO224

SELECTION OF STABLY PRODUCING DROSOPHILA S2 CELLS FOR INCREASED DENGUE VLP PRODUCTION

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Background and novelty: Emerging infectious diseases are increasingly threatening human health due to increased international travel and global warming. Vaccines represents the best way of preventing viral infectious diseases, and Virus Like Particles (VLPs) represent a promising approach to improve vaccine efficacy.

VLPs mimic the natural virus and therefore carries the same capabilities of fusion and entry into host cells as the natural virus. A change in pH causes an irreversibly conformational change to Dengue E-protein¹, which forms part of a PrM-E based Dengue VLP, turning it from a non-fusing particle into a mature fusion particle. This conformational change causes cell fusion during both selection of stable cell lines and during cultivation, leading to much reduced yields or failure to select a cell line.

Experimental approach: In order to overcome the fusion issues caused by decreased pH a ScFv of a pan-flavi neutralizing antibody was co-expressed with the VLP. The aim was to prevent the fusion activity of the VLP by binding a ScFv to the fusion loop of the E protein during selection.

Results and discussion: Western blots of the samples taken from the stable cell lines showed that co-expression of the ScFv improved the expression level of VLP significantly compared to a control cell line selected without ScFv co-expression. The cells also showed improved growth and higher viability during selection. This approach offers a novel solution to the counter productive fusion capabilities of flaviviral VLPs, and should lead to improved VLP production for future vaccine processes.

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Disclosure of Interest: None declared





PO225

EXTENSIVE REARRANGEMENTS BUT HIGH GENOMIC STABILITY IN A BIOTECHNOLOGICALLY ADVANTAGEOUS DERIVATIVE OF MODIFIED VACCINIA VIRUS ANKARA

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Background and novelty: Vectored vaccines based on modified vaccinia virus Ankara (MVA) are reported to be safe, reactogenic, tolerant to pre-existing immunity, and able to accommodate and stably maintain large transgenes. MVA is usually produced on primary chicken embryo fibroblasts but continuous cell lines are being investigated as more versatile alternatives. Using our continuous suspension cell line derived from the muscovy duck (CR.pIX) in chemically defined medium allowed recovery of an isolate of a hitherto undescribed genotype (MVA-CR19). Viruses with this novel genotype induced fewer syncytia in adherent cultures and replicated to higher infectious titers in the extracellular volume of suspension cultures [1].

Experimental approach: MVA-CR19 was enriched by passaging a non-plaque-purified preparation of MVA. We now report new studies with plaque-purified recombinant MVAs containing distinct expression cassettes in deletion site III and unique combinations of the differentiating point mutations of MVA-CR19 in a backbone of wildtype virus.

Results and discussion: No ultrastructural differences were observed between the novel strain and wildtype by electron microscopy. However, the mutation in at least one structural gene was found to facilitate spread of MVA-CR19 in cell cultures, and an extensive genomic rearrangement may be the cause for augmented replication of the novel strain. The left viral telomere was found to be replaced by the right counterpart in this rearrangement that affects 15 % of the viral genome. We confirm the exceptional genetic stability of MVA. Although the genotype of MVA-CR19 is advantageous for replication, all genetic markers that differentiate wildtype and MVA-CR19 were stably maintained in passages of all tested plaque-purified preparations of recombinant viruses, independent of wildtype or MVA-CR19 backbone.

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Disclosure of Interest: None declared





PO226

ON-LINE MONITORING OF MVA AND INFLUENZA VIRUS REPLICATION AT HIGH-CELL-DENSITIES

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Background and novelty: Dielectric spectroscopy has been extensively described as a technique for the on-line monitoring of cell biomass and virus production [1, 2]. Given the crucial importance of on-line monitoring for process control and optimization at high-cell-densities (HCD), on-line measurements of permittivity (ϵ) and membrane capacitance (C_m) were evaluated for influenza and Modified Vaccinia Ankara (MVA) virus production at HCD.

Experimental approach: AGE1.CR.pIX cells were cultivated in 0.6 L bioreactors in batch (4×10^6 cells/mL) and perfusion (50×10^6 cells/mL, with an ATF2 system) and infected with MVA-CR19 or influenza A/PR/8/34 (H1N1) viruses. Cellular dielectric properties were monitored on-line with a capacitance probe (Hamilton). From on-line ϵ and C_m values, the viable cell densities (VCD) were estimated and the virus replication was investigated. In addition, influenza virus-infected cells were analyzed by imaging cytometry with an ImageStream X Mark II (Amnis, EMD Millipore).

Results and discussion: On-line and off-line VCD measurements correlated for all cultivations up to late infection times. Cyclic oscillations of C_m seemed to correlate with the viral infection dynamics. For infections with MVA-CR19 above 50×10^6 cells/mL, two distinctive C_m oscillation cycles were identified exclusively during accumulation of infected cells and infectious virus particles. For influenza virus infections, two and three oscillation cycles were observed at 4×10^6 and 50×10^6 cells/mL, respectively. Imaging cytometry revealed that virus-producing cells occur mainly when C_m increases to its maximum and that newly infected cells appear when C_m decreases to its initial value.

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Disclosure of Interest: None declared





PO227

OPTIMIZED TRANSFECTION EFFICIENCY FOR CHO-K1 SUSPENSION CELLS THROUGH COMBINATION OF TRANSFECTION AND CULTURE MEDIA

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Background and novelty: One of the central issues in pharmaceutical bioprocess engineering is to reduce time to market and production cost. Transient gene expression systems using polyethyleneimine (PEI) are considered to be fast, flexible and cost-efficient for recombinant protein production ¹.

The transfection efficiency depends on the type of media. Certain components in culture medium for cell growth and protein production can inhibit or diminish the transfection efficiency. This issue can be solved by performing the transfection in specific transfection medium followed by feeding of complete culture media².

Experimental approach: In this study, six different media were screened for suitability in transient gene expression of CHO-K1 cells using PEI and pEGFP-N1 plasmids. The transfection efficiency was determined by the percentage of CHO-K1 cells, which were expressing GFP.

Results and discussion: Media screen result exhibits using of Opti-MEM medium around 50% of transfected cells successfully express GFP. To improve the transfection efficiency, basic parameters including cell density, plasmid and PEI concentrations were varied. Further results show that the incubation of CHO-K1 cells in Opti-MEM (transfection medium) for 5 hours followed by addition of CHOMACS CD (culture medium) for further cultivation increased the transfection efficiency up to $85 \pm 2.6\%$.

That optimal transfection condition was applied for TGF- β 1 production using another plasmid. The purified TGF- β 1 was successfully detected by western blot. The bioactivity of produced TGF- β 1 was evaluated by the inhibition of A549 cell proliferation with the help of CTB assay. TGF- β 1 was incubated with the cells for 5 days and 20 % of A549 proliferation decrease was observed compared to the untreated group.

Bibliography, Acknowledgements: I would like to thank DAAD for a Ph.D. grant.

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Disclosure of Interest: None declared





PO228

REGULATION OF RECOMBINANT PROTEIN EXPRESSION DURING CHOBRI/RCTA POOLS GENERATION INCREASES PRODUCTIVITY AND STABILITY

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Background and novelty: In order to deal with the growing demand of large quantities of therapeutic proteins in a timely fashion, expression systems are being optimized to reduce the time of generation of stable clones as well as to increase the levels of protein secretion. This can be achieved by a combination of expression cassette optimization, cell engineering and selection process.

Experimental approach: We have developed a cell line (CHO^{BRI/rcTA}) allowing the inducible expression of recombinant proteins, based on the cumate gene switch, which is a very efficient expression system for protein production.

Results and discussion: We have shown that the cumate-inducible promoter (CR5) was the strongest promoter we had tested so far in Chinese hamster ovary (CHO) cells. With this promoter, we were able to generate stable CHO pools capable of producing high levels of a Fc fusion protein (900 mg/L), outperforming by 3 to 4-fold those generated with CMV5 and hybrid EF1 α -HTLV constitutive promoters. Besides the strength of the CR5 promoter, we demonstrated that the ability to control both the time and the level of expression during pool generation and maintenance gave a real advantage to the inducible expression system. Indeed, we observed that keeping the expression OFF during selection enabled the generation of pools with superior productivity compared with the pools whose expression was maintain ON. Moreover, preliminary results suggest that keeping recombinant protein expression down increase the frequency of producer clones. Knowing that one of the main bottlenecks of the successful bioprocessing of recombinant proteins using CHO cells is the rapid isolation of a high producer, our data suggest that the cumate gene-switch system could be a valuable platform for the generation of stable clones.

Bibliography, Acknowledgements: The authors would like to thank Louis Bisson for performing protein A-HPLC analyses.

Disclosure of Interest: None declared





PO229

HYPER N-GLYCOSYLATED HUMAN INTERFERON-ALPHA2B PRODUCED IN HEK293 CELLS PRESENTS HIGHER ANTITUMOR ACTIVITY THAN THE CHO-K1-DERIVED PROTEIN

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Background and novelty: Glycosylation constitutes an important attribute of glycoproteins since it defines many of their properties. In this context, CHO and HEK cells are interesting hosts for their production as biotherapeutics. The potentiality of using the human cell line over the rodent model to produce a hyper N-glycosylated protein is herein analyzed.

Experimental approach: A N-glycoengineered hIFN- α 2b (IFN4N) was produced in CHO and HEK cell lines and purified. Both molecules were compared in terms of glycosidic profile, pharmacokinetics and *in vitro* and *in vivo* biological activity.

Results and discussion: IFN4N_{CHO} presented higher average molecular mass and more acidic isoforms compared to IFN4N_{HEK}. Accordingly, a significantly higher sialic acid content was found in IFN4N_{CHO} compared to IFN4N_{HEK}. These results were in agreement with monosaccharides quantification and WAX chromatography, which supports the existence of highly sialylated and ramified structures for IFN4N_{CHO} glycans, in contrast with smaller and truncated structures from IFN4N_{HEK}. Mass spectrometry analysis revealed that FA2G2S2, FA3G3S3, FA4G4S4 and FA4G4Lac1S4 were predominant in IFN4N_{CHO} whereas FA2B/FA3, FA2G1 and FA2BG1 were the main IFN4N_{HEK}-bearing glycans. Unexpectedly, those remarkable differences had not a considerable impact on their pharmacokinetic properties, showing quite similar elimination profiles. Also, despite the *in vitro* antiviral specific biological activity of both proteins was the same, IFN4N_{HEK} was more efficient as an antiproliferative agent. Further experiments with nude mice implanted with PC-3 cells confirmed the highest antitumor efficiency of IFN4N_{HEK}. Our results show the importance of an appropriate host selection to set up a bioprocess and potentiate the use of HEK cells for the production of a new hyper N-glycosylated protein-based pharmaceutical.

Bibliography, Acknowledgements: Gugliotta et al. 2017 EJPB 112:119-131

Disclosure of Interest: None declared





PO230

PROCESS DEVELOPMENT BY CONDITIONAL MANIPULATION OF ENDOGENOUS CHO CELL MIRNA

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Background and novelty: With the ability to affect multiple genes and fundamental pathways simultaneously, miRNA engineering of Chinese Hamster Ovary (CHO) cells has significant advantages over traditional gene expression or repression studies. Tri-Omics analysis; mRNA, miRNA and protein, of fast and slow growing IgG producing CHO cell clones, revealed 51 miRNAs to be differentially expressed (Clarke et al, 2013). A subset of candidate miRNA were chosen for validation of impact on industrially relevant CHO cell phenotypes. This work takes the next step in CHO cell engineering with miRNA, by the addition of control, via employment of tetracycline inducible expression systems (Das et al, 2016).

Experimental approach: Stable mixed pools of CHO DP12 cells, each expressing a specific inducible miRNA sponge decoy (Kluvier et al, 2012) were subject to analysis of relevant CHO cell characteristics.

Results and discussion: Profiling data indicated these miRNAs as being associated with growth rate. Of the six miRNA vetted, three improved cell growth; miR-505 23% ($p=0.02761$), miR-455 24% ($p=0.0082$), miR-378 39% ($p=4.3 \times 10^{-5}$), and one increased product titre, miR-338 1.95 Fold ($p=0.019143$). This work aims to identify novel miRNA engineering targets, evaluate inducible miRNA expression systems in Chemically Defined (CD) growth conditions, and outline provisional strategies for conditional multi-miR engineering of CHO cell lines.

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Disclosure of Interest: None declared





PO231

GENERATING MONOCLONAL PRODUCTION CELL LINES WITH ≥ 99.9 % PROBABILITY

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Background and novelty: The main regulatory authorities and organizations demand proof of monoclonality for biotechnological producer cells. With increasing pressure to shorten timelines and increase drug safety, technologically advanced methods have to be established to ensure that production cell lines are derived from a single progenitor cell.

Experimental approach: Sartorius Stedim Cellca's single cell cloning approach is based on one round of fluorescence-activated cell sorting (FACS) using Becton Dickinson (BD) FACSAria™ Fusion cell sorter combined with photo-documentation by SynenTec Cellavista microscopic imaging system. For validation of monoclonality, critical process parameters such as different cell lines, viability and cell aggregation levels were investigated separately to assess their contribution to the probability of monoclonality.

Results and discussion: The probability P(d) of seeding more than one cell per well by the described cell sorting procedure was determined to be 0.6 %. In a second step, the conservative probability P(i) of having cells that are out-of-focus of the Cellavista imaging on day 0 directly after the sorting was determined to be 1.9 %. Moreover, the realistic probability P(k) of having cells that are out-of-focus on day 0 directly after the sorting but are eventually used for research cell bank (RCB) preparation was determined to be 0.6 %. In combination, the final documented probability of having monoclonal cells in RCBs is in average ≥ 99.9 % with a ≥ 95 % confidence level for both the realistic and conservative examination. In summary, Sartorius Stedim Cellca's single cell cloning process provides a well-documented record of cell monoclonality that is in line with regulatory requirements.

Bibliography, Acknowledgements: Special thanks goes to all Sartorius Stedim Cellca employees who contributed to the presented work.

Disclosure of Interest: None declared





PO232

LEGACY CLONING METHODS IN THE MODERN WORLD PART 1: REASSESSING THE CAPILLARY AIDED CELL CLONING TECHNIQUE

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Background and novelty: About 15 years ago the cloning method capillary aided cell cloning (CACC) was employed. This technique was based on the 'spotting' technique of Clarke and Spier (1980) and relied on independent visual confirmation by two scientists of the presence of a single cell in a 1 μ L droplet. This technique was introduced as it enabled a high probability of monoclonality to be achieved in a single cloning round.

Regulatory guidance (ICH Q5D) requires that any cell line used to produce biopharmaceuticals originates from a single progenitor cell. Recently, there has been increased scrutiny of the method(s) used to achieve this requirement, with concerns expressed over certain approaches taken. Here, we review the suitability of the legacy CACC method in light of this changing landscape of expectations.

Experimental approach: We describe the CACC process giving emphasis to understanding potential sources of error and the control measures for the preparation of cells and for the spotting procedure in place to control them.

Results and discussion: A mixing experiment was designed to answer the question 'can droplets which contain more than one particle be distinguished when the CACC method is used'. The study used two very similar cell lines expressing similar antibodies mixed in a 1:1 ratio. Following cloning, only one antibody was observed in samples from the 156 wells verified to contain a single cell and where growth was observed. Analysis of the entire data set showed that a scientist misses a cell infrequently – frequency was in the range 0.4% to 1.3% for this experiment. The observational step using two scientists is appropriate for use with the CACC method. The frequency of observational error does not invalidate use of direct observation methods for cell cloning and supports the conclusion that the single cell seen by both scientists is highly likely to be monoclonal.

Bibliography, Acknowledgements: Hilary Metcalfe and Kunle Onadipe

Disclosure of Interest: None declared





PO233

IMPROVED VECTOR DESIGN EASES CELL LINE DEVELOPMENT WORKFLOW IN THE CHOZN GS^{-/-} EXPRESSION SYSTEM

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Background and novelty: Vector design is a key step in cell line development for the expression of therapeutic biologics. It is essential that the vector design results in high, stable expression of the encoded protein. Other considerations include ease of cloning, stability for propagation in *E. coli* as well as in the mammalian host cell line, and ease of sequence amplification for verification of vector construction and for detection of insertion site and copy number in stably expressing cells. For these reasons, use of the same promoters and polyA tails in dual cassette vectors, as is common for expression of the heavy and light chains of monoclonal antibodies, can be problematic.

Experimental approach: To address these issues, we have modified the promoters, introns, and polyA tails of the light chain and heavy chain expression cassettes in the dual expression vector commonly used for the expression of therapeutic antibodies in the CHOZN GS^{-/-} cell line development platform. The modification of these vector elements minimized sequence similarities between the two expression cassettes. To determine the effect of these modifications, expression levels of fluorescent reporter proteins as well as a recombinant human monoclonal antibody were measured in transiently and stably expressing pools. The top performing vector designs were identified. Stable pools and single cell clones from the lead vector designs and original vector design were generated for comparison of both titer and stability.

Results and discussion: This work has resulted in the identification and characterization of a dual expression vector with minimized similarity between the two expression cassettes easing the cloning, propagation and analysis of vector integration in stable cell lines while maintaining the high, stable expression of the encoded protein of the original vector design.

Bibliography, Acknowledgements: We acknowledge Atum for their contributions to vector design and construction.

Disclosure of Interest: None declared





PO234

OPTIMIZATION OF THE CLC FOR MANUFACTURING PURPOSES USING A NOVEL SINGLE CELL PRINTING TECHNOLOGY

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Background and novelty: To support a rapid drug material supply of an increased number of biotherapeutic molecules that would enter clinical trials, the cell line construction (CLC) process is on the critical path for development of the manufacturing process for drug substance. It also requires compliance with Regulatory Authorities' expectations for the generation of clonal cell lines. Novimmune's approach to support the subcloning is based on a single round of limiting dilution cloning in 96-well plates (96-wpl), associated with imaging of single cells to assure monoclonality. However with the use of limiting dilution, at a low average target number of cells per well (< 1), the proportion of monoclonal cells identified per plate is typically low, as it is dependent on the Poisson distribution. Therefore the throughput using this method can be limited. The use of a single cell printing (SCP) device (Cytena) to replace the limiting dilution process was therefore evaluated. This device dispenses a single cell in each 96-wpl well resulting in an important increase of the number of monoclonal wells obtained compared to the limiting dilution saving both time and resources.

Experimental approach: In order to evaluate the device for use in the subcloning of cells, a mixture of two CHO cell lines exhibiting different fluorescent markers were dispensed using the device to identify the number of wells containing both cell lines in order to demonstrate a high probability of monoclonality and to determine the recovery of cells post subcloning.

Results and discussion: By use of a SCP device, the number of monoclonal cell lines identified could be increased substantially; enabling higher throughput compared to the use of limiting dilution and the resulting cell lines had a high probability of monoclonality.

Bibliography, Acknowledgements: CYTENA GMBH

Disclosure of Interest: None declared





PO235
ANTI-TETANUS NEUTRALIZING HUMAN MONOCLONAL ANTIBODIES

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Background and novelty: Infectious diseases can be treated with antibodies since 1890; today the immunotherapy can move towards to monoclonal antibodies (mAbs). MAbs derived from human B cells are especially important, as generated and tolerized in humans, their safety and efficacy are potentially higher than mAbs from other origin.

Experimental approach: Peripheral blood of donors was collected after revaccination and signed informed consent in accordance with the Human Ethics Committee. B cells were individually isolated by cell sorter in 96-well plates based on the surface immunostaining. The variable regions of the antibodies expressed by single sorted B cells, HC and LC separately, were amplified by RT-PCR and cloned in expression vectors which were used for transient transfection of HEK293-F cells. After purification the recombinant mAbs were analyzed by *in vitro* and *in vivo* neutralization tests.

Results and discussion: Along the work different labeling and sorting strategies were tested for the capture of the rare specific B cells. The staining of the cells with the tetanus toxin (TT) labeled independently with two different markers allowed the separation of specific B lymphocytes. The sequences of variable HC and LC of each pair were analyzed; the VDJ and VJ regions were identified and compared. The expressed mAbs in HEK293-F cells were purified and the binding evaluated by ELISA to TT, tetanus toxoid, recombinant fragment C and the inhibition of the binding of the TT to the ganglioside GT1b. The *in vivo* neutralization assay, performed with Swiss mice in accordance to the potency test used for release of hyperimmune sera, showed that a cocktail of three different mAbs was able to protect the animals by neutralization of the TT when challenged with the antigen.

Bibliography, Acknowledgements: FAPESP grants: FAPESP 2012/14127-0, 2015/15611-0. Fellowships: FAPESP 2011/22334-2, 2016/22402-1, CNPq 311934/2013-7.

Disclosure of Interest: None declared





PO236

A NOVEL SI-RNA AIDED METHOD FOR CHO CELL LINE SELECTION

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Background and novelty: Traditional cell line selection strategies are mainly based on antibiotics resistance selection where the product gene is co-transfected with an antibiotics resistance gene. This approach showed wide success, but still retains some disadvantages: cells are not selected for product expression, but antibiotic resistance. Thus, selection is leaky, as cells expressing the resistance gene, but not the product, will also survive and in fact will have a metabolic advantage. In addition, cells have an translational burden, as they have to designate part of their protein synthesis machinery to the antibiotic resistance.

Experimental approach: Our system utilizes siRNAs that are spliced out from the product gene's mRNA. The siRNAs protect the cells from a suicide gene encoded in an mRNA subsequently transfected which causes non-protected cells to die rapidly. The selection process depends entirely on mRNA transcription of the product to produce the siRNA, thus ensuring their tight co-expression and preventing any leakage. By using an RNA based protection, no translation of a protein is required, allowing more of the translational machinery to be available for the protein of interest in a production environment.

Results and discussion: Transfection with the suicide gene proved to be 100% lethal within 2 days, with no outgrowth over two weeks. Protection by expression of the siRNA was shown to be efficient. Currently a comparison of stable cell line development programs based on siRNA selection and neomycin selection is ongoing. The novel selection system should speed up cell line development, as the system kills rapidly and directly selects for cells transcribing the product gene on a high level. We expect to see more high producers earlier in the process, which will allow for an easier and faster selection in the following steps.

Bibliography, Acknowledgements: Austrian BMWFW, BMVIT, SFG, Standortagentur Tirol, Government of Lower Austria and Business Agency Vienna through the Austrian FFG-COMET- K2.

Disclosure of Interest: None declared





PO237

LEGACY CLONING METHODS IN THE MODERN WORLD PART 2: VALIDATING THE STATISTICAL MODEL FOR CAPILLARY AIDED CELL CLONING

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Background and novelty: The capillary aided cell cloning (CACC) method was introduced to shorten development timelines by achieving a high probability of monoclonality in 1 cloning round instead of the many needed with the limiting dilution method. Regulatory guidance (ICH Q5D) requires that any cell line used for producing biopharmaceuticals originates from a single progenitor cell. Recently, the methods used have faced increased scrutiny, with concerns expressed over certain methods. In this poster we discuss the suitability of the statistical model used with the legacy CACC method in light of this changing landscape of expectations.

Experimental approach: The method is based on the 'spotting' technique (Clarke & Spier, 1980) and relies on independent visual conformation by 2 scientists that there is only 1 cell in a 1 μ L droplet.

Results and discussion: Observations in the visual confirmation step are: 0 cells, 1 cell or >1 cell. Each well has 2 observable outcomes: growth or no growth. The 6 frequencies are compared against values predicted by a statistical model to assess the model's accuracy. The predicted frequency is calculated using a probability that a particular outcome is observed. This probability is a mathematical function of: number of cells per droplet; cloning efficiency; and a probability describing the reported outcome given the actual number of cells in the well. These probabilities are estimated using a function maximisation algorithm. The probability of monoclonality is estimated using the probabilities from the best-fit model. Review of 24 cloning experiments revealed that the probability of monoclonality ranged from 0.98 to 1.00. The industry trend is to combine imaging with cloning to support the claim of monoclonality. The applicability of the statistical model used with for CACC in such a situation will be outlined.

Bibliography, Acknowledgements: Authors acknowledge Drs Hilary Metcalfe & Kunle Onadipe in developing the method.

Disclosure of Interest: None declared





PO238

NEW SINGLE CELL DEPOSITION TECHNOLOGIES WITH INTEGRATED IMAGE ANALYSIS FOR CELL LINE DEVELOPMENT OF PRODUCTION CELL LINES

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Background and novelty: To ensure monoclonality of production cell lines the most common single cell deposition methods in cell line development are two rounds of limited dilution or FACS technologies. A single round of cloning is only accepted by regulatory authorities with images showing a single cell per well after seeding (d0 picture) and additional supportive data i.e. statistical method validation. To overcome the limitations of Limited dilution and FACS (e.g. low cloning efficiency) several new devices for single cell deposition combined with high resolution imaging are currently under investigation or recently reached the market. We tested a single cell printer already on the market as well as a not yet launched new device combining single cell deposition with direct imaging.

Experimental approach: We evaluated the devices for their cloning and single cell deposition efficacy by using CHO cells producing an IgG. We tested freshly transfected cells as well as cell pools also using different fluorescent labelled cells and started a first statistical evaluation to calculate the probability of monoclonality. In addition we developed an integrated image data management and a visualization tool to facilitate the right clone selection based on image data.

Results and discussion: Cloning and single cell efficacy data will be presented showing a much higher single cell efficacy as compared to limited dilution. Cloning efficacy had to be improved and could be reached by optimized protocols. Our statistical calculation shows a good probability of monoclonality. The importance of a high image resolution and the value of an image data base combined with a visualization tool will be shown.

Finally, the results of setting up a single round of cloning with the new technology will be presented.

Bibliography, Acknowledgements: We thank our partners from Cytena GmbH and Solentim Ltd for continuous support.

Disclosure of Interest: None declared





PO239

DESIGN OF A PLATFORM FOR THE EXPRESSION, PURIFICATION AND IN VITRO ASSESSMENT OF SOLUBLE GLYCOSYLATED RECOMBINANT HUMAN STEM CELL FACTOR PRODUCED IN HEK293 CELLS

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Background and novelty: Stem cell factor (SCF) is a growth factor that promotes proliferation, differentiation and survival of hematopoietic stem cells (HSCs). Its potential therapeutic applications include anemia treatment, HSCs mobilization for recovery and transplant, and improvement of transduction efficiency on gene therapy.

Most HSCs *ex vivo* expansion protocols use *Escherichia coli*-derived SCF. However, protein glycosylation is of paramount importance to the efficacy and manufacturing of therapeutic glycoproteins. The aim of this work was to produce and purify glycosylated recombinant human SCF (rhSCF) using HEK293 cells as host.

Experimental approach: Two rhSCF-producing cell lines were generated using different promoter sequences through lentiviral transgenesis of HEK293 cells. Antibiotic selection was performed in order to increase productivity. A C-terminal HisX6 tag was used to purify rhSCF from cultures supernatant by Immobilized Metal-ion Affinity Chromatography (IMAC). Gel densitometry, spectrophotometry quantification and isoelectric focusing were performed. CD34⁺ HSCs were isolated from umbilical cord blood and cell proliferation assays in presence of glycosylated and *E.coli* rhSCF were performed.

Results and discussion: All cell lines culture supernatants evidenced the presence of rhSCF (18 to 48 kDa) in SDS PAGE followed by western blot analysis. rhSCF was produced using highest productivity cell line. After IMAC, rhSCF presented 98% purity and most contaminants were removed with no significant rhSCF loss. Isoelectric focusing showed prevalence of acidic and neutral isoforms (pI between 3 and 7). HSCs culture reached a ten-fold expansion when stimulated with purified rhSCF and a six-fold expansion when supplemented with *E.coli* rhSCF, thus demonstrating an improved proliferative capacity of glycosylated rhSCF.

Bibliography, Acknowledgements: Lennartson and Ronsstrand. Am Physiological Soc (2012)

Disclosure of Interest: None declared





PO240

STUDYING MITOCHONDRIAL METABOLISM IN CHO CELL LINES CONTAINING DIFFERENT HETEROPLASMY VARIANTS.

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Background and novelty: Mitochondria generates chemical fuel (ATP) for the cell through several metabolic cycles. Implications of mitochondria energy generation in established cell lines used by industry has been studied during the last years, using different approaches (1). Differences in respiration have been proved to influence product yield in productive CHO cell lines. Some recent studies have also evidenced the strong influence of mitochondrial heteroplasmy(2), the existence of different mitDNA copies inside the same mitochondria, in cell metabolism. In this study, using heteroplasmy data obtained by previous sequencing, mitochondrial content, activity and gene expression of several industrial productive CHO-cell lines, which exhibited differences in growth rates and different levels of mitochondrial heteroplasmy, were analyzed.

Experimental approach: Five different CHO-cell lines, with varying growth phenotypes were cultured in serum-free media in batch mode and samples were collected daily. Cell growth was monitored by flow cytometry. Mitochondrial content was assessed using Mitotracker green probe. Oxygen consumption was measured using a respirometry assay. Finally, the expression of different genes belonging to respiratory chain complexes such as COX2 was evaluated by qPCR and Western Blot.

Results and discussion: Differences in mitochondrial content between fast and slow growing cell lines were observed. Different cell lines exhibited different levels of oxygen consumption with no apparent relationship with their observed growth phenotype. Finally, COX2 expression levels also showed a distribution independent of the cell line growth phenotype. These results represent a first step into establishing a relationship between heteroplasmic mutations and changes in metabolism or phenotype.

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2. Casoli *et al.*,. Front Aging Neurosci, 7:pe 142.

Disclosure of Interest: None declared





PO241

APPLICATION OF LARGE SCALE MODELLING OF THE PROTEIN BIOMASS OBJECTIVE IN CHO CELLS USING ENHANCED PULSE SILAC AND PROTEOMIC RULER APPROACH

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Background and novelty: Although Chinese hamster ovary (CHO) cells have been used to produce recombinant proteins (rPs) for decades, there is still limited understanding of the regulation of their intracellular activities. It is important to quantitatively characterize functional behaviour of CHO cells in the culture to improve both growth and productivity.

Experimental approach: In this study we have applied large scale modelling of the protein biomass objective in a parental and a stable monoclonal antibody producing CHO cell line by integrating enhanced pulse SILAC¹ derived protein turnover data with the estimation of individual protein copies using proteomic ruler approach². Downstream bioinformatics processing was performed using publicly available databases including Uniprot and CHOgenome.org. The proteins have been further functionally annotated using Gene Ontology and crucial pathways have been examined in detail using KEGG (Kyoto Encyclopedia of Genes and Genomes).

Results and discussion: CHO cells turn over the majority of the proteome within their generation time. By integration of the protein turnover and protein copy data, we have established where CHO cells focus most of their synthesis and degradation machinery to achieve specific biomass objective – whether it is purely the growth and division or the secretion of the monoclonal antibody. Further linking of the rates of protein turnover with transcriptomic data will enable the development of novel genetic and metabolic engineering strategies.

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Disclosure of Interest: None declared





PO242

CHO CELL CLONE STABILITY ASSESSMENT USING FUNCTIONAL PHENOTYPE ARRAY PROFILING

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Background and novelty: Instability of recombinant protein production from CHO cell lines remains a serious bioprocess concern [Mariati et al, 2014]. Stability is loosely defined as a loss of titer over a defined period of time (typically >60 generations). There is no consensus definition of an “unstable” cell line in industry, nor are there clear regulatory guideline on what constitutes a stable cell line. Many companies find that a substantial proportion of their candidate clones fail due to instability problems, having initially demonstrated good production performance in small-scale bioreactors.

Experimental approach: It was hypothesized that unstable phenotypes could be predicted by observing time dependent changes in CHO cell responses to an evidence-based panel of small molecule chemical stresses e.g. Sodium Butyrate Rapamcin, BrefeldinA, Cycloheximide, and NaCl. To investigate this, a panel of CHO cell lines which had been previously characterized as being stable [n=16] and unstable [n=16] were obtained and cellular responses [should we be more specific here] to a suite of chemical stressors were measured at various time points from generation 14 to 49. This data was used in a multivariate modeling approach to discriminate stable and unstable phenotypes.

Results and discussion: Using this approach, we demonstrated that by measuring changes in chemical stress response over time it was possible to differentiate stable and unstable producing cell lines using a simple LDA modeling approach. It is predicted that this phenotypic stress response stability screening coupled with our modeling approach could potentially shorten and simplify stability screening studies. Using a simple model, an 85% separation success could be achieved which correctly classified 27/32 clones as either stable or unstable.

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Disclosure of Interest: None declared





PO243

CHO CELL CLONE IDENTIFICATION AND TRACKING USING FUNCTIONAL PHENOTYPE ARRAY PROFILING

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Background and novelty: Chinese hamster ovary (CHO) cells remain the most popular choice of production host for biopharmaceuticals. There is a growing pressure to guarantee cellular identity from regulators and publishers. Current methods [e.g. SNP, STR, CO1 barcoding, etc] are expensive, time consuming and may not be able to differentiate phenotypically different cells with a common parental origin, such as those derived through clonal selection.

Experimental approach: It was hypothesized that 'phenotypic fingerprinting', as derived from CHO cell responses to an evidence-based panel of small molecule chemical stresses e.g. one of the high ranking chemicals contributing to ValitaID Cobalt Chloride, could be used as a rapid and facile technique for cell line identification and identity assurance. To investigate this, the "phenotypic fingerprints" of a panel of CHO cell lines were taken and discriminatory models were built and 'blind tested' for cell identification.

Results and discussion: Using novel shrinkage discrimination identification algorithms we demonstrate that CHO cells can be uniquely identified down to a clonal level with confident identification possible between clonal derivatives of a common parent. Using our SDA models we were able to uniquely identify 17 out of 21 distinct clones of varying degrees of relatedness. We believe this technology could have utility for demonstrating the identity of 'genetically similar' CHO clones and moreover, it may be useful to track the "phenotypic identity" of a clone through stages of bioprocess development such as scale up or changed media/feed regime to ensure process changes don't substantially impact clone phenotype, i.e the cell develops a "non-self" phenotype.

Bibliography, Acknowledgements: We gratefully acknowledge the support of MedImmune during this work.

Disclosure of Interest: None declared





PO244

HIGH-THROUGHPUT QUANTITATION OF FC CONTAINING RECOMBINANT PROTEINS IN CELL CULTURE SUPERNATANT BY FLUORESCENCE POLARIZATION SPECTROSCOPY

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Background and novelty: ‘Quality by design’ concepts are becoming increasingly important in biomanufacturing to gain control of critical quality attributes of the product. This necessitates the ability to rapidly measure multiple bioprocess attributes simultaneously in a high-throughput manner. This study highlights the suitability of FP assays for use in bioprocess development and control due to the simplicity of assay development and the ease of use to enable measurement of molecular interactions (typically a specific ligand and a molecule of interest such as IgG, host cell protein, glycoform etc) in the liquid phase, to give instant quantitation of a molecule of interest with no need for washing, lengthy incubation periods or complex equipment.

Experimental approach: Here we describe a simple, rapid (<3 min per 96 samples) assay that enables high-throughput quantitation of recombinant IgG, and Fc containing IgG derivatives, in mammalian cell culture supernatant using microplate fluorescence polarization (FP) spectroscopy. The solution-phase FP assay is based on the detection of immunoglobulin Fc domain containing analyte binding to FITC-conjugated recombinant Protein G ligand to measure analyte concentration dependent changes in emitted fluorescence polarization.

Results and discussion: Air-dried assay microplates containing pre-formulated ligand that is re-solubilized on addition of analyte containing solution did not affect assay performance, typically yielding an across plate coefficient of variation of <1%, and a between-plate standard deviation below 1%. Comparative assay of the same samples by FP and other commonly used IgG assay formats yielded a coefficient of determination >0.99 in each case.

Bibliography, Acknowledgements: We would like to gratefully acknowledge the support of MedImmune during this work.

Disclosure of Interest: None declared





PO245

CELL LINE ENGINEERING USING MIRNAS IN A NOVEL HUMAN PRODUCTION CELL LINE

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Background and novelty: Protein-based drugs, biopharmaceuticals, are most frequently produced in Chinese hamster ovary (CHO) cells, because these cells are quite robust, grow in defined serum-free media and produce high product titers. However, the post-translational modifications are non-human and CHO cells struggle with complex glycoproteins or new complex formats. Therefore, novel human production cell lines, such as CEVECs amniocyte production (CAP), are under development for the production of such difficult-to-express proteins. A novel cell line engineering strategy involves endogenous small RNAs such as microRNAs (miRNAs) that are able to regulate whole cellular pathways without adding further translational burden to the cell. We recently developed a high-content screen to identify pro-productive miRNAs. The top pro-productive miRNAs are currently in use for the first time to engineer the novel CAP cell line.

Experimental approach: Out of the high-content screen, 12 miRNAs were chosen to establish and analyze stable miRNA overexpression cell lines using CAP. The pre-miRNA was cloned into a modified miRNA expression vector, transfected into CAP-mAB, and stable cell pools selected using Puromycin treatment. miRNA overexpression was analyzed by qPCR and the effect on productivity tested in batch experiments. For the best pro-productive miRNAs the mechanism of action is currently analyzed by transcriptomic approaches.

Results and discussion: For most of the 12 miRNAs an overexpression of the mature miRNA could be achieved. Batch experiments identified several miRNAs that improved volumetric productivity. In addition, one miRNA turned out to show cytostatic effects as growth and productivity were severely inhibited. Analysis of the targets of the miRNAs by transcriptomic approaches will soon reveal the mode of action of pro-productive miRNAs as well as target genes that might be beneficial for productivity.

Bibliography, Acknowledgements: -

Disclosure of Interest: None declared





PO246

DEVELOPING ALTERNATIVES TO CHINESE HAMSTER OVARY (CHO) MEDIA FOR SINGLE CELL CLONING

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Background and novelty: Single-cell cloning is an essential step used by the biopharmaceutical industry in the upstream development of transformed cell lines for therapeutic protein production. Decreased cloning efficiencies cost the industry time & money and limit the pool of candidate colonies for large-scale culture. To address this problem, we aimed to develop a highly-efficient serum-free media suitable for optimising single cell cloning by studying CHO conditioned media.

Experimental approach: Conditioned media was collected from attached and suspension cultures growing in the commercial media CHO-S SFM-II (Gibco). Samples were assessed for efficacy using a Limited Dilution Cloning assay, examining the CHO-K1 colony formation, which allowed for the identification of the highest-performing products. CHO-K1 cells were chosen as the test line due to their widespread use in industrial antibody production. Successful media candidates were subsequently screened using additional CHO cell lines.

Results and discussion: From the CHO-K1 tests, four successful conditioned media samples were isolated:

SFMII media conditioned by suspension CHO-K1; SFMII media and Poly Vinyl Alcohol conditioned by suspension DG44; SFMII media conditioned by suspension CHO-S and SFMII media conditioned by adherent CHO-S.

The CHO-K1 product is the most well-characterised to date, improving cell cloning efficiency for DG44 cells (avg. increase>50%) and CHO-S cells (avg. increase>3-fold) and also the adherent CHO-K1 cell line growing in ATCC+5%FBS, indicating that this product works well for attachment-independent lines, as well as for multiple cell types.

We feel that these early-stage products may increase cloning efficiencies during upstream CHO cell line development, resulting in financial savings for the industry.

Bibliography, Acknowledgements: U Ming Lim et al, Identification of Autocrine Growth Factors Secreted by CHO Cells for Applications in Single-Cell Cloning Media, *J. Proteome Res.* 2013, 12, 3496–3510

Disclosure of Interest: None declared





PO247

EXPEDITING UPSTREAM STAGES OF PROTEIN BIOMANUFACTURE THROUGH THE USE OF UBIQUITOUS CHROMATIN OPENING ELEMENTS - UCOES

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Background and novelty: The main rate-limiting step in the upstream stages of protein biomanufacture is the isolation of stable, high producing cell clones. Ubiquitous Chromatin Opening Elements (UCOEs) consist of at least one promoter region with associated methylation-free CpG island from housekeeping genes; they possess a dominant chromatin opening capability and thus confer stable transgene expression. UCOE-viral promoter based plasmid vectors reduce the time it takes to isolate high, stably producing cell clones. Although some UCOE-viral promoter combinations have been tested, they have not been evaluated in Chinese hamster ovary (CHO) cells.

Experimental approach: Plasmid vectors containing combinations of either the human HNRPA2B1-CBX3 UCOE (A2UCOE) or murine Rps3 UCOE linked to different viral promoters (hCMV, gpCMV, SFFV) driving expression of an eGFP reporter gene were functionally analysed by stable transfection into CHO-K1 cells and expression analysed by flow cytometry and qPCR to determine vector copy number.

Results and discussion: The results at 21 days post-transfection and selection clearly indicate that the Rps3 UCOE-gpCMV and -hCMV combinations give the highest transgene expression. The A2UCOE-hCMV/gpCMV constructs were the next efficacious but 2-fold lower than the Rps3 UCOE vectors. The SFFV promoter linked with either of the two UCOEs was the least effective with expression levels 17-fold lower than the Rps3-CMV constructs. The Rps3 UCOE-gpCMV/hCMV constructs are now being further modified to include elements that will provide optimal post-transcriptional pre-mRNA processing (splicing, polyadenylation, transcription termination, mRNA stability) thereby maximising stable cytoplasmic transgene mRNA levels and protein production. Results from these second generation Rps3-CMV vectors will also be presented.

Bibliography, Acknowledgements: Antoniou, M., et al (2003). *Genomics* 82(3):269-279.

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Disclosure of Interest: None declared





PO248

NEW POOL GENERATION PROCESS CLD 2.0

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Background and novelty: The production of recombinant proteins in a heterologous host is highly important in modern biotechnology, with Chinese Hamster Ovary cells being the most commonly used system. Through recent advances, especially in media development and process optimization, product titers as high as 10 g/L were achieved in the pharmaceutical industry. Nevertheless, there is a substantial number of difficult-to-express products for which it is very challenging to generate good producing cell lines. Based on our CHO DG44/DHFR platform technology, we developed an improved cell line generation approach in order to specifically deal with such molecules.

Experimental approach: Our standard cell line development approach comprises a selection phase applying very low selective pressure (selective medium or 2.5 nM MTX, respectively) followed by an amplification step with 30 nM MTX. We innovated our cell line development process by increasing initial selection stringency using 2.5, 5 and 10 nM MTX, thereby allowing the omission of the 30 nM MTX amplification step. The new approach was developed to specifically target difficult-to-express proteins, which we observed to be in many cases more susceptible to increased MTX levels and thus not amplifiable with 30 nM MTX.

Results and discussion: By omitting the additional 30 nM MTX amplification, reliability of cell line development for difficult-to-express proteins as well as the obtained titers were clearly increased. As a positive side effect, the timeline for the pool generation process was reduced by around 35 % and the workload was decreased by around 40 %. Moreover, we demonstrated that the production stability of cell lines generated with the new approach is not hampered by the applied lower MTX levels.

Bibliography, Acknowledgements:

Disclosure of Interest: None declared





PO249

LEVERAGING THE CHO CELL TOOLKIT TO ACCELERATE BIOTHERAPEUTICS INTO THE CLINIC

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Background and novelty: High quality biopharmaceuticals, in particular in areas of unmet need, need to be delivered to patients as quickly as possible. As cell line development historically has been a rate limiting step, multiple approaches, including utilization of uncloned pools and targeted integration, have been utilized to produce toxicological and even clinical drug substance in order to decrease timelines.

Experimental approach: We have addressed this challenge differently by developing a dual selection GS (-/-) and DHFR (-/-) double knock-out CHO host cell line.

Results and discussion: The dual selection host is capable of reaching uncloned pool titers that are up to 10 fold higher than our previous host. Ultimately, the final clones are about 50% more productive than any from the single selection host systems in our host toolkit. This dual selection host also has potential to offer flexibility to bioprocess development through the amplification process, which may allow for increased specific productivities and lower biomass. By having a productive host line, timelines can be shortened by utilizing the highly productive pool or a mixture of high performance clones. This host also offers the advantage of a consistent product quality profile throughout the development process, such that use of material from these early cell populations will yield very comparable product quality to the final clones. This lends itself to enabling a 'fast to tox' strategy whereby toxicology studies can be performed with representative material from an earlier cell population, thus speeding up the clinical timelines.

Bibliography, Acknowledgements: Our new dual selection host offers robust and consistent performance that enables a highly productive, flexible process and faster clinical timelines.

Disclosure of Interest: None declared





PO250

HIGH-THROUGHPUT PLATFORM FOR DESIGN OF MULTI-COMPONENT SYNTHETIC DNA ASSEMBLIES FOR CHO CELL ENGINEERING

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Background and novelty: Engineering cellular functions relevant to the performance of CHO cell factories requires the simultaneous modulation of multiple genetic effectors. To create multi-component synthetic DNA assemblies, two fundamental design criteria should be addressed; (i) selection of the specific and minimal combination of components that operate in synergy to achieve the desired objective, and (ii) determination of their optimal relative functional stoichiometry.

Experimental approach: We report the development and validation of a standardised high-throughput platform enabling simultaneous, micro-scale, transient transfections of CHO cell cultures, each with multiple plasmids (utilising a common backbone) encoding different genetic effectors at varying relative stoichiometry, interfaced with rapid evaluation of transfected cell growth/productivity. Microplate electroporation was optimised for DNA load, cell number and viability to achieve high transfection efficiency (99%), reproducibility (CV~10%) and titratable gene expression.

Results and discussion: To quantitatively confirm multi-plasmid transfection of individual cells at the pre-specified stoichiometry, co-transfections with three plasmids, each encoding a fluorescent protein, were analysed by flow cytometry. After statistical normalisation with respect to the inherent fluorescent protein brightness, analysis of the data showed a high proportion of transfected cells conformed to the expected ratio, validating the utility of the platform to assess the combined effect of multiple co-transfected effectors. The platform has been utilised to test potential genetic effectors of IgG expression either individually or in combination using Design of Experiments. We show enhanced productivity is dependent on the relative titration of co-expressed genetic effectors. The platform is an effective strategy to design novel multigene assemblies for CHO cell engineering.

Bibliography, Acknowledgements: BRIC, MedImmune

Disclosure of Interest: None declared





PO251

GENOME EDITING TO CREATE ENHANCED MAMMALIAN BIOMANUFACTURING PLATFORMS

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Background and novelty: Biopharmaceuticals are predominantly manufactured in Chinese Hamster Ovary (CHO) cells, and whilst media and platform optimisations have been successfully implemented to boost cell line productivity to the g/L range, the demand for cost-effective biopharmaceuticals continues to increase. The aim of this project is to use genome-editing tools to create a toolbox of engineered CHO cells with enhanced biomanufacturing capabilities.

Experimental approach: A new commercially available CHO-K1 GS^{-/-} host cell line (HD-BIOP3, Horizon Discovery) has been used to generate gene knockouts for Bax, Bak and a metabolic enzyme (ME1) both singly and in combination using CRISPR/Cas9 technology.

Results and discussion: Single knockout clones showed an extension to culture longevity (on average: Bax+1, Bak+2, ME1+2 days) with no effect to VCD. Bax/Bak double knockouts were subsequently generated by re-targeting Bak clones, which produced an additive effect to longevity (+5 days) and showed evidence of inheritability in both growth and metabolic properties (e.g. Glucose, Lactate). GC-MS metabolomics analysis identified changes to metabolite profiles that were found to be both incremental and additive (e.g. citrate and succinate in Bax/Bak edits), but also gene-specific changes such as increased malate in Bax and decreased waste products in WE1. A 1.6-fold increase in transient IgG titre was observed in Bak and WE1 edits over a 10-day batch culture, whilst the Bax edit decreased titre by 50%. The Bax/Bak edit showed no increase in titre, however both Bax/Bak and WE1 showed an increase in specific productivity of 1.6 and 1.7-fold respectively. These data highlight the importance of metabolic networks in biopharmaceutical production and an understanding of the molecular basis for these phenotypic changes will help define future engineering targets.

Bibliography, Acknowledgements: This collaboration is funded as part of the IB Catalyst Programme with financial support from the BBSRC and Innovate UK.

Disclosure of Interest: None declared





PO252

CELL PRINTING: A FLUIDICS APPROACH TO SINGLE CELL CLONING FOR MANUFACTURING CELL LINES.

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Background and novelty: Cell line development processes deliver cell lines that secrete high yields of biotherapeutic proteins with the required product quality. To meet regulatory requirements, manufacturing cell lines need to be shown to have been derived from a single progenitor cell ie monoclonal. Traditional approaches to single cell cloning include limiting dilution cloning, plating into semi-solid media and fluorescence activated cell sorting. However, advancements in microfluidics are now being harnessed for cell culture applications that include cloning and have led to the development of a new generation of instruments. The microfluidics-based Single Cell Printer (SCP) from Cytena integrates single cell imaging and deposition into microwell plates and offers a simplified workflow for cell cloning.

Experimental approach: In this presentation, we combine the power of the SCP with a high content imaging system (Cellavista, Syntec) to develop a novel cell cloning platform that provides a viable alternative to more traditional cloning methods. We describe the strategy for method development and optimisation including investigation of the culture parameters for the starting transfectant pools, optimisation of the recovery medium and imaging protocols.

Results and discussion: The evaluation results show that the SCP platform can achieve efficient deposition of single CHO cells into microwell plates and monoclonality can be validated by imaging post-printing. Moreover, colony recovery from the single cells was efficient and fitted with automation workflows, whilst the cartridges used for the microfluidic cloning were disposable allowing cost-effective segregation between projects. The implementation of this novel technology results in the isolation of cell lines with a high level of monoclonality with a single round of cloning, and provides significant advantages over traditional methods with respect to cost, simplicity, timings and flexibility.

Bibliography, Acknowledgements: N/A

Disclosure of Interest: None declared





PO253

ULTRA-DEEP NEXT GENERATION MITOCHONDRIAL GENOME SEQUENCING REVEALS WIDESPREAD HETEROPLASMY IN CHINESE HAMSTER OVARY CELLS.

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Background and novelty: Recent sequencing of the Chinese hamster and Chinese hamster ovary (CHO) cell genomes has dramatically advanced our ability to understand the biology of mammalian cell factories for biopharmaceutical production. In this study, we have focused on the powerhouse of the CHO cell, the mitochondrion.

Experimental approach: Utilizing a high-resolution next generation sequencing approach we sequenced the Chinese hamster mitochondrial genome for the first time and surveyed the mutational landscape of CHO cell mitochondrial DNA (mtDNA).

Results and discussion: Depths of coverage ranging from ~3,319X to 8,056X enabled accurate identification of low frequency mutations (>1%) revealing that mtDNA heteroplasmy is widespread in CHO cells. The frequency of common predicted loss of function mutations varied significantly amongst the clones indicating that heteroplasmic mtDNA variation could lead to a continuous range of phenotypes and play a role in cell to cell, production run to production run and indeed clone to clone variation in CHO cell metabolism. Experiments that integrate mtDNA sequencing with metabolic flux analysis and metabolomics have the potential to improve cell line selection and enhance CHO cell metabolic phenotypes for biopharmaceutical manufacturing through rational mitochondrial genome engineering.

Bibliography, Acknowledgements: The authors gratefully acknowledge funding from Science Foundation Ireland (grant refs: 13/SIRG/2084, 13/IA/1841 and 13/IA/1963) and the eCHO systems Marie Curie ITN programme (grant ref: 642663). The authors would also like to acknowledge Lin Zhang (Pfizer Inc.), Scott Estes, Chapman Wright and Brian St. Germaine (Biogen Inc.) for access to CHO cell lines.

Disclosure of Interest: None declared





PO254

NEXT GENERATION RNA SEQUENCING REVEALS EXTENSIVE ALTERNATIVE SPLICING OF THE CHO CELL TRANSCRIPTOME FOLLOWING TEMPERATURE SHIFT.

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Background and novelty: While progress towards rational genetic engineering to improve manufacturing efficiency has accelerated following publication of the Chinese hamster (CHO cell) genome in 2011, the assembly and annotation of this sequence is at a relatively early stage. The most widely used physiological change in industrial cell culture is a reduction of temperature from 37°C to 31°C to extend production. In this study, we have utilised RNA-Seq to gain a deeper understanding of “temperature shift” and improved the characterisation of the CHO cell transcriptome.

Experimental approach: Acquisition of >50 million 150bp paired-end reads per sample enabled a high quality *de novo* transcriptome to be assembled using *Trinity* and annotated using *Trinotate*. Reads were aligned to the Chinese hamster genome and transcriptome using *HISAT2*. *DESeq2* and *DEXSeq* were used for the identification of differentially expressed genes and exon usage respectively. The *StringTie* and *Ballgown* algorithms were utilised to identify differential transcript expression.

Results and discussion: We have identified alterations in both gene and isoform expression upon induction of mild hypothermia in CHO cells. >2,000 differentially expressed transcripts were detected following temperature shift and ~1,500 genes exhibited differential exon usage. Through this analysis we have also improved annotation of the transcriptome, assembling >60,000 CHO cell transcripts and have highlighted a number of potential cell line engineering routes.

Bibliography, Acknowledgements: The authors wish to acknowledge funding through the Science Foundation Ireland SIRG programme (13/SIRG/2084) and the Marie Curie ITN programme (grant ref: 642663).

Disclosure of Interest: None declared





PO255

GENOME-SCALE MODELING APPROACH FOR IN SILICO ANALYSIS OF CHO CELL METABOLIC NETWORK

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Background and novelty: Chinese Hamster Ovary (CHO) cells are the predominant expression system for large-scale production of complex biopharmaceuticals. However approaches to enhance production yield remain limited due to a lack of understanding of CHO intracellular activities and industrial strategies for upstream process development are mainly based on empirical results. The introduction of simulation by computer is an efficient way of characterizing CHO cells internal metabolic behaviors.

Experimental approach: Genome scale models (GSMs) are reconstructed from the information present in the genome and literature. The predictive capabilities of the model can be examined by constraints-based flux analysis. The model used in this study is a GSM for CHO DG44 cells, consisting of 4526 reactions¹. The objective was to test the robustness of the model to be able to simulate the behavior of cells under different conditions and to get a preview on the impact of different feeding strategies on growth rate and antibody production in fed batch cultures.

Results and discussion: The robustness of the model was evaluated by increasing the specific productivity and observing the other fluxes variations, which were abnormally high. Some fluxes were responsible for futile cycles, transporting metabolites back and forth in different cell compartments. These irregularities were corrected manually with modifications of reversibility of reactions involved in central metabolism, by deleting multiple transport reactions aiming at importing amino acids from extracellular medium and by using parsimonious Flux Balance Analysis. This preliminary study allowed to identify the most important corrections needed to confront the model with experimental data.

Bibliography, Acknowledgements: 1. Hooman Hefzi et al., "A community genome-scale model of Chinese Hamster ovary cell metabolism identifies differences in the efficiency of resource utilization for various bioprocesses" in "Cell Culture Engineering XV", 2016

Disclosure of Interest: None declared





PO256

IDENTIFICATION OF INTEGRATION SITES OF CHO GENOME FOR THE GENERATION OF HIGH PRODUCER CELLS BY CRISPR/CAS9 MEDIATED TARGET INTEGRATION

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Background and novelty: CHO cells have become the most popular platform for production of therapeutic proteins. However, the generation of high-producer cells is a time-consuming and labor-intensive process that requires the screening of large amount of cells to get a clone of high titer and stability. Since the expression titer and stability of clone is highly dependent on the site of integration, we demonstrated a new cell line development strategy by using NGS to identify the integration site and using CRISPR to generate the target integrated high producing cell lines.

Experimental approach: To identify the high expression sites in the CHO cells, we employed NGS to analyze the integration sites of a high producing cell line (titer > 3g/L). The pair-end reads with one read mapped to the vector and the other read mapped to the CHO reference genome are extracted to identify the integration sites. To test the expression activity of the integration sites, we employed CRISPR/Cas9 to specifically integrate the antibody gene into CHO genome for expression.

Results and discussion: Our data showed 4 integration sites are in the high producing cell line. Among the 4 integration site, IS1 integration site was tested by CRISPR/Cas9 for target integration of antibody gene for expression. The IS1-target integrated cell pool present higher expression titer than cell pool generated by target integration into other integration sites. Furthermore, IS1-target integrated cell pool had less copy numbers than other integration sites. After normalization with copy numbers, The IS1 integration site can reach 130 mg/L/copy which is higher than other integration sites. This approach will cost less time and labor to generate a high producing cell lines. The active integration site will serve as a platform like a cassette player for therapeutic antibody production.

Bibliography, Acknowledgements: This study is funded by Ministry of Economic Affairs of ROC

Disclosure of Interest: None declared





PO257
SYNTHESIS OF THE CHO CELL PROTEOME

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Background and novelty: We have used pulsed stable isotope labelling with amino acids in cell culture (pSILAC) MS-based proteomics to measure the turnover of >4000 individual proteins in CHO cells over five orders of magnitude. Mining this new resource will enable targeted identification of redundant genes/pathways, underpin directed cell and vector engineering and inform computational model based prediction of CHO cell functional performance.

Experimental approach: CHO cells sampled through batch culture containing proteins labelled in either light or heavy isotope-labelled Arg and Lys were media exchanged (i.e. heavy to light and vice versa). Post isotope switch the rate of turnover of individual proteins was determined by measurement of isotopic ratio in tryptic peptides and intensity based absolute protein quantification using a Q-Exactive HF mass spectrometer.

Results and discussion: (i) Protein biosynthetic activity in CHO cells is significantly biased towards relatively few essential proteins which account for a significant proportion of protein biosynthetic activity.

(ii) Several proteins synthesized at a relatively high rate have reported functions apparently irrelevant to CHO cell function *in vitro*.

(iii) The relative stoichiometry at which discrete proteins are synthesised within functional groups has been analysed during batch culture.

(iv) Utilisation rates of individual isoacceptor tRNAs and amino acids generally align with CHO genomic codon use bias and media amino acid composition although fractionation of the proteome according to protein synthetic rate revealed specific alterations in component utilization rate.

Bibliography, Acknowledgements: BBSRC Bioprocessing Research for Industry Club

Disclosure of Interest: None declared





PO258

METABOLIC PROGRAMMING OF CHO CELLS VARYING IN CELLULAR BIOMASS ACCUMULATION DURING FED-BATCH CULTURE

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Background and novelty: CHO cell based biopharmaceutical manufacturing process development still relies on intensive empirical screening of engineered clonal isolates to select production cell lines ideally capable of both rapid proliferation and extended maintenance of high viable cell biomass *in vitro*. However, there is still a relatively poor understanding of the underpinning metabolic programs that enable CHO cells to achieve both rapid proliferation and high capacity biomass synthesis, both of which support high volumetric product titer in biphasic production processes.

Experimental approach: Using microplate based measurement of oxygen consumption and extracellular acidification we comparatively dissect both mitochondrial and glycolytic functions of numerous discrete CHO subclonal lineages derived from extended evolutionary subculture (>200 generations) which vary in their ability to create and maintain cellular biosynthetic capacity during fed-batch culture.

Results and discussion: CHO cells capable of the optimal combination of rapid exponential proliferation and extended maintenance of high cell biomass concentration utilise an infrequent yet conserved metabolic program that significantly increases cellular glycolytic and mitochondrial oxidative capacity at the onset of stationary phase culture. Our data reveal that for the vast majority of isolated subclonal populations, metabolic phenotype and culture performance drift over extended subculture, with only a small proportion of subclones exhibiting functional stability. Taken together, we show that (i) exploitation of functional diversity in metabolic programs arising through evolutionary subculture is a logical route to obtain parental CHO hosts with significantly improved ability to synthesize biomass and (ii) cell engineering strategies to holistically improve CHO cell culture performance should target control of mitochondrial biogenesis and oxidative metabolism.

Bibliography, Acknowledgements: Conacyt

Disclosure of Interest: None declared





PO259

CHO MEDIA PLATFORM FACILITATES INTEGRATED CELL LINE DEVELOPMENT AND MEDIA OPTIMIZATION

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Background and novelty: The development process for a biotherapeutic begins with generating a high-performing stable cell line which can be used for manufacturing. After identifying top clones, optimization of the culture media is essential to maximize performance. Together this labor-intensive work typically requires the longest timeframe during development and strategies to shorten and streamline this work are in demand. Here we present an accelerated approach which integrates cell line development with the media optimization by implementing a CHO media platform.

Experimental approach: GS-CHO cells adapted in BalanCD CHO Growth A (BCGA) media were transfected with Trastuzumab and Rituximab using Transfectory CHO media. Stable pools were established by removal of Glutamine and treatment with MSX in BCGA. Clones were generated using semi-solid cloning in 96-well plates in CloneMedia CHO Growth A. Approximately 200 single-cell wells were identified per antibody using the CloneSelectImager. A liquid media overlay was assayed for secreted antibody by ELISA. The top 50% of clones based on titer were expanded in BCGA and a subsequent screening was performed in 24-well plates. The top 20 clones per antibody were evaluated under platform fed-batch protocol in shake flasks for productivity and product quality. Feed media strategy was optimized using spent media and metabolite analysis.

Results and discussion: The semi-solid cloning efficiency was ~12%, facilitating reasonable throughput while providing imaging data for monoclonality. The top five Trastuzumab clones achieved harvest titers between 1.8 – 2.3 g/L under fed-batch conditions and Rituximab clones between 1.4 - 1.8 g/L. Optimization of the feed media strategy further increased the titer of a top clone. Clones were selected in BCGA eliminating the need to adapt and survey growth media. This integrated process from gene to top clones with optimized media strategy was completed within 8 months.

Bibliography, Acknowledgements: .

Disclosure of Interest: None declared





PO260

HIGH-THROUGHPUT LIPIDOMIC AND TRANSCRIPTOMIC ANALYSIS TO COMPARE SP2/0, CHO, AND HEK-293 MAMMALIAN CELL LINES

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Background and novelty: A combined lipidomics and transcriptomics analysis was performed on mouse myeloma SP2/0, Chinese hamster ovary (CHO), and human embryonic kidney (HEK) cells in order to compare widely used mammalian expression systems. These mammalian expression systems are the preferred cell types for biologic drug production. However, the production yields and efficiency remain a challenge for biopharmaceutical manufacturing. It is therefore valuable to explore the physiological pathways of these cells for future exploitation and enhancement.

Experimental approach: In this study, multiple lipid profiling techniques, including thin layer chromatography (TLC) and mass spectrometry (MS) were applied for the characterization of key lipid metabolites and pathways. Also transcriptomics was used to find out the differentially expressed genes and key enzymes in between these three cell lines.

Results and discussion: Initial TLC analysis indicated that phosphatidylethanolamine (PE) and phosphatidylcholine (PC) were the major lipid components in all cell lines with lower amounts of sphingomyelin (SM) in SP2/0 compared to CHO and HEK, which was subsequently confirmed by MS analysis. HEK contained 4–10-fold higher amounts of lyso phosphatidylethanolamine (LPE) and 2–4-fold higher amounts of lyso phosphatidylcholine (LPC) compared to SP2/0 and CHO cell lines. The SP2/0 cell line exhibited 30–65-fold lower amounts of SM principally in the amount of 16:0. By mapping the transcriptomics data to KEGG pathways, we found expression levels of key enzymes contributing to the differences in LPE, LPC and SM. Results provided insights that will aid understanding of the physiological and secretory differences across recombinant protein production systems.

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Disclosure of Interest: None declared





PO261

THE DEVELOPMENT OF A TARGETED INTEGRATION CHO HOST FOR CLINICAL & COMMERCIAL CELL LINE DEVELOPMENT

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Background and novelty: Unlike conventional random integration (RI) cell line development, the targeted integration (TI) cell line development process introduces the transgene at a predetermined “hot-spot” in the CHO genome with a defined copy number. TI cell line development is a more controlled and predictable process compared to RI, and the TI cell lines developed are likely to exhibit fewer of the undesired phenotypes that are often associated with RI cell lines.

Experimental approach: In this study, we performed a genome wide screening and established a CHO TI host with a CRE/LoxP recombinase-mediated cassette exchange (RMCE) system for mAb expression.

Results and discussion: Using a novel two plasmid RMCE strategy, we have created TI cell lines that have productivity similar to conventional RI cell lines. In addition, our TI cell lines have a better stability profile and lower chance of sequence variants than RI cell lines.

Bibliography, Acknowledgements: Amy Shen, Brad Snedecor, Michael Laird

Disclosure of Interest: None declared





PO262

MODEL-DRIVEN STRAIN DESIGN OF CHO CELLS FOR BIOTHERAPEUTIC PROTEIN PRODUCTION

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Background and novelty: Chinese hamster ovary (CHO) cells are the preferred choice for biotherapeutic protein production as more than 5 of the top 10 drugs are produced using them. While the biopharmaceutical industry has overcome the low product yields of the early years via bioprocess optimizations to increase cell culture density and longevity, ensuring consistent high product quality still remains a major challenge as the molecular basis of protein production in CHO cells are yet to be discerned completely.

Experimental approach: In order to overcome such issues, recently we propose the “Mammalian Systems Biotechnology” paradigm which employs integrative multi-omics data and model-driven approaches to study and rationally design/engineer mammalian cells for the efficient production of recombinant therapeutic proteins.

Results and discussion: To demonstrate the successful application of proposed approach, here we exploited the transcriptome based in silico strain design strategies developed by our group earlier, namely Transcriptomics based strain optimization tool (tSOT) [1] and Beneficial Regulator Targeting (BeReTa) [2]. These methods combine high throughput omics data and genome-scale model [3] to identify both local and global cellular engineering targets that can be manipulated for the overproduction of desired product such as recombinant proteins. The initial validation of model-driven analysis targets show a significant increase in product yields in silico, highlighting the useful nature of newly designed computational tools.

Bibliography, Acknowledgements: Acknowledgements: This work was supported by Next-Generation BioGreen 21 Program [SSAC, No. PJ01109405], the Rural Development Administration (Republic of Korea)

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Disclosure of Interest: None declared





PO263

SINGLE CELL CHARACTERISATION OF CHINESE HAMSTER OVARY CELLS

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Background and novelty: Biopharmaceuticals are a class of large-scale molecules including antibodies and antibody derivatives, and are generally produced from mammalian cells line via secretion directly into the media.

Biopharmaceutical manufacturing at MedImmune requires the generation of Chinese hamster ovary (CHO) clonal cell lines capable of producing biopharmaceutical product at commercially relevant quantities with optimal product quality. MedImmune's cloning process, based on random single cell deposition, provides a heterogeneous panel of expressers which are screened for desirable productivity and then product quality later in the process.

Experimental approach: This project will investigate correlative links between product titre and product quality with cell attributes at the single cell level, with the aim of integrating a cell attribute-based deposition step into the cloning process, increasing the proportion of high-producing clones with the desired product quality, and potentially reducing timelines and resource requirements.

Results and discussion: To identify clones for this study, a panel of twenty parents expressing a recombinant monoclonal antibody, were characterised in terms of growth, productivity, and intracellular recombinant protein and mRNA amounts. The clones covered a range of low, mid and high expressers. Assays were developed to investigate cell attributes and were used to investigate correlative links between clonal cell attributes, cell productivity and product quality. The developed assays are expected to allow a greater mechanistic understanding of CHO cells. Moreover, outcomes from this study have the potential to not only integrate into the cell line development clonal selection process, shortening timelines and reducing cost and resource requirements, but also inform host cell engineering projects with the potential to develop an improved CHO host.

Bibliography, Acknowledgements: We would like to thank G. Pettman, C. Godfrey, A. Mason, L. Chakrabarti and G. Davies.

Disclosure of Interest: None declared





PO264

A CROSS-SPECIES HIGH-THROUGHPUT SIRNA SCREEN FOR SUSPENSION CHO CELLS

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Background and novelty: Chinese Hamster Ovary (CHO) cells are the most frequently used mammalian cell factory for the production of human-like recombinant proteins. Due to existing limitations in growth and protein production, genetic optimization of CHO cell lines may significantly enhance bioprocess productivities. Knockdown of genes by siRNAs is a standard method to identify genes involved in a desirable phenotype, either because their knockdown improves or degenerates the property. As at least 13000 different transcripts are present in a cell at any time¹, it is of interest to develop a method that is able to efficiently test the effect of gene knockdown at an appropriate throughput and scale. Here we describe a high-throughput and small scale siRNA screening assay for suspension CHO cells that produce a secreted fluorescent protein.

Experimental approach: After method development and optimization, a whole genome siRNA library of *Mus musculus* was used to conduct the screen. Cells were allowed to grow for 4 days in 384 well plates post siRNA delivery by lipofection. Then, laser cytometry was used to detect the total fluorescence per well. In addition, the viable cell density was determined by the CellTiter Glo® Luminescent Cell Viability Assay. SiRNA sequences were mapped to two CHO transcriptomes^{2,3}, and all siRNAs with ≤ 1 mismatch to a transcript were kept for further processing. The transcripts were mapped to their respective genes and off-target effects were addressed by applying seed-based correction⁴.

Results and discussion: Though part of the screening results had to be excluded because of sequence dissimilarities between *Mus musculus* and CHO, 13666 genes could be targeted. Genes were identified, that, upon knockdown, effect the productivity and/or the growth of CHO cells. The genes with the largest positive effect on total productivity are now chosen for a validation screen.

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²Rupp et al., 2014

³O'leary et al., 2016

⁴Jackson et al., 2006

Disclosure of Interest: None declared





PO265

USE OF AN AUTOMATED CELL SCREENING SYSTEM FOR THE GENERATION OF STABLE HIV-1 PACKAGING CELL LINES FOR THE MANUFACTURE OF LENTIVIRAL VECTORS

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Background and novelty: Large-scale production of lentiviral vectors (LV) for therapeutic applications in gene therapy is necessary to achieve the full potential of this technology. One way of achieving this is through the development of packaging and producer cell lines (PaCL/PCL) reducing costs and providing improved consistency between batches.

Experimental approach: To generate PaCL/PCLs, isolation of stably transfected clones by cloning by limiting dilution (LDC) in antibiotic selective media is required. Oxford BioMedica has developed a bespoke Automated Cell Screening System (ACSS) for the isolation of clones (>3000) by automated LDC. Furthermore, the ACSS can perform routine passage and high-throughput (HTP) clonal LV production and evaluation of productivity using various screening methods. The ACSS was used to isolate and screen >1000 HIV-1 packaging clones with the aim of isolating cell lines that can produce high titre LV when transiently transfected with an HIV-1 vector genome. Inducible packaging plasmids (HIV-1 Gag/Pol, HIV-1 Rev and VSV-G envelope) were transfected into a HEK293T cell line that stably expresses the TetR protein. The ACSS was used to LDC the stably transfected cells in 96-well plates containing antibiotic selective media. These plates were monitored to ensure monoclonality and a HTP screen performed for LV production on semi confluent clones.

Results and discussion: A number of the clones that were selected using the ACSS were further expanded and screened at larger scale. The best clones produced higher titre LV than obtained using manual processes, and demonstrated higher titres than achieved using the current HEK293T transient transfection process. In conclusion, the isolation and screening of larger numbers of clones using the ACSS leads to the selection of higher titre LV producing clones than previously achieved when performing manual screening.

Bibliography, Acknowledgements: N/A

Disclosure of Interest: None declared





PO266

IDENTIFYING OPPORTUNITIES IN CELL ENGINEERING FOR THE PRODUCTION OF 'DIFFICULT TO EXPRESS' RECOMBINANT PROTEINS

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Background and novelty: There is a growing demand for production of recombinant proteins of different structural varieties, either as therapeutics or for protein characterisation. However, certain recombinant proteins are "difficult to express" requiring extensive cell line and process optimisation. The Tissue Inhibitors of Metalloproteinase (TIMP) protein family, TIMP-2, -3 and -4, are naturally secreted proteins that share significant structural homology, but show profound differences in their secretion in mammalian expression systems. This study has investigated the molecular mechanisms that selectively restrict expression of recombinant proteins of extensive sequence similarity.

Experimental approach: The molecular steps that limit expression have been defined step-by-step by polymerase chain reaction, proteomic analyses and protein localisation studies.

Results and discussion: The data showed all three TIMPs were detectable at the mRNA and protein level within the cell but only TIMP-2 was secreted effectively into the culture medium. Localisation studies showed intracellular protein for all three TIMPs, mainly co-localised in the organellar fraction. TIMP-3, which was not secreted, was detected within the cell in an immature partially glycosylated form and non-glycosylated form. Knockout of the N-glycan site did not result in successful secretion. These data suggest that the post-translational processing of poorly expressed TIMPs limits transit through the secretory pathway. Further, computational analyses reveal unique sequences present in the N-terminus of TIMP-3 cause this block in secretion. To overcome this challenge, cell engineering of limiting components could enhance production of these recombinant proteins.

Bibliography, Acknowledgements: We gratefully acknowledge the BBSRC and AstraZeneca for the funding and the contributions of Dr Jim Warwick and Dr Manuel Alejandro Carballo Amador as part of the computational work.

Disclosure of Interest: None declared





PO267

COMPLETE KNOCKOUT OF LACTATE DEHYDROGENASE IN CHINESE HAMSTER OVARY (CHO) CELLS

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Background and novelty: Toxic bioproducts such as lactate and ammonia have posed considerable challenges in bioprocessing since they limit cell growth and impact critical quality attributes by altering the regulation of biosynthetic enzymes. To mitigate the negative effects of lactic acid accumulation and control the culture pH, base is added to the media during the course of a bioprocess. However, the base addition increases osmolarity over time, which also negatively impacts the bioprocess by inhibiting growth and shortening the length of time in which the cells can produce the recombinant protein. Thus, it is of great interest to reduce or eliminate lactate production. We report the first complete knockout of lactate dehydrogenase (Ldha) in a mammalian cell line and concomitant elimination of lactate production.

Experimental approach: Knockout of Ldha and other necessary genes was accomplished via CRISPR/Cas9 and verified at multiple levels. The knockout was introduced into both nonproducing and producing CHO cell lines.

Batch and fedbatch were carried out for both producing and nonproducing cell lines to characterize the phenotypic impact of the knockout on important cellular attributes.

Results and discussion: In fedbatch, nonproducing cells had a prolonged growth period to higher VCDs than the wildtype CHO-S cells, likely due to lower osmolarity as base addition to maintain optimal pH was unnecessary. Characterization of the nonproducers in batch and the producers in batch and fedbatch are currently underway. We will present results on the effect of the knockout on growth, protein production, product quality and nutrient utilization for all experiments.

Bibliography, Acknowledgements: We acknowledge generous funding through the Novo Nordisk Foundation Center for Biosustainability at the Technical University of Denmark (NNF16CC0021858).

Disclosure of Interest: None declared





PO268

DEVELOPMENT OF A TRANSPOSON-MEDIATED INTEGRATION SYSTEM TO GENERATE HIGH YIELD PRODUCING CELLS WITH LOW COPY NUMBER OF INTEGRATED TARGET ANTIBODY GENE

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Background and novelty: Productivity and stability are key factors for the selection of cell line in protein drugs production. Large amount of target gene integrated in cell genome could lead to the instability of production. Therefore, cells with low copies of target gene integrated in high yield sites could be an ideal production cells for manufacturing. It is known that the transposon system can control the integrated copy number of target gene and can generate high yield producing cells, it could be a great approach to generate stable high yield producing cell lines carrying low copies of target gene through transposon system.

Experimental approach: We developed a platform to generate high yield producing cell lines carrying 1-2 copy of the integrated target gene using transposon system. Two CHO cell lines, CHO-S cells and DXB11 cells, have been applied. Cells were co-transfected with transposon and target gene expression plasmids. After drug selection, the cell pool with highest productivity per target gene copy was applied to single cell cloning. The productivity and copy number of cell clones were further determined.

Results and discussion: In the stable pools of CHO-S and DXB11 cells, the productivities per integrated target gene copy were about 11-13 mg/L/copy and 68-75 mg/L/copy in a batch culture, respectively. After single cell cloning, the integrated copy numbers in most cell clones were less than three copies per cell. In CHO-S and DXB11 cell clones, the productivities per integrated target gene copy were 30-60 mg/L/copy and 140-150 mg/L/copy in a batch culture, respectively. These results suggested that our platform is capable to develop high yield producing cells with 1-2 copy of integrated target antibody gene and can be applied to identify high yield integration sites in a high through-put scale.

Bibliography, Acknowledgements: This study is funded by Ministry of Economic Affairs, ROC.

Disclosure of Interest: None declared





PO269

A NEW CELL LINE DEVELOPMENT PLATFORM FOR HIGH EFFICIENCY SINGLE CELL DEPOSITION WITH IN-SITU IMAGE VERIFICATION

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Background and novelty: Deposition of single cells and associated seeding efficiency is currently regarded as a discrete step in the sequential cell line development workflow. After seeding, the cells then need to be independently verified in the wells of a microplate using an imager to confirm single cell origin.

Many labs currently use FACS or the limiting dilution method for single cell deposition, each of which have their own drawbacks. For FACS, it is possible to get very high seeding efficiency, however, many of the cells fail to grow following the harsh treatment resulting in low cloning efficiency. For limiting dilution, the reverse is the case; good cell survival but low seeding efficiency. Novel cell printing approaches appearing on the market are claiming efficiency improvements, however, these approaches still need a separate well image verification step.

In this poster, we introduce a new approach called **Verified In-Situ Plate Seeding (or VIPS™)**, which combines single cell deposition with concurrent in-situ image verification of the single cell in a well.

Experimental approach: We will demonstrate the performance of the new VIPS technology using CHO suspension cells for assessing seeding and cloning efficiencies in 96 and 384 well plates. Cells are seeded in standard cell culture media. All images of single cells in the wells of the plates are documented along with colony outgrowth.

Results and discussion: Data will be presented for the seeding and cloning efficiencies as well as examples of in-situ well images.

Benefits of verified in-situ seeding will be discussed including:

higher seeding efficiencies; real-time verification using image-based documentation of a single cell in a well; higher cloning efficiencies; reduction in the number of image time points required; complete elimination of plate and well edge effects for imaging; and QC of cell pool prior to seeding

Bibliography, Acknowledgements: None

Disclosure of Interest: None declared





PO270

CELL CYCLE AND TRANSCRIPTOME ANALYSIS USING RNA-SEQ FOR BETTER UNDERSTANDING OF CHO-K1 SUSPENSION CELL LINE BIOPROCESSING

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Background and novelty: Knowing how Chinese Hamster Ovary (CHO) cells behave during bioprocessing has until now relied heavily on empirical results with a limited knowledge of the intracellular dynamics. With the recent advances in techniques for decoding the CHO cell genome, it is now possible to leverage the genomic resources to better understand and further improve CHO cell bioprocessing. The aim of this work was to study the distribution of cell cycle phases and to perform transcriptome analysis during the culture stages of a typical industrial bioprocess of suspension-adapted CHO-K1 cells.

Experimental approach: Three replicates of CHO-K1 cells were cultured during 20 days in 1 L bioreactors. Identical operating conditions were used and the temperature was shifted gradually from 37°C to 31°C. For cell cycle study, a daily sample was taken and DNA content was determined by propidium iodide staining followed by flow cytometry analysis. Furthermore, RNA samples were collected on day 1, and during the exponential (days 7 and 8) and the stationary (days 14 and 15) growth phases for strand-specific RNA-Sequencing analysis. Differential gene expression (DE) between culture phases, Gene Ontology enrichment and pathways analysis were performed.

Results and discussion: Due to temperature shift, it was observed an S phase cell population reduction and G0/G1 arrest over culture time. One of the applications of this RNA-Seq study was to detect and understand the effect of low culture temperature on gene expression behavior. A number of key regulatory genes and pathways involved in modulating this response could be identified. The DE analysis showed that at 31°C 443 genes were up-regulated and 182 genes were down-regulated. These genes can be utilized as targets for cellular and metabolic engineering to improve CHO cell bioprocessing.

Bibliography, Acknowledgements: C. Chen *et al.* Biochem Eng J. 107 (2016) 11-17

Disclosure of Interest: None declared





PO271

A FLUX MODEL THAT QUANTIFIES THE PARTITION OF METABOLIC RESOURCES BETWEEN CELLULAR AND mAb GLYCOSYLATION IN GS-CHO CELLS

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Background and novelty:

Production cell lines consume metabolic and energetic resources to simultaneously glycosylate their components and the therapeutic proteins (TPs) they secrete. In order to establish mechanistic links between both processes, we present a novel metabolic flux analysis (MFA) model that describes how GS-CHO cells partition energetic and metabolic resources between cellular and mAb glycosylation.

Experimental approach:

IgG4-producing GS-CHO cells were cultured using three different amino acid feeding strategies. Data for viable cell density, nutrients, metabolic by-products, intracellular nucleotide sugar (NS) concentrations, and mAb glycosylation were collected. Computationally, an existing MFA [1] was expanded to include the aspartate-malate shuttle and a detailed description of ATP and NADH balances. NS demand towards cellular [2] and mAb glycosylation were included in the corresponding stoichiometric equations. The underdetermined MFA was solved using multi-objective optimisation, where the error between experimental and calculated fluxes was minimised and ATP synthesis per flux unit was maximised.

Results and discussion:

During initial phases of culture, cellular glycosylation consumes higher amounts of NS biosynthetic precursors (ATP, glucose, and glutamine). As growth ceases, a larger proportion of resources are allocated to mAb glycosylation, but total NS consumption decreases. This suggests that cellular glycosylation is the larger metabolic 'sink' within our cell line, a result that is consistent with the intracellular NS accumulation observed towards the end of culture. With further refinement, our MFA framework can be used to design optimal feeding strategies to control mAb glycosylation while simultaneously minimising negative impacts on cell growth and productivity.

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Disclosure of Interest: None declared





PO272

COMPARISON OF GLUCOSE-LACTATE METABOLISM OF THREE DIFFERENT MAMMALIAN CELL LINES USING FLUX BALANCE ANALYSIS

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Background and novelty: Mammalian cells show an inefficient metabolism characterized by high glucose uptake rate and the production of high amounts of lactate, which inhibits cell growth. Recently, we have observed a different glucose-lactate metabolism. While some cell lines are unable to metabolize lactate, others can co-metabolize glucose and lactate under certain culture conditions even during the exponential growth phase. These metabolic differences have been studied by means of Flux Balance Analysis (FBA).

Experimental approach: Three different cell lines were studied in a 2-liter bioreactor: a CHO, a HEK293 and a hybridoma. For the FBA, two genome-scale metabolic models were used: a reconstruction of *Mus musculus* for CHO and hybridoma, and a reconstruction of Human metabolic model (Recon 2) for HEK293.

Results and discussion: In pH-controlled cultures, the three cell lines produce large amounts of lactate due to their high glucose consumption rates. Interestingly, when pH dropped below 6.8 due to acid lactic secretion, concomitant consumption of glucose and lactate was observed in CHO and HEK293, even during the exponential growth phase (pH detoxification strategy). Conversely, hybridoma cells are unable to co-consume lactate and glucose. FBA showed that lactate is produced because only a small amount of pyruvate is introduced into TCA through Acetyl-CoA, and the excess is converted to lactate to fulfill the NADH regeneration requirements in the cytoplasm. Under such conditions, a better balance between glycolysis and TCA cycle fluxes is reached. Therefore, lactate is transported into the mitochondria to be further oxidized to pyruvate, reducing glucose uptake and yielding a more efficient substrate consumption.

Bibliography, Acknowledgements: Liste-Calleja, Leticia, et al. "Lactate and glucose concomitant consumption as a self-regulated pH detoxification mechanism in HEK293 cell cultures." *Applied Microbiology and Biotechnology* 99.23 (2015): 9951-9960.

Disclosure of Interest: None declared





PO273

PECTOPRO®: A POWERFUL HIGH YIELD TRANSFECTION SOLUTION FOR TRANSIENT CHO AND HEK-293 EXPRESSION SYSTEMS

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Background and novelty: Transient protein expression is a common bioproduction process used to produce milligram to gram quantities of recombinant proteins and antibodies for a wide range of applications. However, when compared to stable expression platforms, the overall yield of transient protein expression remains weak, limiting its use in bioproduction processes.

Experimental approach: One of the manufacturing strategies for improving the transient productivity is then to increase transfection efficiency. We have developed a novel technologically advanced transfection solution, FectoPRO®, which outperforms currently available PEI-based and lipid-based transfection reagents. This poster presents data and protocol leading to remarkable protein and antibody yields in different transient CHO and HEK-293 expression systems.

Results and discussion: FectoPRO-mediated transfection leads to higher transfection efficiency than any other currently available transfection reagents, and to very high protein yields. Transient transfection is now reproducible and easily scalable. In addition, FectoPRO is compatible with a number of different media, including the Expi293 and ExpiCHO cell systems.

Bibliography, Acknowledgements: We would like to acknowledge the whole biology and chemistry R&D teams from Polyplus-transfection.

Disclosure of Interest: None declared





PO274

ROBUST AND RELIABLE TRANSIENT PROTEIN PRODUCTION WITH PEIPRO®, A WELL CHARACTERIZED PEI OPTIMIZED FOR TRANSFECTION

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Background and novelty: Transient protein expression in mammalian cell lines has gained increasing relevance as it enables fast and flexible production of high-quality eukaryotic protein. Milligram amounts of protein can be produced within several days, meaning a significant shortening of process time in comparison to protein production from stable clones. However, to ensure the robustness of the process, it is absolutely necessary to have a reliable transfection solution. That's why we developed PEIpro®, an enhancement of the gold standard PEI, optimized for transient protein expression and perfectly suitable for the development of bioproduction processes with great scale-up predictability. In this poster, we present experimental data showing the benefits of using PEIpro® for protein production, and its efficiency in comparison to other PEIs.

Experimental approach: Suspension HEK-293 and CHO cells were transfected with different PEIs. PEIpro was tested in CHO cells and HEK-293 cells grown in different media, at different scales. Protein yields were assayed 48h after transfection.

Results and discussion: PEIpro is an optimized PEI especially developed for protein and virus production in HEK-293 and CHO cells grown in synthetic media. It shows extremely reliable and reproducible results in a wide variety of media and at different scales. PEIpro is provided with extensive QCs and is ideal for the development of bioproduction processes. Polyplus-transfection also provides the highest quality PEI available with extra Quality Controls (identity, potency, safety and purity) and supplied with extensive documentation, PEIpro®-HQ: Ideal for use in GMP processes.

Highest quality PEI available with extra QualityControls (identity, potency, safety and purity)
and supplied with extensive documentation,
PEIpro®-HQ: Ideal for use in GMP proces

Bibliography, Acknowledgements: Polyplus-transfection would like to thank the whole chemistry and biology R&D teams.

Disclosure of Interest: None declared





PO275

IDENTIFICATION AND CHARACTERIZATION OF POTENTIAL MICRO-RNAS AS PHENOTYPE ENGINEERING TARGETS TO DELAY THE ONSET OF CELL DEATH.

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Background and novelty: MicroRNAs have gained considerable attention as a breakthrough in cell and molecular biology as a new class of endogenous non-coding small RNAs to control gene expression at post-transcriptional levels. They are engineering alternatives, in comparison to gene-based methods, because of their ability to simultaneously regulate the expression of multiple genes. However, their role in several cellular pathways is unclear and uncertainty in their target recognition still remains a challenge.

Experimental approach: Our objective is to demonstrate their role as potential engineering tools to genetically enhance cellular phenotypes in CHO cells, widely used in the biopharmaceutical industry production. We aim to better understand the molecular mechanisms of promising miRNAs candidates on cell death mechanisms, with a particular emphasis to identify the specific molecular targets of these novel miRNAs, to achieve an extended culture lifespan.

Results and discussion: Our miRNA engineering approach has generated a significant amount of novel information on the role of microRNAs on cell death in CHO cells. Our data suggests a new knowledge on how the expression of specific miRNAs such as miR-31*, miR-125b, miR-155, miR-128a, miR-326 or miR-365, could exert an impact on bioprocess-relevant phenotypes.

The effect of these miRNAs in different pathways such as cell cycle and apoptosis, demonstrate the utility of how the fine-tuning of these novel engineering targets could be used to improve the most relevant phenotypes, making the industry more sustainable and driving down the cost of and access to medicines to patients.

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Disclosure of Interest: None declared





PO276

COMPARISON OF BICISTRONIC AND TRICISTRONIC EXPRESSION STRATEGIES FOR TRASTUZUMAB AND TRASTUZUMAB-INTERFERON-A2B PRODUCTION IN CHO AND HEK293 CELLS

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Background and novelty: Monoclonal antibodies are widely used in anticancer therapies, being produced by mammalian cell lines. Their conjugation to biological molecules for enhancing their antitumor activity opens a new powerful tool for tackling the disease. We have assessed the production of commercially approved Trastuzumab (Tzmb) and its fusion with interferon- α 2b by transfecting CHO and HEK293 cells with two bicistronic or a single tricistronic plasmids. The in vitro efficacy of both antibodies has been tested and compared side by side.

Experimental approach: Firstly, Tzmb heavy and light chains were cloned in two bicistronic plasmids (pIRESpuro3 and pIRESneo3). Secondly, they were both cloned in a tricistronic plasmid derived from pIRESpuro3. The fusion protein Tzmb-interferon- α 2b was also cloned in the same fashion than non-modified Tzmb. Purified products (using protein A chromatography) were quantified by both ELISA and SDS-PAGE. Antigen binding test was performed in Sk-br-3 breast cancer cell line by means of flow cytometry analysis. The biological activity of the different candidates was tested with MTT assay.

Results and discussion: Both Tzmb and the fusion protein Tzmb-interferon- α 2b have been successfully produced in CHO and HEK293 cells with both cloning strategies tested. In both cell lines and for both proteins produced, the tricistronic strategy resulted in the most efficient strategy, showing a 3-fold increase in terms of productivity with respect to the bicistronic double-transfection strategy for Tzmb-interferon- α 2b in CHO cells and a 7-fold increase in HEK293 cells. The differences in biological anticancer activity between Tzmb and Tzmb-interferon- α 2b will be discussed.

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Disclosure of Interest: None declared





PO277

SIALIC ACID AND BEYOND: ENGINEERING VIRAL RESISTANCE IN CHO CELLS

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Background and novelty: Contamination by the parvovirus minute virus of mice (MVM) remains a significant threat to CHO biomanufacturing. Recently, we reported the creation of a novel MVM resistant CHO host cell line. Deletion of the CMP-sialic acid transporter Slc35a1, abrogated cell surface binding and internalization of the MVM virus and resulted in complete resistant to MVM infection. Here we describe the application of the Slc35a1 cell lines for the production of a recombinant monoclonal antibody and also, further study the MVM virus-CHO host cell line interaction.

Experimental approach: Slc35a1 mutants were compared to the wild-type (WT) cells for growth, productivity and product quality at a 2L benchtop bioreactor scale. In more complex glycoproteins, elimination of sialic acid could have a clinical impact. Since MVM preferentially binds to α -2,3 sialylated glycans we replaced all α -2,3 sialylation with a α -2,6 sialylation phenotype and tested for resistance. Gene knockouts targeting virus entry, virus transport and replication were also tested for MVM resistance. *In-vitro* MVM-pull down assays, whole genome Crispr screens etc are ongoing to identify other targets for engineering MVM resistance.

Results and discussion: Slc35a1 mutant cell lines were found to be similar to WT cells lines, and the monoclonal antibody products, while devoid of terminal sialic acid, had comparable product quality attributes to WT produced proteins. Replacing the α -2,3 linked sialic acid with α -2,6 linked sialic acid maintained complete resistance to MVM infection. Additionally, Dynamin family knockouts had a significant effect on MVM resistance indicating an endocytic mechanism of viral entry. As efforts to create better and safer CHO host cell lines continues, the incorporation of viral resistance in host cell lines results in adding greater assurance of production of safely delivered cell derived products.

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Disclosure of Interest: None declared





PO278

IMPROVED PLATFORMS FOR CHO CELL LINE DEVELOPMENT

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Background and novelty: Isolation of highly-productive stable cell lines is an obstacle in the process of therapeutic protein production. Methods such as gene amplification and minipool clone selection have been somewhat successful in overcoming this barrier; however, these methods affect key drivers such as timelines, stability and resources. Platforms have been introduced to the industry to address these drivers; however there is a need for further improvements.

Experimental approach: Ubiquitous Chromatin Opening Element (UCOE[®]) technology was introduced to the CHOZN[®] cell lines and evaluated for titer and frequency of highly-productive clones. Recombinant CHOZN[®] GS cells were selected by both minipool and bulk pool selection. With CHOZN[®] CHO K1 cells, vectors were developed to contain UCOE[®] technology, transcription and translation enhancers and bulk pools and single cell clones were selected using antibiotic selection. Titer, stability and time metrics were evaluated.

Results and discussion: Improvements in recombinant antibody titer, timelines, resources and expression stability were made when using UCOE[®] technology. In the CHOZN[®] GS system, UCOE-containing clones derived using a bulk pool approach had comparable titers to those isolated using the minipool approach. However, clones derived using the bulk pool approach required substantially less resources and time. With CHOZN[®] CHO K1 cells, the addition of UCOE[®] technology greatly enhanced antibody expression relative to control transfected cells in stably transfected bulk pools and single cell clones. Further, expression enhancing elements boosted antibody expression relative to control UCOE[®] vectors. Additionally, stably transfected CHOZN[®] CHO K1 cells with UCOE[®] vectors possessed superior expression stability relative to non-UCOE[®] controls.

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Disclosure of Interest: None declared





PO279

PRODUCTION OF THERAPEUTICALLY RELEVANT LENTIVIRAL VECTORS FOR CLINICAL STUDIES

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Background and novelty: The genetic engineering of patient-specific T cells with lentiviral vectors (LVV) expressing chimeric antigen receptors (CAR) for late phase clinical trials requires the large-scale manufacture of high-titer vector stocks. The state-of-the-art production of LVV is based on 10- to 40-layer cell factories transiently transfected in the presence of serum. This manufacturing process is extremely limited by its labor intensity, open-system handling operations, its requirements for significant incubator space plus costs and patience risk due to presence of serum. To circumvent these limitations, this study aims to develop a stable and serum-free process to produce LVV with PEI-mediated transfection. In addition, this study also focuses on the development of a production system not only using a GFP marker but also a therapeutically relevant transgene (CD20-CAR).

Experimental approach: Therefore, three different cell lines (HEK 293, 293T, 293FT) were investigated concerning their productivity of LVV and their growing behavior in the *in-house* serum-free medium TransMACS. As part of this, Design of Experiment was used to investigate the optimal conditions for PEI/DNA-transfection. Furthermore, this statistical approach was used focusing an ideal ratio between the 3rd generation plasmids (transfer plasmid CD20-CAR or GFP, envelope plasmid, packaging plasmids). In addition, different enhancers were investigated concerning their effects on productivity comparing HEK cultures producing LVV encoding for GFP-marker or CD20-CAR.

Results and discussion: The outcome of these experiments enabled the development of a robust HEK 293T based process to produce clinical relevant LVV under serum-free conditions. Furthermore, it provides an insight how therapeutic genes and the expression of its transgene can influence cell productivity.

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Disclosure of Interest: None declared





PO280

PURSUING IMPROVED CONDITIONS FOR GENETIC MODIFICATION OF HUMAN CELLS FOR REPO PRODUCTION UNDER SUSPENSION SERUM-FREE CONDITIONS

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Background and novelty: Human cell lines have attracted great interest for recombinant protein production since they are capable of producing glycosylated proteins in a more similar way to native human proteins, reducing the potential for immune responses. This work aims to establish improved conditions to genetically modified the human cell lines HUH-7, HKB-11, SK-HEP-1, under suspension serum-free conditions, for recombinant erythropoietin (EPO) production.

Experimental approach: Firstly, experiments were conducted to establish improved conditions for transient transfection and/or transduction in suspension serum-free conditions using a lentiviral vector encoding GFP expression. The transient transfection were performed using PEI (25 kDa linear) as a cationic donor and different amounts of total DNA/10⁶ cell (1.5; 3.0; 4.5 and 6 µg DNA/10⁶ cells) and DNA:PEI ratios (1:2, 1:3, 1:4, 1:6 e 1:8). Transduction experiments were conducted using a MOI (multiplicity of infection) of 1.

Results and discussion: The GFP expression levels obtained by transient transfection in suspension serum-free cultures were not satisfactory, even testing different transfection conditions. SK-HEP-1, HKB-11 and HUH-7 cells presented a maximum transfection efficiency of 6.5, 6.6 and 13.2% (1, 6 and 3 µg DNA/10⁶ cells and 1:3 DNA:PEI ratio), respectively. By using lentiviral transduction, the efficiency were considerably higher (52.4, 26.4 and 24.8 for SK-HEP-1, HKB-11 and HUH-7 cells, respectively). Based on this, genetic modification by transduction was chosen to generate the cells for EPO production. rEPO-producing SK-HEP-1, HKB-11 and HUH-7 cells presented an efficiency of approximately 41.4; 36.5 and 63.1%, respectively. Experiments are being conducted to characterize cell growth and rEPO production kinetics in each cell line in order to chose the best host human cell line for EPO production.

Bibliography, Acknowledgements: CAPES and FAPESP (2012/04629-8)

Disclosure of Interest: None declared





PO281

MOLECULAR IMPACT OF THE MIRNA 23 CLUSTER ON BIOPROCESS ATTRIBUTES OF CHO CELLS

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Background and novelty: Chinese Hamster Ovary (CHO) cells are the prominent cell line used in biopharmaceutical production. Although optimisation efforts have led to a increase in productivity, CHO cells yield less than other expression systems[1]. To improve yields and find beneficial bioprocess phenotypes genetic engineering plays an essential role in recent research. The miR-23 cluster was first identified as differentially expressed during temperature shift suggesting its role in proliferation and productivity[2]. Previous work showed that the depletion of the miRNA-23 cluster or single members can result in beneficial bioprocess phenotypes.

Experimental approach: In this work we aimed to disrupt miRNA function using sponge decoy technology and furthermore, to implement the recently developed CRISPR/Cas9 system to knockout the same miRNAs. In addition, we aimed to compare the molecular impact of miRNA sponges and CRISPR/Cas9 by targeting miR-24 with both technologies using LC-MS/MS

Results and discussion: Depletion of miR-23 cluster and miR-24 as well as miR-27 individually in a CHO-S cell line expressing a Fc fusion elevated titers up to 1.5 fold in batch culture. For the purpose of comparing the sponge approach and CRISPR/Cas9, miR-24 was either stably depleted or targeted using CRISPR/Cas9. Targeting miR-24 using CRISPR/Cas9 resulted in increased proliferation and ultimately boosted IgG titers in mixed cell populations. However, depletion using a sponge for miR-24 showed no increase in proliferation suggesting a dosage dependent effect. This is an important consideration when choosing the appropriate tools for gene engineering.

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Disclosure of Interest: None declared





PO282

INTRACELLULAR SECRETION ANALYSIS OF RECOMBINANT THERAPEUTIC ANTIBODIES IN ENGINEERED CHO CELLS AIMING TO ESTABLISH HIGH PRODUCER

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Background and novelty: The Chinese Hamster Ovary (CHO) cell is widely used for the production of therapeutic antibodies. Various post-translational approaches have been performed to increase the productivity [1]. However, the secretion process in genetically modified CHO cells for recombinant antibody production has not fully been investigated. As it might be a bottleneck in the production, we analyzed the secretion process, not the productivity, in engineered CHO cells.

Experimental approach: To analyze the antibody secretion detail, chase assay was performed [2]. CHO-HcD6 cells, a high producer of recombinant IgG derived from CHO-K1, were incubated in a serum-free medium with translation inhibitors. The amount of IgG, both which remained in the cells and secreted to the medium, were measured by quantitative western blotting. The localization of IgG was analyzed by immunofluorescence microscopy. Furthermore, aggregation analysis was performed by size exclusion chromatography (SEC).

Results and discussion: The amount of IgG in the cells cultured in a plate with IMDM decreased until 2 h, and the IgG in the medium reached a plateau at about 2 h as well. When the cells were cultured in a shaking flask with in-house mTop2 medium, IgG in the cells reached a plateau at around 4-6 h. In both cultures, IgG were not fully secreted, and 30-40% of the IgG still remained in the cells. Results from immunofluorescence microscopy showed that remaining IgG co-localized mainly with the endoplasmic reticulum (ER). SEC analysis revealed that some IgG heavy chains aggregated. The most IgG in cells seemed to form tetramer even after the secretion reached a plateau. In summary, engineered CHO cells may have a bottleneck in the ER, especially in the maturation of tetramer.

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Disclosure of Interest: None declared





PO283

DIFFERENTIAL LONG NON-CODING RNA EXPRESSION INDUCED BY HYPOTHERMIC SHOCK IN CHINESE HAMSTER OVARY CELLS

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Background and novelty: Modulation of Chinese hamster ovary (CHO) cell mRNAs and microRNAs has been successfully utilised to improve beneficial bioprocess phenotypes such as productivity and growth rate. Long non-coding RNAs (lncRNAs) - transcripts longer than 200 nucleotides that do not code for proteins - are a potential new avenue for genetic engineering due to their regulatory roles in cellular processes such as apoptosis. In this study, we sought to improve CHO cell lncRNA annotation as well as identify variations in lncRNA expression following temperature shift.

Experimental approach: Transcriptomic analysis was conducted using Illumina next generation sequencing of rRNA depleted total RNA from 4 biological replicates of CHO cells cultured at 37°C and 31 °C. Following strand specific library preparation, >50 million 150bp paired-end reads were acquired for each sample. A combination of *de novo* and reference-guided transcriptome assembly as well as an *in-silico* assessment of protein coding potential was used to classify putative lncRNAs. Transcript level differential expression analysis was utilised to determine if the abundance of the identified lncRNAs were affected by the change in culture temperature. Antisense lncRNAs that had evidence of differential expression to that of the corresponding protein coding gene on the sense strand were selected as high priority targets for qPCR validation.

Results and discussion: This study has improved the annotation of lncRNAs in CHO cells and identified a cohort of differently expressed lncRNAs following temperature shift. We have also identified potential *cis* regulatory networks between antisense lncRNA and sense coding RNA, such as the mitochondrial matrix gene *Oxct1* and its antisense lncRNA.

Bibliography, Acknowledgements: The authors wish to acknowledge funding through the Science Foundation Ireland (SFI/13/SIRG/2084) and the Marie Curie ITN program (642663)

Disclosure of Interest: None declared





PO284

EVALUATION OF DIFFERENT GENOMIC SITES AND INTEGRATION APPROACHES FOR RECOMBINANT GENE EXPRESSION

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Background and novelty: Discovering a recombinant cell line harboring all desired features that are of interest for most scientific and industrial projects is still a costly and time-consuming issue. Today, different techniques can be applied to generate a unique cell line that fulfills all these characteristics like high transgene expression as well as an assured stability over time. The main drawback here is that this includes a laborious screening procedure and has to be reapplied for each new transgene to be expressed.

Experimental approach: For the identification of cell lines that allow the reliable expression of a certain transgene various genomic integration technologies were applied. With this comprehensive approach different genomic sites were utilized. The efficiency of the cell line generation as well as the expression level/stability was determined and compared between the approaches and sites.

Results and discussion: The developed cell lines are based on HEK293 cells and each harbors a versatile transgenic expression cassette, which allows selection as well as the visualization of expressing cells. The resulting cell lines show integration site dependent expression patterns on a clonal level. Three different genomic loci were analyzed based on their expression and stability levels resulting in reproducible characteristics. The well characterized AAVS1-locus was used besides indicating a homogenous but comparably low expression. We envision to transfer our knowledge to other relevant cell lines to obtain a universal applicable cell modification technology.

Bibliography, Acknowledgements: .

Disclosure of Interest: None declared





PO285

ZIKA MONOCLONAL ANTIBODY DISCOVERY BY HIGH-THROUGHPUT SEQUENCING OF PAIRED HEAVY AND LIGHT CHAINS FROM SINGLE B CELLS

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Background and novelty: Zika virus is a flavivirus primarily transmitted to humans by *Aedes* mosquitoes, but sexual transmission is also possible. Only about 20% of infected humans are symptomatic, and symptoms are mostly mild. However, zika is a trigger of Guillain-Barré syndrome, and infection during pregnancy can lead to fetal malformations. Monoclonal antibodies are important tools that can assist countermeasures as research reagents, for diagnostic purposes and as therapeutics. Thus, in the present work, we used high-throughput single B-cell sequencing for discovery of mouse and human mAbs.

Experimental approach: For mouse mAbs, BalbC and C57BL6 mice were immunized by electroporation with a plasmid encoding zika premembrane (prM) and envelope (E) proteins, and then boosted in the footpad with prM-E virus-like particles (VLPs) produced in HEK293 cells. CD138+ antibody-secreting cells obtained from the popliteal lymph node were then isolated. For human mAbs, peripheral blood mononuclear cells (PBMCs) from convalescent patients were used and CD27+ cells were selected. Single human and mouse B cells were isolated by a high-throughput technique enabling capture of mRNA from single cells. VH:VL amplicons were then generated by reverse transcription and overlap extension PCR, and sent for next-generation sequencing.

Results and discussion: The mouse genes encoding the highest frequency VH:VL pairs identified via bioinformatics were synthesized, and mAbs were expressed in HEK293 cells. The human paired VH:VL genes were synthesized and cloned in a yeast display library which was then sorted using labelled zika VLPs as probes to select for mAbs with desired specificity. The resulting recombinant mouse and human mAbs were then characterized by ELISA and neutralization assays. This workflow allows for the discovery of dozens of mAbs within a time frame of 4-6 months.

Bibliography, Acknowledgements: Assistance of P. Darrah and G. Lynn is gratefully acknowledged.

Disclosure of Interest: None declared





PO286

RATIONAL ANTIBODY HUMANIZATION ASSISTED BY MOLECULAR DYNAMICS SIMULATIONS

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Background and novelty: Humanized monoclonal antibodies (mAbs) are among the most promising drugs, but defined strategies for their modification are still not available. Our work deals with humanization of murine mAb2/3H6. The superhumanization approach leads to a loss of binding affinity which was partially restored by a single human-to-mouse backmutation (T98^hR). This residue was selected by synergistic combination of sequence analyses of antibody framework regions and structural information using novel *in silico* simulations. For structural stabilization, a conglomeration of tyrosine residues surrounding T98^hR was identified, the so called “tyrosine cage”¹. In a recent series of experiments we tried to enhance binding affinity by three new variants with backmutations in the variable light chain (VL). Originating from T98^hR, residues in the VL were selected based on their spatial proximity to the CDR3 loop of the variable heavy chain.

Experimental approach: Analysis of the tyrosine cage was done by alanine scanning mutations with a double mutation variant T98^hR + Y27^hA (BM09) and a triple mutation variant T98^hR + Y27^hA + Y32^hA (BM10). Affinity improvement of T98^hR was evaluated by VL double backmutation variants T98^hR + F46^lL (SU01) and T98^hR + Q49^lS (SU02) and a triple backmutation variant T98^hR + F46^lL + Q49^lS (SU03). All five variants were expressed transiently in HEK293-6E cells and binding affinities were investigated in two individual settings with bio-layer interferometry.

Results and discussion: Alanine scanning of the tyrosine cage demonstrated a reduction of binding affinity (BM09) and a severe loss of binding (BM10), concluding that the tyrosine cage plays an important role for supporting a correct CDR loop conformation. Further affinity improvements of the single mutation variant T98^hR were reached via mutations in the VL. It demonstrates the underestimated role of the VL for the interaction with its binding partner.

Bibliography, Acknowledgements: ¹J.Mol.Recognit. 2016;29:266–275

Disclosure of Interest: None declared





PO287

ACCELERATED HOMOLOGY-DIRECTED TARGETED INTEGRATION OF TRANSGENES IN CHO CELLS VIA CRISPR/CAS9 AND FLUORESCENT ENRICHMENT

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Background and novelty: Targeted gene integration into site-specific loci can be achieved in CHO cells via CRISPR/Cas9 genome editing technology and the homology-directed repair (HDR) pathway⁽¹⁾, and can potentially overcome the unstable and variable transgene expression caused by random integration. However, the low efficiency of HDR often requires antibiotic selection, limiting targeted integration of multiple transgenes at multiple sites due to a limited number of selection markers. We improve the HDR-mediated targeted integration platform without the use of selection markers, by applying fluorescent enrichment of transfected cells.

Experimental approach: The improved system is based on a GFP-linked Cas9, a sgRNA towards the integration site and donor DNA harboring a mCherry gene outside homology regions. Involvement of fluorescent markers in constructs confers FACS enrichment of cells transfected with both Cas9 and donor DNA. Subsequent selection of non-fluorescent clonal cells excludes cells with randomly integrated donor DNA.

Results and discussion: Introducing GFP-linked Cas9 and sgRNA expression vectors together with donor plasmid enabled precise targeted integration of large transgenes encoding model proteins, following transient expression and FACS enrichment. The approach provides a simple and fast strategy for selection marker-free targeted integration of GOI. Further improvement in targeted integration efficiency was assessed by chemical treatment toward cell cycle arrest or nonhomologous end joining inhibition combined with fluorescent enrichment. Taken together, the present platform has a huge potential to accelerate targeted generation of stable production cell lines in a rational way.

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Disclosure of Interest: None declared





PO288

MS-SILAC APPROACH FOR PHOSPHO PROTEOMICS OF IGF SIGNALING IN PRODUCER CHO CELLS

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Background and novelty: Engineering of stable CHO cells with high product titers remains challenging without knowing underlying cellular effects in detail. Analyzing signaling pathways in modified cells by phospho proteomics-MS uncovers switch-points characterizing high producer cell lines. Here, we compare the signaling of IGF (insulin growth factor) on producer and parental CHO based on SILAC-MS quantification.

Experimental approach: Cells were cultivated in triplicates in SILAC-CDM with heavy or light Lys and Arg with an IGF induction at VCD of appr. 5×10^6 cells/ml. After 5 min entire protein was extracted and digested. Phospho-peptides were enriched via TiO_2 -tips and measured by nanoLC-ESI Orbitrap MS. Data evaluation was performed by MaxQuant and Perseus.

Results and discussion: A viability stimulating effect of IGF was observed for parental and producer cells, as well as a short term increase in S-phase cells. Only for one producer line an increased overall growth, but for both producer CHO cells a decreased qP and product titer was detected.

MS identification, based on 17.000 compounds, yielded 800 CHO-proteins via SwissProt-DB compared to more than 900 with the more redundant NCBI-DB. Almost 90% turned out as phosphoproteins, indicating an efficient TiO_2 -purification strategy.

For parental and producer lines 160-270 significantly regulated phosphorylation sites were calculated based on CHO-specific entries. GO and interaction evaluation illustrated the fast but nevertheless wide spread signaling events depending on IGF as growth hormone in CHO cell cultures. We determined shifted phosphorylation in cellular events such as cell cycle, cytoskeleton, metabolism, and interestingly transcription and translation. A final modeling of signaling will be performed after further bioinformatics evaluation, serving as a basis for application of activators or inhibitors to boost cell productivity.

Bibliography, Acknowledgements: We would like to thank the AIBN (Brisbane) for providing the CHO clones.

Disclosure of Interest: None declared





PO289

SK-HEP-1 AS PLATFORM FOR EXPRESSION OF NOVEL RECOMBINANT HUMAN FACTOR IX WITH AUGMENTED CLOTTING ACTIVITY

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Background and novelty: Hemophilia B (HB) is a bleeding disorder due to a defect in factor IX (FIX). The main treatment for the coagulopathy is replacement therapy using FIX concentrates. Prophylactic treatment in HB is very effective but is limited by cost issues. Production of a recombinant human FIX (rhFIX), in human cell lines, with enhanced clotting activity, offering the possibility of fewer infusions and lower costs with similar efficacy, is one of the current challenges for HB treatment. This study focused on development of mutated rhFIX with augmented clotting activity expressed in SK-Hep-1.

Experimental approach: Using site-directed mutagenesis in specific sites of the FIX domains, three rhFIX mutants were developed and produced in the SK-Hep-1 cells. The quantification of the expression of rhFIX was analysed using ELISA and chromogenic assays.

Results and discussion: FIX antigen levels of the variants was 2-3-fold lower than wild-type FIX (FIX-WT). However, the clotting activities of FIX variants were higher than the FIX-WT ranged from 5.2 to 7.3 UI/mL. FIX-ALL showed the highest biologic activity (7.3 UI/mL), which was 6.6-fold higher than FIX-WT (1.1 UI/mL). FIX-YKALW (6.8 UI/mL) and FIX-LLW (5.2 UI/mL) showed 6.2-fold and 4.7-fold increment in clotting activities than FIX-WT, respectively. FIX-ALL showed 20-fold higher specific activity (U/mg) compared with the FIX-WT. FIX-YKALW showed 12-fold increment in specific activity and FIX-LLW showed 9.2-fold increase in specific activity compared with the FIX-WT. We have engineered and characterized three improved FIX proteins with enhanced in-vitro activity expressed in a human cell line. Future studies are required to evaluate in-vivo activity of these novel recombinant FIX mutants.

Bibliography, Acknowledgements: The authors would like to acknowledge the Capes, CNPq and the INCTC for financial support.

Disclosure of Interest: None declared





PO290

NEW RED-SHIFTED FLUORESCENT BIOSENSOR FOR MONITORING INTRACELLULAR REDOX CHANGES IN MAMMALIAN CELL LINES

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Background and novelty: Mammalian cell lines have become the dominant system for the production of recombinant proteins for clinical applications. During cell culture, cells experience oxidative stress, which hampers proliferation and productivity. Monitoring of intracellular redox changes can be useful to guide intervention strategies aimed at optimizing cell-based production processes. Redox-sensitive variants of green fluorescent proteins (roGFP2 and rxYFP) able to report the pool of reduced and oxidized glutathione have been developed [#]. Here we present the generation and characterization of a new red-shifted redox biosensor that allows the detection of redox changes in cells in a non-invasive, dynamic, real-time and in-situ manner. The new redox biosensor will turn of great utility for multiparametric, high-content analysis of cells.

Experimental approach: Based on the computer-aided theoretical models a new variant of mRuby2 was designed incorporating two cysteine residues that confer sensitivity towards redox potential. The new rxmRuby2 biosensor was expressed, purified and characterized biochemically and biologically in different cellular systems (HeLa and HEK-293 cells).

Results and discussion: Spectrofluorimetric analysis reveals that the biosensor responds in a reversible manner to redox stimuli at different pH along the physiological range. HeLa and HEK293 redox reporter cell lines showed a sensitive and reversible response to different redox stimuli as assessed by flow cytometry. Future studies with this reporter cell lines aim at detecting metabolic deficiencies associated with cellular redox balance that may help optimizing industrial processes as well as its validation for in vivo imaging or deep-tissue imaging applications.

Bibliography, Acknowledgements:

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This project is funded by ANII-FMV1-2014-1-104000.

CP and FS have contributed equally to this work.

Disclosure of Interest: None declared





PO291

INCREASING CHO FED BATCH PRODUCTIVITY THROUGH SMALL MOLECULE TARGETING OF EPIGENETIC MACHINERY

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Background and novelty: Increased demand for recombinant proteins warrants continued efforts to improve cell-specific productivity. While process development has successfully increased harvest titer, these gains are often derived from an increase in cell mass which can complicate purification efforts. Host cell engineering has the potential to increase per cell productivity, but these efforts are confounded by the pleiotropic nature of this phenotype. Increases in productivity may require multiple genetic alterations with each risking unknown consequences on cellular functionality. Small molecules have the capacity to target functional networks and positively alter cellular phenotypes, while retaining the ability to tune the magnitude and timing of the alteration. Epigenetics is a rapidly evolving field pertaining to changes in gene expression caused by modifications to the genome, rather than the genetic code itself. The pliability of these modifications makes them appealing targets for chemical perturbation.

Experimental approach: We screened chemical compounds known to inhibit epigenetic pathways and found that compounds targeting a class of Bromodomain containing proteins (BRD) had a corresponding increase in fed batch performance. BRDs bind acetylated histones and tend to amplify the euchromatic local environment promoted by acetylation.

Results and discussion: We have identified a compound that increased the fed batch titer of three manufacturing-grade CHOK1 cell lines by 13-35%, while the per cell productivity increased by 21-42% without altering product quality. Preliminary transcriptomic data suggest that these benefits take place through silencing of apoptotic pathways, as well as inducing translational biosynthetic and protein transport pathways. When dosed into fed batch cultures the BRD compounds have the capacity to further increase the cellular productivity of highly productive manufacturing cell lines.

Bibliography, Acknowledgements: Chapman Wright

Disclosure of Interest: None declared





PO292

ASSESSMENT OF GENOMIC REARRANGEMENTS IN CHINESE HAMSTER OVARY (CHO) CELLS

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Background and novelty: CHO cells are the most important mammalian cell line for the manufacturing of therapeutic proteins, as their adaptability to a variety of culture conditions enables efficient production of biopharmaceuticals. On the down side, this adaptability can result in genomic and phenotypic instability caused by variations in chromosome structure and number, resulting in prolonged screening-efforts to isolate a suitable industrial producer clone. Therefore, the aim of this study was the development of methods to assess genomic rearrangements in CHO cells.

Experimental approach: Two cytogenetic methods, chromosome counting and chromosome painting, were established to analyse genomic instability. Chromosome counting determines the distribution of the chromosome number in individual cells within the population, based on metaphase spreads, microscopic and statistical analyses. Chromosome painting uses a mixture of fluorescently labelled painting probes covering the entire Chinese hamster genome. Images were processed using the in-house developed software "ChromaWizard" to visualise larger scale chromosomal rearrangements.

Results and discussion: Chromosome counting revealed a large spread in numbers, not only within the same population over time, but also between different CHO cell lines, with a trend to more divergent, near-tetraploid counts with increasing time in culture. Large-scale rearrangements were evaluated in detail by chromosome painting, and were found to occur in each population analysed, whether subclone or pool. Repeated thawing from the same MCB or parallel, independent cultivation after splitting resulted in new variants. Thus these frequent structural and numeric chromosomal aberrations appear and disappear on a continuous basis over time.

Bibliography, Acknowledgements: This work has been supported by the Austrian BMWF, BMVIT, SFG, Standortagentur Tirol, Government of Lower Austria and Business Agency Vienna through the Austrian FFG-COMET-Funding Program

Disclosure of Interest: None declared





PO293

CONSTRUCTION OF A SYSTEM FOR RAPID EVALUATION OF PRODUCTION ENHANCER GENE IN CHO ANTIBODY PRODUCTION

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Background and novelty: To improve antibody production in CHO cell expression system, it seems to be useful to up- or down-regulate the gene of interest (GOI) regarding antibody folding, secretion, cell metabolism and so on. However, identifying the production enhancer gene is the rate-limiting step for CHO cell engineering, because a conventional method requires a series of experiments including genomic integration of GOI, selection of stable clones and cell culture of several clones. In this study, we suggest a novel approach for rapid evaluation of production enhancer gene based on an episomal expression system.

Experimental approach: Plasmid vector carrying the EBNA1 was transfected into CHO cell lines producing IgG1 antibody. After G418 selection and single colony picking, EBNA1 expression was checked with capillary electrophoresis system. Expression vector for production enhancer genes was prepared by inserting an oriP sequence into plasmid vector, resulting in pOTC vector.

Results and discussion: We constructed CHO cell lines stably expressing EBNA1. In capillary electrophoresis analysis, we observed a clear peak corresponding to the EBNA1 expression in all cell lines. pOTC-GFP vector was transfected into the cell lines with PEI. The number of GFP-positive cells continued to increase after transfection resulting in 70-80% of GFP-positive ratio. These results suggest that the pOTC-GFP plasmid was stably retained by EBNA1/oriP system in recombinant CHO cell lines. We also prepared shRNA vector carrying oriP for down-regulation. The constructed system makes it possible to evaluate the effectiveness production enhancer genes in a rapid manner. In the presentation we will report test evaluation of candidate genes.

Bibliography, Acknowledgements: This research is partially supported by the developing key technologies for discovering and manufacturing pharmaceuticals used for next-generation treatments and diagnoses both from METI and AMED, Japan.

Disclosure of Interest: None declared





PO294

GS SYSTEM FOR INCREASED EXPRESSION OF DIFFICULT-TO-EXPRESS PROTEINS

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Background and novelty: 2G UNic™ technology comprises a set of protected genetic elements that improve protein production by acting on transcription as well as on translation. The elements can either be inserted into existing (platform) vectors or be provided as complete ready-to-use vectors. The technology can be used in stable and in transient transfection to boost protein production for product development and is being applied in CLD for pharmaceutical proteins. In combination with antibiotic selection or DHFR selection, 2G UNic™ technology routinely results in 2-3 fold increase in expression of client antibodies or fusion proteins, both in pools and after clonal selection.

We have successfully combined 2G UNic™ technology with glutamine synthetase (GS) selection and the CHO GS null cells of Horizon Discovery, resulting in clonal cell lines producing > 5 g/l of a biosimilar mAb in fed-batch assay. Here we present the further development of vectors based on the combination of 2G UNic™ technology and the GS system for increased expression of difficult-to-express proteins.

Experimental approach: CHO GS null cells (Horizon Discovery) were transfected with expression vectors comprising different variants of 2G UNic™ genetic elements (ProteoNic). Expression of well-expressed and difficult to express target proteins was measured in batch cultured stable bulk pools to screen for the best performing vectors. Expression in clonal cell lines was measured to confirm bulk pool data under fed-batch production conditions.

Results and discussion: I will present case studies of the application of 2G UNic™ technology in expressing difficult-to-express pharmaceutical proteins in the CHO GS platform. The data show that the 2G UNic™ genetic elements can be successfully used to obtain a significant increase in titers of difficult-to-express proteins, including fc-fusion proteins and large multi-subunit complex biotherapeutics.

Bibliography, Acknowledgements:

Disclosure of Interest: None declared





PO295

STRENGTHENING THE UTILITY OF FACS WHEN CLONING CELLS

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Background and novelty: Over the past decade fluorescence activated cell sorting (FACS) has been used to quickly isolate single cells, with a high probability of monoclonality achieved in a single cloning round instead of the multiple rounds required with the limiting dilution method.

Experimental approach: Recently, there has been increased scrutiny of the methods used to achieve monoclonality, with concerns expressed over certain approaches taken. Typically, there has been reliance upon the vendor's data and recommendations to support FACS set-up for single-cell sorting. However, we have identified gaps in the FACS set-up and introduced controls that ensure that any resultant cell line has a high probability of being monoclonal.

Results and discussion: This poster will show how a reproducible high probability of monoclonality (≥ 0.99) has been achieved. Following careful instrument set-up, a representative sample of cells is fluorescently stained and single cell sorted, onto a 96-well plate-lid, using a series of gates to exclude cell debris, non-viable cells and cell aggregates. These 96-well plates are visually inspected using fluorescent microscopy; observations of 0 cells, 1 cell or ≥ 2 cells are recorded. The number of observations for each category is used to estimate the probability of monoclonality using a prior to posterior Bayesian analysis. Since use of the FACS assumes each droplet contains a cell, statistical methods based upon random distribution of cells in the droplets are not appropriate. System performance is monitored at regular intervals in the cloning session. If instrument performance drifts, appropriate control strategies are used to return the FACS to its desired performance envelope. Use of such control strategies increases the confidence that a well contains a single cell. With this increased control over the method, we believe that use of the FACS is a highly appropriate method for generating cell lines for bioprocessing uses.

Bibliography, Acknowledgements: Thank you.

Disclosure of Interest: None declared





PO296

MAMMALIAN SYSTEMS BIOTECHNOLOGY REVEALS GLOBAL CELLULAR ADAPTATIONS IN A RECOMBINANT CHO CELL LINE

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Background and novelty: Developing efficient host cells for therapeutic protein production is often a laborious process due to the poor characterization of cellular transfection.

Experimental approach: Here, we unraveled the overall changes during the transformation of Chinese hamster ovary (CHO) K1 cell line into a high-producer using systems biotechnology approach.

Results and discussion: At global-level, sequencing data revealed extensive rearrangements in few targeted loci linked to transgene integration sites and , and the perturbation of perturbed gene expression of DNA damage repair and cellular metabolism gene expression through “hard-wired” changes in the gene copy numbers. Subsequent integration of transcriptome with genome-scale in silico model indicated an overall reduction in cellular fitness at the expense of recombinant protein synthesis. Finally, metabolomics/lipidomics and glycomics analyses further highlighted new characteristics of high- producers: elevated levels of energy and long-chain lipid metabolites, and surprising stability of N-glycosylation profiles. Collectively, our findings provide a global picture of cellular adaptations upon transgene integration, thus suggesting potential targets an integrative mammalian systems biotechnology framework for new vector designs and improved cell line engineering.

Bibliography, Acknowledgements: This work was supported by the Academic Research Fund (R-279-000-476-112) of the National University of Singapore, Biomedical Research Council of A* STAR (Agency for Science, Technology and Research), Singapore, and a grant from the Next-Generation BioGreen 21 Program (SSAC, No. PJ01109405), Rural Development Administration, Republic of Korea.

Disclosure of Interest: None declared





PO297

LAB-AUTOMATION & DATABASE INTEGRATION: STREAMLINING THE CELL LINE DEVELOPMENT PROCESS

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Background and novelty: The generation and data management of stably expressing cell lines through traditional cell line development (CLD) work flows remains both challenging and labor intensive. Furthermore, continued growth of the biopharmaceutical industry demands increased production, thereby requiring the implementation of rapid high-throughput solutions. In an effort to overcome these limitations, an automated cell line development platform and streamlined work flow integrating robotic cell culture handling technologies with software-based data handling applications was developed.

Experimental approach: An automated CLD platform was developed using a core liquid handling workstation integrated with both incubation and cell imaging functionalities for seeding, incubation, clone selection, cell counting, screening, and passaging. In parallel, a software-based data handling application was piloted and leveraged to enable complete system data integration.

Results and discussion: Through laboratory robotics implementation and software-based data handling integration we have worked to develop an automated cell line development platform. As a result, we have demonstrated reduced operator labor and increased throughput capacity in support of the cell line development process and subsequent generation of stably expressing cell lines. Results have demonstrated comparable candidate clone screening between manual and automated methodologies for parallel projects as determined by key selection criteria. Furthermore, database mediated rank ordering and subsequently automated clone identification and selection has reduced manual rate limiting processing steps and expedited work flow time lines. Detailed proof of concept case study results will be presented.

Bibliography, Acknowledgements: -

Disclosure of Interest: None declared





PO298

EXPEDITING PROTEIN BIOMANUFACTURING THROUGH THE UCOE® GENE EXPRESSION PLATFORM

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Background and novelty: Ubiquitous chromatin opening elements (UCOE®) from housekeeping gene loci are compact and easy to manipulate genetic regulatory elements, which provide highly reproducible and stable expression irrespective of transgene integration site within the host cell genome. UCOE®-based vectors markedly expedite the production of stably transfected clonal cell lines capable of high (grams/liter) levels of protein production. No transgene amplification step is required leading to shortening of the generation of desired high producing clonal cell lines to just 2 months.

Experimental approach: UCOE®-augmented and non-UCOE® CMV-based plasmid vectors driving expression of a monoclonal antibody were stably transfected CHO cells. Side-by-side comparisons of UCOE® vectors with other expression systems containing MAR, STAR and insulator elements were undertaken in stably transfected CHO cells.

Results and discussion: Results show a striking superiority (>10-fold) of the UCOE®-CMV vector in terms of antibody productivity in bulk cell pools and especially cell clones. High expressing cell clones were readily isolated. UCOE® vectors significantly outperform those based on MAR, STAR and insulator elements. Cell lines expressing a wide range of protein products such as antibodies, receptors, enzymes, cytokines, etc. can readily be produced with industry standard platforms employing, for example, CHO cells, CMV promoters, plasmids, transfection agents, media, etc. Thus the UCOE® platform is seeing increasing commercial success. The presentation will provide (i) a brief basic science understanding of UCOE® systems, (ii) describe a range of different expression vector systems and (iii) exemplify how UCOE®-based vectors can be used to rapidly generate high protein producing clonal cell lines and their progression to industrial scale manufacture.

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Disclosure of Interest: None declared





PO299

TARGETED EPIGENETIC MODULATION OF EXPRESSION CASSETTES REVERTS SILENCING OF TRANSGENE EXPRESSION

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Background and novelty: Epigenetic silencing of promoters is influenced by both, the chromosomal integration site and the design of the incoming cassette. The crosstalk leading to epigenetic silencing remains elusive and is largely unpredictable. We aimed to elucidate the mechanisms underlying expression instability in specific integration sites and to develop strategies to overcome transgene inactivation

Experimental approach: We investigated transgene silencing upon epigenetic remodeling which naturally occurs during differentiation of embryonic stem cells. Targeted integration of various cassettes with different promoters (viral, cellular, synthetic) was achieved in two defined chromosomal sites by Flp mediated cassette exchange (RMCE).

Results and discussion: Upon differentiation all of the integrated promoters were inactivated while the flanking chromosomal sites remained fully active. Further analysis revealed that silencing was associated with pronounced DNA methylation of the transgene promoters while the endogenous promoters remained methylation free. We evaluated if chromosomal elements previously described to stabilize transgene expression could overcome silencing at these sites. The UCOE element, could stabilize expression for combinations of promoter and integration sites to a certain extent. However, they could not restore transgene expression. To specifically activate transgene expression we designed a synthetic biology approach aiming at actively and site specifically erasing DNA methylation marks at the transgene promoter. To this end, a synthetic epigenetic modulator protein was constructed to recruit DNA hydroxymethylase activity to the silenced promoter. Strikingly, expression of the epigenetic modulator reduced DNA methylation of the transgene promoter and could restore transgene expression. Thus, by targeted epigenetic modification transgene expression can be stabilized.

Bibliography, Acknowledgements: The UCOE construct was a gift from Thomas Moritz

Disclosure of Interest: None declared





PO300

CRISPR-BASED TARGETED EPIGENETIC EDITING IN CHO CELLS

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Background and novelty: Extensive knowledge has been gathered by applying –omics techniques towards a holistic understanding of the Chinese Hamster Ovary (CHO) cell's regulatory network. However, these data are far from fully explanatory. The epigenome, i.e. the genetic signature that contributes to modulation of gene expression, has not been fully explored yet. Interestingly, DNA methylation has been reported to result in a loss of productivity in CHO cells¹. Additionally, a genome-wide study showed that DNA methylation effects are site-specific and dependent on the gene element². Routinely, epigenetic control in cells is executed by exposure to small inhibitor or enhancer molecules¹. These approaches are feasible when genome wide epigenetic signature changes are desired, but inappropriate when epigenetic modulators should target single loci. Because of recent developments within the CHO field, e.g. genome-sequencing and application of genome editing tools, a new and more elegant way to study epigenetic effects on CHO performance parameters seems possible.

Experimental approach: The present study aims to explore epigenetic targets from genome wide methylation and CHIP-seq data with CRISPR-based tools to target specific loci and to induce site-specific DNA methylation or demethylation. The required constructs are tested for their effect on gene expression of both endogenous and exogenous targets using specific guideRNAs against the respective promoter.

Results and discussion: For proof of concept, the current design targets a recombinant cell line as a reporter for transgene expression modulation. This epigenetic tool set would allow to build a new layer of cell control to complement existing techniques and may even allow for a more sensitive investigation of gene function by induction and repression of genes without altering the DNA sequence.

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Disclosure of Interest: None declared





PO301

GENERATING HIGH PRODUCING SINGLE CELL CLONES BY UTILIZING AN OPTIMIZED SELECTION STRATEGY

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Background and novelty: For the production of biotherapeutics, suspension cells such as the human CAP cell line have become a highly valuable tool, offering the necessary scalability for industry scale production. However, generating high producer single cell clones (SCCs) can be time consuming, as it involves the production of a stable pool as a first step, followed by an extensive single cell cloning and screening approach. Stable pools are mixtures of non-, low-, and high-producer clones. Usually, high-producers show a growth disadvantage over low producer clones and can be overgrown easily during pool generation. Therefore, a huge amount of SCCs has to be analyzed in order to identify high-producing clones when using standard protocols.

Experimental approach: We developed a fast and robust method to generate SCCs from CAP cells by skipping the pool generation step and plating the newly transfected cells directly into semisolid medium. Thereby, we minimize the risk of losing high-producer clones. In addition, by using a modified expression vector, which harbors an optimized antibiotic resistance cassette, we can apply very rigid selection conditions to the diluted cells immediately post transfection. This ensures that only high producing SCCs can survive and propagate in the semisolid medium.

Results and discussion: Modified expression vectors were engineered for the selection markers blasticidin, neomycin, or puromycin. Using optimized selection conditions, our new approach shortens the timelines for cell line development by around 20 days. In addition, we were able to significantly increase the number of high producers after transfection. This allowed us to screen considerably less single cell clones as most of the present ones were high producers with up to four times higher expression levels compared to single cell clones generated by standard protocols.

Bibliography, Acknowledgements: -

Disclosure of Interest: None declared





PO302

CHARACTERIZATION OF ANTIBODY-PRODUCING CHO CELLS WITH CHROMOSOME ANEUPLOIDY

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Background and novelty: Chinese hamster ovary (CHO) cells have chromosome number distribution. Previously, DG44-SC20 and DG44-SC39 cell lines with modal chromosome numbers 20 and 39 were isolated from parental CHO-DG44 cells, and IgG3-expressing cell lines named IgG3-SC20 and IgG3-SC39 were established, respectively. Even though all of the IgG3-SC20 clones and half of the IgG3-SC39 clones contained the same number of vector integration sites (one each), antibody productivities of IgG3-SC39 clones were higher than those in any of the IgG3-SC20 clones [1]. In this study, we performed transcriptome analysis to investigate the characteristic of high producer cells with chromosome aneuploidy.

Experimental approach: Transcriptome analyses using HiCEP and *de novo* mRNA-seq were performed to DG44-SC20, DG44-SC39, IgG3-SC20, and IgG3-SC39. To compare cell lines with different numbers of chromosomes, transcriptome data from mRNA-seq were adjusted for cell number using RNA reference materials. Pathways related to differentially expressed genes were searched by KeyMolnet (KM Data).

Results and discussion: High chromosome number cells showed bigger cell diameters by Vi-CELL (Beckman Coulter) measurement. Based on the analysis of gene expression levels per cell volume, 90 % of genes showed lower expressions in both DG44-SC39 and IgG3-SC39 compared to DG44-SC20 and IgG3-SC20, respectively. This reduction may produce extra energy for the recombinant protein production in high chromosome number cells. In addition, the number of genes whose expression level was decreased per cell in IgG3-SC39 (vs. DG44-SC39, vs. IgG3-SC20) was larger than those that increased. The results of the comparisons between IgG3-SC20 and IgG3-SC39 indicate that differentially expressed genes were mainly related to cell growth, apoptosis, lipid metabolism, and epigenetic histone modification pathways. The effect of these pathways on antibody production should be examined in future.

Bibliography, Acknowledgements: [1] Yamano *et al.*, JBB, 2016

Disclosure of Interest: None declared





PO303

QUANTITATIVE PHOSPHOPROTEOMIC ANALYSIS OF RECOMBINANT CHINESE HAMSTER OVARY CELLS IN RESPONSE TO REDUCED CULTURE TEMPERATURE

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Background and novelty: The reduction of culture temperature during the exponential phase of a culture is a strategy that is commonly employed by biopharma to increase product yield. Phosphorylation is one of the most important post-translational modifications, playing a crucial role in regulating many cellular processes. However, its role in regulating CHO bioprocess-relevant phenotypes has not been studied in detail to date.

Experimental approach: A recombinant CHO-SEAP cell line was grown at 37°C and then subjected to a reduction in temperature to 31°C. Cells were harvested 36 hours post-temperature shift. Protein samples were digested and phosphopeptide enrichment was carried out using TiO₂ and Fe-NTA spin columns. Enriched phosphopeptide digests were separated using an Ultimate 3000 RSLCnano system interfaced with an LTQ-Orbitrap mass spectrometer. Differential phosphopeptide expression analysis between the two culture conditions was carried out using Progenesis Q1 for Proteomics.

Results and discussion: Quantitative label-free LC-MS/MS phosphoproteomic analysis resulted in the identification of 700 differentially expressed phosphopeptides following a reduction in temperature from 37°C to 31°C. Gene ontology analysis showed a significant enrichment of biological processes related to ribosomal biogenesis, RNA processing and growth, and molecular functions related to RNA binding and transcription factor activity. We have also characterised over 3500 CHO-specific phosphosites to date.

This data suggests the importance of including the post-translational layer of regulation, e.g. phosphorylation, in CHO 'omics' studies. This study also has the potential to identify phosphoprotein targets that could be modified using cell line engineering approaches to improve recombinant protein production.

Bibliography, Acknowledgements: This work is supported by funding from Science Foundation Ireland Investigator Programme Award, Grant no. 13/1A/1841.

Disclosure of Interest: None declared





PO304

PROCESS DEVELOPMENT AND OPTIMIZATION FOR THE PRODUCTION OF CLINICAL GRADE HIV-1 ENVELOPE GLYCOPROTEIN VARIANTS

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Background and novelty: Soluble native-like trimers (NLT) of the HIV-1 envelope glycoprotein (env) applied as antigens are among the most promising approaches to elicit a broadly neutralizing antibody response fighting HIV-1 infection. In the last decade, remarkable progress has been made in development and design of NLT HIV-1 env protein variants. In this project we aim to determine factors influencing the efficient production of the env protein in CHO cells for the purpose of clinical grade protein production.

Experimental approach: Stable CHO cell clones co-expressing human furin protease (mediates efficient cleavage of the env precursor) and variants of HIV-1 env were generated by BAC vector technology [1]. Stable transfection pools were then single-cell cloned to obtain clonal populations. Screening for trimeric env specific expression was performed by ELISA with mAb PGT145 recognizing native trimeric env selectively. Further, integration of the human furin gene was assessed by PCR. The top producers were then compared under small lab-scale production conditions to get an insight in clone specific protein production behaviour.

Results and discussion: Native PAGE analysis of culture supernatants from the top producing clones indicated a trimer specific signal at the expected molecular mass. Western blotting of crude culture supernatants against gp41 and gp120 of a non-reduced and reduced SDS-PAGE suggested that mainly fully cleaved env protein was produced. Further, PCR analysis confirmed that top producers were positive for furin but also a small set of furin negative clones showed to produce NLT env. In further investigations furin negative clones will be evaluated for their env processing efficiency in absence of co-expressed furin. This will give us a more profound understanding of the actual level of co-expressed furin needed for HIV-1 env processing and its limitations.

Bibliography, Acknowledgements: [1] Zboray et al., Nucl. Acids Res. (2015)

Disclosure of Interest: None declared





PO305

WHOLE GENOME SEQUENCING TO SURVEY GENETIC CHANGES IN STABLE CHO CELL LINES

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Background and novelty: Chinese hamster ovary (CHO) cells represent the most frequently applied host cell system for industrial manufacturing of recombinant protein therapeutics. Generating and identifying high producing clones in a fast and efficient way that do not lose their expression capability over time has been a major focus of the industry.

Experimental approach: Using Next-Generation Sequencing technologies combined with proprietary bioinformatics tools called SUREscan™, we now have the ability to quickly analyze the whole genome of any generated cell line.

Results and discussion: We find that phenotypic changes in growth behavior and metabolism that are typically caused by cellular stress such as adaptation to a different media are associated with a rise in single nucleotide polymorphism (SNP). A total of more than 300 SNPs were detected using a single SNP regression approach. This study provided a framework upon which to identify the causal mechanisms underlying the various growth traits. Importantly, in light of the FDA and EMA recent concerns regarding establishment of clonality for IND and BLA submissions, these SNPs could be used to assess monoclonality demonstrating that SUREscan™ can be used to improving traceability of RCBs, MCBs and WCBs. Singularly, karyotype analysis of over 60 RCBs revealed that our CHO lineage is chromosomally stable indicating that the critical stages of a cell line production platform (transfection, recovery, selection and expansion) do not induce chromosomal changes. This contrasts with previous studies that have shown large chromosomal rearrangements in CHO cell lines [1, 2].

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Disclosure of Interest: None declared





PO306

DYNAMIC CHANGES TO THE PHOSPHOPROTEOME OF RECOMBINANT CHINESE HAMSTER OVARY CELLS DURING GROWTH IN SUSPENSION CULTURE.

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Background and novelty: Little work has been carried out on the phosphoproteomic analysis of CHO cells despite the importance of phosphorylation in regulating many cellular processes including cell proliferation, transcription, translation, apoptosis and cell survival pathways. During CHO cell culture it can be expected that the phosphoproteome will change and that such changes may give indications on the cellular state in terms of growth, viability and productivity, for example. In this study we applied mass spectrometry based phosphoproteomics to monitor changes in expression patterns of the phosphoproteome during lag, exponential, and stationary phases of the growth curve in recombinant CHO cell suspension cultures.

Experimental approach: CHO DP12 cells were cultured in serum-free media at 37°C. Samples were collected at different time points during the different growth phases, i.e. lag, exponential, stationary and death phase. Phosphopeptide enrichment was performed by MAOC (TiO₂) chromatography in parallel with IMAC (Fe-NTA) chromatography prior to quantitative label-free LC-MS/MS phosphoproteomic analysis. Differentially expressed phosphopeptides were identified using quantitative label free data analysis software, Progenesis Q1 for Proteomics. PhosphoRS filters were set at >75% for assignment of site-specific phosphorylation. Gene Ontology was carried out using DAVID functional annotation software.

Results and discussion: Phosphoproteomic comparison between the growth phases revealed differentially phosphorylated proteins with potential regulatory significance, including transcription factors, cell cycle modulators and key signalling molecules. These results provide a functional understanding of the CHO cell phosphoproteome during growth in suspension culture.

Bibliography, Acknowledgements: Funding from Horizon 2020 Marie Curie ITN programme - eCHO systems (grant ref: 642663).

Disclosure of Interest: None declared





PO307

ECHO SYSTEMS – ENHANCING THE CHO CELL FACTORY THROUGH SYSTEMS BIOLOGY

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Background and novelty: Chinese hamster (CHO) ovary cells are the production host for a +50 billion €/year biopharmaceuticals market. Current CHO production platforms date to 1980 and are based primarily on media and process optimisation with little consideration to improving the cellular machinery. Fortunately, with the recent sequencing of the CHO genome, an opportunity has opened to significantly advance the CHO platform. This ITN graduate training programme - eCHO Systems - blends conventional molecular, cellular, and synthetic biology with genome scale systems biology, including 'omics data acquisition, biological network modelling, and genome engineering, in order to make a big step in the improvement of the CHO cell factory.

Experimental approach: The eCHO Systems ITN consists of three Work Packages with different but complementary experimental approaches: first, to identify and utilise essential published genomic, transcriptomic, proteomic, metabolomics, and epigenetic data sets and to generate additional complimentary datasets, to be integrated into computational network models to describe and simulate the phenotypic behaviour of different CHO production lines. Second, to develop sophisticated synthetic biology tools that will permit large-scale genome engineering of the CHO chassis. Finally, to apply these computational and genome engineering tools to improve the capabilities of the CHO platform by targeting a number of pathways including secretion, glycosylation and metabolism.

Results and discussion: After having completed 2 years of the programme, one of its main objectives has already been fulfilled: to train a talented and motivated group of young scientists. In addition, at this stage, all different ESR projects are mature and have produced the first scientific results on improving the CHO cell platform, which are being presented at different international events, including ESACT.

Bibliography, Acknowledgements:

Disclosure of Interest: None declared





PO308

EVALUATION OF MICRORNA-BASED GENETIC SWITCHES AS TRANSGENE EXPRESSION MODULATORS IN CHO CELLS

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Background and novelty: Current methods for controlled gene expression in CHO cells include a few inducible promoters/transcription factors, most of which require the addition of chemicals to the culture medium to modulate gene expression (e.g. TET-Promoter-responsive elements). MicroRNAs (miRNAs) play an important role in the regulation of protein expression at a post-transcriptional level. The ability of these molecules to regulate complex gene networks has made them very interesting targets for cell engineering. In this study, a novel use for endogenous CHO microRNA as potential transgene expression modulators using miRNA-responsive cassettes was investigated.

Experimental approach: miRNA targets were identified from next generation sequencing analysis of pre and post temperature shifted CHO cells culture. A selection of miRNAs found to be differentially expressed were subsequently validated via qPCR. Synthetic genetic constructs containing miRNA-responsive elements complementary to the validated targets and a reporter transgene were constructed. The generated plasmids were transiently and stably transfected in different CHO cell lines. These cell lines were then evaluated to assess the expression of the transgene after temperature shift. miRNA expression and transgene mRNA levels were measured by qPCR. Transgene expression at a protein level was measured by flow cytometry or another Luciferase reporter assay.

Results and discussion: Different cell lines containing different miRNA-responsive cassettes were generated. Correlations between miRNAs levels and transgene expression at the mRNA and protein level were established for each of the cell lines as well as the ability of the different constructs to link miRNA expression to miRNA changes after temperature shift. These results represent a first step towards developing cell lines with specific switchable pathways using miRNAs as endogenous triggers.

Bibliography, Acknowledgements:

Disclosure of Interest: None declared





PO309

THE GENERATION OF A PROPRIETARY NEW CHO HOST CELL LINE WITH ENHANCED BIOMANUFACTURING QUALITY ATTRIBUTES

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Background and novelty: The industrial production of recombinant proteins relies upon the ability of having a host cell line that has the desired quality attributes. Depending upon the production platform such attributes include; full documented pedigree, industrial compatibility with current bioproduction processes, robustness and the ability to produce high yields of complex polypeptides, antibodies and fusion proteins with high fidelity expression of the required mammalian post translational modifications.

Experimental approach: A novel proprietary CHO cell line, (NeuCHO®), has been developed that is both robust and has superior growth characteristics when compared to a parental CHO-S cell line. The NeuCHO® cell line was generated by the stable transfection of parental CHO-S cells with a proprietary pNeu® vector (an optimised vector, designed for enhanced expression, complementary to NeuCHO® cells) which expresses a growth factor.

Results and discussion: Extensive characterisation experiments have shown that the NeuCHO® cell line maintains a higher viability in both fed-batch and mini-bioreactor culture environments, than the parental CHO-S cell line. In addition, the NeuCHO® cell line also reaches higher viable cell densities compared to the CHO-S, allowing for increased yields at harvest. This prolonged viability and increased IVCD is particularly promising in other types of production processes such as perfusion or continuous integrated bioprocessing platforms.

A panel of clones were derived from the NeuCHO® pool via FACS. A subset of clones was used as parental cells and subsequent stable transfections were performed with a mAb biosimilar. New experimental data has shown comparable and in some cases, favourable results, compared to CHO-S, in terms of product quality and titre. We have also elucidated the intracellular mechanisms leading to possible new cell engineering strategies.

Bibliography, Acknowledgements: None

Disclosure of Interest: None declared





PO310

GENETIC ELEMENT COMBINATIONS TO IMPROVE EXPRESSION LEVEL OF CELL LINES FOR MONOCLONAL ANTIBODY PRODUCTION IN CHO CELLS

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Background and novelty: The establishment of a high expressing cell line for therapeutic protein production remains challenging. It is well known that the expression level of production cell lines can be improved at genetic level by expression constructs including variety of regulatory elements and their positions and directions.

Experimental approach: The goal of this work was the optimization of our in-house expression vector system for improved monoclonal antibody expression. The focus was put on the (i) promoter activity at our CHO cells, (ii) arrangement of expression cassette of heavy chain, light chain and selection marker, (iii) signal peptide combinations for model IgG1 molecules. The expression level was studied by quantification of the IgG titer in the supernatant and relative amount of transcripts.

Results and discussion: In our host cell line, well established viral constitutive active promoters gave the high mRNA levels than endogenous PGK promoter. The data demonstrated that the promoter for selection marker also affected heavy and light chain transcript level and IgG titers at transient expression systems. Furthermore IgG promoters also highly influence selection gene expression level and stable pool titers. As for signal peptide, the naturally occurring IgG signal peptide worked best as long as we tested. The codon for signal peptide is also affected IgG titer in supernatant and also intracellular accumulated protein. By testing each vector component, high expressing cell line could be established with as high production rate as high as 50 pg/cell/day. From these data, for the construction of high expression vector, it is beneficial not only by transient expression evaluation system but also stable cell evaluations.

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Disclosure of Interest: None declared





PO311

SIALYLATION OF O-LINKED GLYCANS IS CRUCIAL FOR SIGNIFICANT PROLONGED PLASMA HALF-LIFE OF RECOMBINANT C1 INHIBITOR.

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Background and novelty: The development of therapeutic proteins has accelerated immensely over the past years. However, the recombinant expression of highly glycosylated recombinant therapeutic proteins, like e.g. serum proteins, has remained a challenging task.

C1-Inhibitor is glycosylated with 7 N- and 8 O-glycans. Plasma derived C1-Inh as well as recombinant C1-Inh from transgenic rabbits are approved for the treatment of acute attacks in patients with hereditary angioedema. However, the recombinant product shows dramatically reduced plasma half-life in comparison to the plasma-derived versions.

Experimental approach: We have developed the CAP-Go protein expression platform, an expression system based on human cells, to confer optimal glycosylation to complex glycoproteins such as C1-Inh.

Results and discussion: The CAP-Go.1 cell line has been modified to facilitate expression of proteins with fully sialylated N-glycans. Several recombinant proteins produced with CAP-Go.1 show a significantly prolonged serum half-life in rats. However, expression of rhC1-Inh in CAP-Go.1 cells had no positive impact on the pharmacokinetic profile.

However expression of C1-Inh in CAP-Go.2 cells, which in addition addresses the O-linked glycosylation patterns, results in a significantly increased serum half-life which is indistinguishable from the plasma-purified protein. O-glycan analysis shows that rhC1-Inh expressed by CAP-Go.2 cells contains only highly sialylated core1 O-glycan structures, highly comparable to plasma-derived Berinert. C1-Inh expressed from CAP-Go.2 cells, matches serum-derived C1-Inh in specific activity, serum half-life, and glycosylation pattern and offers the advantage of being manufacturable at large scale on a safe platform.

In conclusion, our results demonstrate that in addition to N-glycosylation, the structure of O-glycans plays a crucial role in bioavailability and pharmacokinetic properties of glycoproteins.

Bibliography, Acknowledgements: /

Disclosure of Interest: None declared





PO312

WHAT NGS CAN TELL US: SPONTANEOUS EXPRESSION OF A SLEEPING GENE IN CHO CELLS LEADS TO CRITICAL CHANGES IN PRODUCT QUALITY

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Background and novelty: The genetic configuration of a cell mainly defines its phenotypic status. Advances in NGS technologies have enabled new opportunities to gain deeper insights into the complex nature of CHO cell biology. MAbs produced by CHO cells exhibit complex N-glycosylation pattern with low sialylation levels. We have recently identified a CHO cell line producing mAbs with abnormally high levels of immunogenic NGNA sialic acids. Hence, we investigated these spontaneous changes in mAb sialylation using an integrated genomic, transcriptomic and miRnomic analysis approach.

Experimental approach: We established a NGS analysis workflow combining DNA and RNA sequencing with extensive process and product related analytics for CHO cells. In a case study, 2 different clonal CHO cell lines producing the same mAb but with different NGNA levels were cultivated in fed-batch processes. At different time points during cultivation, bioreactor samples were subjected to DNA and RNA sequencing and compared to process and product related analytics.

Results and discussion: Although genome sequences of both cell lines were found to be highly similar, cell lines showed considerable differences in gene and miRNA expression. Strikingly, increased NGNA levels could finally be attributed to the spontaneous expression of a single gene in one of the cell lines which was commonly believed not to be expressed in CHO cells. These results underscore that NGS is capable of accurately deciphering cellular behavior and thus represents a potent tool for advanced cell line development. This case study clearly points out that critical genetic events might occur much more frequently in CHO cells than previously expected with a profound influence on process performance and product quality. A careful cell line selection strategy including *a priori* genetic characterizations might thus be the key to successful cell line development in the future.

Bibliography, Acknowledgements: -

Disclosure of Interest: None declared





PO313

MIRNA KNOCKOUT USING CRISPR/CAS9 TO ENHANCE CHO CELL BIOPHARMA PHENOTYPE

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Background and novelty: Chinese Hamster Ovary (CHO) cells are the workhorse of Biopharma industry for the production of mAbs and therapeutic proteins or biologics, because of their inherent ability to produce human-like post-translational modifications and glycosylation patterns which make them bioactive in humans without any further processing. Even though CHO cells are in use for the production of biologics from last three decades, there is a huge requirement for further optimisation of CHO cells to manufacture the next generation biologics; there is an increasing global demand for these biologics as a treatment for a broad range of diseases. miRNAs are small noncoding RNAs which act as endogenous regulators of gene expression. miRNA knockdown will result in regulation of different gene pathways without any translational burden on cell line and has a beneficial impact on CHO cell phenotype. Improving the productivity of CHO cells will help to decrease the cost of production and ultimately ensure the availability of therapeutics for a broad range of diseases.

Experimental approach: Stable depletion of a miRNA achieved by transfecting a 'sponge' plasmid to sequester endogenous miRNA and divert it away from its natural target mRNAs. However, this conventional approach does not achieve complete knock down of miRNA and requires the overexpression of another transgene (the sponge or shRNA) in the producer cell line. Complete miRNA knockdown achieved by targeting genome using CRISPR/Cas9 technology which will disrupt or delete the miRNA from genome will result in a complete knock down of miRNA and its natural function.

Results and discussion: Initial results after miRNA-24 knockout showed enhanced cell proliferation and further analysis of knockout done in different CHO cells by analysing CHO cell phenotypes viable cell density, viability and productivity.

Bibliography, Acknowledgements: SFI TIDA grant 16/TIDA/4127

Disclosure of Interest: None declared





PO314

DEFINITION AND REMOVAL OF BOTTLENECKS IN CURRENT CHO PRODUCTION CELL LINES WITH REGARD TO COMPLEX BIOLOGICAL FORMATS

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Background and novelty: With the advance of complex biological formats such as bispecific antibodies or fusion proteins, mammalian expression systems often show low performance. Described determining factors may be accumulation or haltering of heterologous proteins within the different cellular compartments and thereby disturbing transport or secretion. Therefore, the description of production and transport routes of complex biologics within Chinese hamster ovary (CHO) host cell systems is the primary aim of the current study which will finally lead to the identification of repressive or saturated organelle structures hampering efficient protein processing.

Experimental approach: A set of recombinant CHO cell lines either producing a classical easy-to-express monoclonal antibody (mAB) or more complex biological formats will be used for organelle staining and co-localization of the produced recombinant protein analyzed by confocal microscopy to describe distribution/accumulation within the distinct cellular compartments. Organelles include the nucleus, endoplasmic reticulum (ER), the cis-Golgi and the trans-Golgi network (TGN) as well as lysosomes and endosomes.

Results and discussion: We have established a staining protocol that enables us to trace a recombinantly expressed protein from its origination to its release traveling through distinct cellular compartments of CHO production cell lines. Currently, a set of recombinant CHO cell lines producing more complex biological formats such as bi-specific antibodies or fusion proteins are analyzed for distribution/accumulation within the described cellular compartments. This will finally lead to the identification of repressive or saturated organelle structures hampering efficient protein production in the CHO host cell system. Consequently, cellular engineering approaches can be applied to establish superior cell systems with improved properties.

Bibliography, Acknowledgements: -

Disclosure of Interest: None declared





PO315

CHO-GLYCO-ENGINEERING USING CRISPR/CAS9 MULTIPLEXING TO DEVELOP CELL LINES WITH HOMOGENEOUS N-GLYCAN PROFILES FOR PHARMACEUTICAL DRUG PRODUCTION

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Background and novelty: Although chinese hamster ovary (CHO) cells' strength is the production of similar *N*-glycans compared to human glycosylation¹, non-engineered CHO cells display a broad variety of *N*-glycans which often includes *N*-glycan structures, that are of no benefit or even have an undesired effect on e.g. efficacy or lectin-mediated clearance of the glycoprotein.

Combining the CHO-K1 draft genome², identified CHO glycosyltransferases³ and the power of multiplexing gene knock-outs with CRISPR/Cas9⁴, we generated several CHO-S cell lines producing homogeneous *N*-glycan profiles which are beneficial to many therapeutic glycoproteins on the market, including tri- and tetra-antennary *N*-glycans for increased EPO glycosylation and bi-antennary *N*-glycans for IgG type molecules.

Experimental approach: The *N*-glycan secretome of the described clones was first deconstructed by targeting several glycosyltransferases with CRISPR/Cas9 multiplexing and afterwards successfully re-built by introducing human-like alpha-2,6-linked sialic acids.

Motivated by the efficient genome editing tool, ten single guiding RNAs were simultaneously co-transfected with Cas9 to attempt knock-out of ten gene targets involved in *N*-glycan build-up, glutamine synthesis, nucleotide sugar synthesis and apoptosis in an IgG producing CHO-S clone.

Results and discussion: Our presented *N*-glycan engineering approach for the secretomes of non-producing CHO host cells offers a flexible tool box to produce several commercial glycoproteins with a selected *N*-glycan structure for improved *in vivo* functions during glycoprotein based therapy and highlights the accelerated genome editing possibilities in CHO cell line engineering.

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Disclosure of Interest: None declared





PO316

PATHWAY MODULATOR BOOSTS YIELDS FOR THERAPEUTIC PROTEIN PRODUCTION

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Background and novelty: Today, typical limitations of transcription and expression stability are addressed either by the use of specific vector design or target gene integration at preferred loci. In addition, optimal nutrient supply provides high cell densities and production capacity. Nonetheless, the protein secretion process may still limit the overall production yield.

Experimental approach: In order to eliminate potential secretion bottlenecks, we employed a mutant of *cdc42*, a rho GTPase known to affect Golgi vesicle transport among multiple other functions. The enzyme was stably expressed in different CHO strains: CHO-S, CHO-K1 and CHO-DG44 to modify host cells or producer clones. Target protein expression and N-glycan profiles, as well as modulator expression stability were studied.

Results and discussion: To our surprise, we found specific productivities and titers enhanced not only for antibodies and proteins with inherently lower expression, but also for those already expressed in the g/l range. In fact, for the majority of the products tested, upstream yields were substantially improved. The higher expression level did not affect the glycan profile. For modified subclones and unmodified producer cell lines, we observed similar N-glycan structures. In CHO-S host cells *cdc42* expression was well tolerated and stably maintained for more than 90 population doublings. We also engineered our own DG44 platform. The *dhfr*-deficient phenotype of this strain is one of the main challenges for such a strategy. Unlike transformants which express a recombinant DHFR version, host cells are not susceptible to cloning or fed batch processes. We solved the issue through defined media adjustments which rescues most of the metabolic blocks. Hence, single cell cloning is now feasible. Furthermore, considerably increased cell numbers can be achieved. This enables studying fed batch performance which is an important criterion to consider when selecting modified host clones.

Bibliography, Acknowledgements: ProBioGen

Disclosure of Interest: None declared





PO317

INVESTIGATION OF FACTORS INFLUENCING RECOMBINANT HUMAN BMP2 EXPRESSION IN MAMMALIAN CELLS

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Background and novelty: Human growth factors have an enormous therapeutic potential. Among them, the bone morphogenetic protein-2 (BMP-2) can induce *de novo* bone formation endowing the protein a high therapeutic potential. However, finding a suitable recombinant production system for such a protein still remains a challenge.

Experimental approach: Recombinant expression of hBMP2 was investigated in transiently transfected HEK-293 cells and in stable clones established in CHO-K1 cells. Protein stability and interaction of the hBMP2 with the producer cells were investigated *in vitro* using commercially available rhBMP2. In addition, we investigated a cell-free protein synthesis system harboring translocationally active microsomal structures, hence having the potential to perform post-translational modifications, as an alternative production method.

Results and discussion: We showed that growth rates and viabilities of the rhBMP2-producing cells were similar to those of the parent cell line, while entry into the death phase was delayed in case of the recombinant cells. The maximum rhBMP2 concentration detected in the culture supernatant was low for stable clones but can be improved by more than 10-fold (~ 300 ng/mL) combining the HEK-293 cells transient expression system and batch reactor cultivation which reflects a better compatibility of the codon usage in the human cells. hBMP2 protein is sensitive to slightly acidic pH and to a lesser extent to proteases and binds to both producers cell lines – All this could incidentally contribute to the low product titers. Upon cell-free protein synthesis, the hBMP2 yield was almost 100-fold higher than the best one in the HEK-293 cells demonstrating that the cell-free expression system is most suitable compared to mammalian cell expression method for the production of glycosylated human BMP2.

Bibliography, Acknowledgements: -

Disclosure of Interest: None declared





PO318

SYSTEMATIC INVESTIGATION OF PDMAEMA-FUNCTIONALIZED MAGNETIC NANOPARTICLES FOR THEIR UTILIZATION IN BIOTECHNOLOGY

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Background and novelty: In the recent years, the development of novel non-viral vectors for gene delivery into mammalian cells has increasingly become on focus. Recently, we reported on novel PDMAEMA-based superparamagnetic nanostars, being superior to polycations used to date and allowing magnetic separation of the cells. We present a characterization of the influence of structure and composition on the function of these polymers using a library of highly homogeneous, paramagnetic nanostars with varied arm lengths and densities.

Experimental approach: The different variants were analyzed for their ability to complex pDNA using various physicochemical methods. Transfection efficiency/cytotoxicity in CHO-K1 cells were determined by flow cytometry. Transfected cells were placed in a magnetic field and the influence of the polymer architecture on the magnetic separation was investigated. Non-parametric Spearman analysis was used to correlate between arm length/arm densities, magnetic properties of the cells and transfection efficiency.

Results and discussion: Transfection efficiencies and cytotoxicities varied systematically with the nanostars architecture, with viability showing a more pronounced dependency on the characteristics of the transfection agent than the transfection efficiency itself. The arm density was particularly important, with values of approximately 0.06 arms/nm² yielding the best results. Moreover, only a certain fraction of the cells became magnetic during transfection. The gene delivery potential of a nanostar and its ability to render the cells magnetic did not correlate. End-capping the polycation arms with PDEGMA significantly improved the serum compatibility. In future optimized, blood-compatible, nanostars, which can be retained / directed by magnetic fields, could become options for non-viral gene delivery *in vivo*.

Bibliography, Acknowledgements: This research was supported by the Upper Franconian Trust, grant P-Nr.: 03847.

Disclosure of Interest: None declared





PO319

INTEGRATED 'OMICS STUDY OF A CONTINUOUS MANUFACTURING CHO PRODUCTION PROCESS FOR MONOCLONAL ANTIBODIES

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Background and novelty: Biotherapeutics such as monoclonal antibodies (mAb) are commonly produced using Chinese hamster ovary (CHO) cell lines. Most of them are manufactured in fed-batch mode, with the exception of unstable proteins prone to enzymatic or chemical degradation. However, using continuous cultivation can be beneficial for the mAb production as the stable operational conditions favour the reduction of product quality inhomogeneity over time. The so-called steady-state, where environmental and process conditions are constant is assumed to be mirrored in the intracellular status over the entire process duration. Rising 'Omics techniques enable the analysis of thousands of intracellular molecules, which could give important insights on the consistency of steady-state assumptions.

Experimental approach: In this study, we investigated the time evolution of the transcriptome and proteome of a mAb producing CHO cell line continuously cultivated in a perfusion bioreactor for 27 days. By the application of high-throughput methods, several thousands of transcripts and proteins could be identified for all time points.

Results and discussion: The fold change of all transcripts and proteins with respect to the steady-state was investigated for each individual dataset proving intracellular conformity. Functional annotation was performed with the help of bioinformatics databases, enabling the identification of potentially important pathways. In addition, the integrated analysis using data mining tools was conducted for all shared transcripts and proteins. This revealed differences between the gene expression and protein abundance as their correlation is not necessarily given. Furthermore, key transcripts and proteins related to N-glycosylation processing were analysed to visualize the role of the intracellular transcript and protein abundance on this important product quality attribute.

Bibliography, Acknowledgements: Acknowledgements: Merck Biopharma and Biognosys Ltd.

Disclosure of Interest: None declared





PO320

UNRAVELLING THE LACTATE METABOLISM OF CHO CELLS ENGINEERED TO GROW IN GALACTOSE

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Background and novelty: Biopharmaceutical sales represent a 100 billion dollar industry. Around 60% of biopharmaceuticals are produced using mammalian cells, being Chinese hamster ovary (CHO) cells the most widely employed.

Although glucose is the preferred carbon source for mammalian cell culture, glucose metabolism leads to high lactate production even under aerobic condition, which inhibits both growth and production. This phenomenon is known as the Warburg effect.

Here the metabolism of a recombinant t-PA producing CHO cell line that over-express galactokinase (CHO-Galk1)[1] is characterized. CHO-Galk1 cell line is able to grow using galactose as the sole sugar source producing virtually no lactate, making this cell line ideal for studying the Warburg effect. A deep understanding of CHO-Galk1 metabolism could provide new tools to improve biopharmaceuticals production in mammalian cells expression systems.

Experimental approach: CHO-Galk1 cells were grown in medium containing either galactose or glucose as the sole sugar. External metabolites were measured throughout the cultivation, and proteins were quantified at mid-exponential phase with SWATH. The metabolic phenotype was characterized using a dynamic metabolic flux analysis method based on B-spline fitting (B-DMFA)[2].

Results and discussion: More than 1,000 proteins were identified, almost 800 were found active in both conditions. Only eleven proteins were found differentially expressed. Under both medium conditions maximum cell concentration and growth rate were comparable. B-DMFA revealed that on galactose cell growth was supported almost exclusively by amino acids consumption, resulting in reduced alanine synthesis, glycine synthesis, higher ammonia accumulation, and trivial lactate accumulation.

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Disclosure of Interest: None declared





PO321

DOING MORE WITH LESS: HIGHER PRODUCTIVITY IN CHO CELLS WITH LOWER SELECTION PRESSURE

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Background and novelty: A DHFR-based CHO platform and flow cytometric reporter based system (FLARE) can be utilized to rapidly isolate and screen for high expressing clones that are suitable for manufacturing of biologics. Beyond this traditional FLARE methodology in cell line development, new approaches that leverage FLARE were designed to increase pool and clone productivity while reducing overall timelines using little to no methotrexate (MTX).

Experimental approach: Two independent sorting methodologies were developed. 1) Rapid Bulk Sorting was applied to quickly (1 week) boost pool productivity or in a sequential fashion to further increase productivity. This method utilizes FLARE sorting to enrich pools for higher productivity, not by killing cells with low transgene expression (as MTX does), but by isolating the minority of high expressing cells in the population. 2) In EPIC (Early Post-transfection Isolation of Cells) Sorting, a small subpopulation of transfected cells are bulk isolated based on reporter expression level prior to initiating pool selection. EPIC generated pools have significantly improved productivity compared to traditional transfection/selection methods.

Results and discussion: Rapid Bulking and EPIC sorting both yield CHO pools which are 2 to 4-fold more productive than traditional MTX selection methods. Each methodology generated pools making up to 0.5 g/L (unfed batch) while offering an overall time savings of 3 to 4 weeks. Clones generated from these pools had titers similar to those generated from the traditional (and longer) MTX selection process, with titers up to 2g/L unfed batch). More importantly, these gains in productivity have been achieved in the complete absence of MTX, questioning the need for MTX for generating highly productive pools and clones. Proof of concept studies will be presented.

Bibliography, Acknowledgements: --

Disclosure of Interest: None declared





PO322

BIOMANUFACTURING OF HEPARAN SULFATE GLYCOSAMINOGLYCANS USING ENGINEERED CHO CELLS

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Background and novelty: This project addresses an unmet commercial need for high quality heparan sulfate (HS), that is, a library of heparan sulfates with reproducible and defined compositions and protein-binding properties. HS consists of polysaccharide chains that are important components in cellular and physiological processes impacting multiple aspects of development, homeostasis and pathology. In these processes, HS chains function as cell surface receptors for specific proteins that dictate physiologic functions at the cellular level. Thus, HS polymers with defined compositions are critical reagents for a wide range of biological research areas. The objective here is to generate a series of physiologically relevant novel HS structures in cell culture that is scalable so as to enable industrial applications.

Experimental approach: HS from cultured cells displays the relevant size and structural composition seen in animal tissues. The HS chains are synthesized and modified by over 25 specific enzymes in the HS biosynthetic pathway. In turn, the HS compositions in different cell types and tissues appear to be the result of distinct expression patterns of the enzymes in the HS biosynthetic pathway. Thus, the composition of HS chains in cultures of Chinese Hamster Ovary (CHO) cells is being engineered by altering the expression pattern of the biosynthetic enzymes by transfection and/or mutation.

Results and discussion: The bioreactor conditions are being presently optimized for production using engineered CHO cells, these conditions can ultimately be scaled up to produce sufficient material for medical applications. Successful cellular expression will facilitate production of HS in a reproducible manner at a scale that will allow investigators to examine biological properties of HS and to fractionate and identify biologically relevant sequences

Bibliography, Acknowledgements: National Science Foundation, SUNY Polytechnic

Disclosure of Interest: None declared





PO323

UPR-MEDIATED INCREASE IN IGG PRODUCTIVITY IN RCHO CELLS ADAPTED UNDER MILD TUNICAMYCIN STRESS

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Background and novelty: The increasing demand of Mabs has necessitated the need to increase the productivity of current industrial cell lines. The study focuses on understanding the role of ER stress-mediated modulation of UPR in affecting the IgG productivity.

Experimental approach: Anti-rhesus IgG-secreting CHO cells [1] were cultured in SF-CDM in 125 mL shake flasks. The cells were subjected to ER-stress by treatment with tunicamycin (Tm) at varying concentrations (30-1250 ng/mL) and durations (12 hrs or till culture end). The cells were adapted by growing them in presence of low concentration of tunicamycin for more than 25 passages. IgG titers and mRNA expression levels were quantified using ELISA and qRT-PCR respectively.

Results and discussion: Screening CHO cells at different concentrations of ER-stress inducer such as Tm demonstrated a direct correlation between induction of chaperones (Grp78, CRT) and key transcription factor, XBP1s. However, increased ER stress also leads to apoptosis through induction of CHOP, a pro-apoptotic protein. In this work, we attempt to modulate the ER stress so as to prevent activation of apoptosis while increasing chaperone levels, by varying both Tm concentration and the time of treatment. Lower concentration Tm treatment resulted in a 1.5-2 fold increase in productivity without severely affecting the viability. Similarly, a ~2-fold increase in productivity can be achieved by reducing the Tm treatment time to 24 hrs. Predicting a UPR-mediated adaptive response in these cells, the CHO cells were adapted in the presence of lower concentrations of Tm over 25 passages, which resulted in cells with higher productivities. The basal UPR gene expression levels in these long term adapted cells were much higher than control suggesting mild UPR induction to be the basis of increased productivity.

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Disclosure of Interest: None declared





PO324

COMPARISON BETWEEN FACS-SINGLE CELL SORTING AND LIMITING DILUTION TO OBTAIN CLONAL CHO CELL LINES: VALIDATION OF SINGLE CELL CLONING BY FACS AND HIGH-RESOLUTION IMAGING

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Background and novelty: The development of therapeutic molecules in mammalian cells requires the selection of cell lines derived from a single cell population to ensure quality and stability over time and generations. At least two rounds of limiting dilution cloning is the most common accepted method to obtain clonal cell lines, but due to cell behaviour on aggregation and doublet formation, there is no perfect statistical model to achieve single cell distribution. Development of therapeutic molecules in mammalian cells can be time and resource-consuming. Strategic approaches must be applied to increase production efficiency as well as to satisfy regulatory requirements.

Experimental approach: Here we compared the probability of obtaining clonal cell lines from Fluorescence Activated Cell Sorting (FACS) vs. limiting dilution cloning (LDC). We utilised a high-resolution imaging system, Cell Metric ® CLD (Solentim Inc), to validate the clonality of the cell lines.

Results and discussion: FACS can significantly increase the probability of obtaining a single cell per well. (99% using FACS vs. 67% using LDC). Cloning efficiency was compared between FACS vs. LDC and was assessed by the ability of single cells to form colonies. Cloning efficiency using either method was similar. Interestingly, we observed a higher proportion of clones sorted using FACS having greater productivity than those obtained from LDC. Automated single cell cloning using FACS combined with high resolution imaging is a proven superior cell cloning method allowing for documentation and validation to meet regulatory requirements.

Bibliography, Acknowledgements: ¹ These authors contribute equally to this work

Disclosure of Interest: None declared





PO325

ENHANCEMENT OF ANTIBODY PRODUCTIVITY IN RECOMBINANT CHO CELLS CONSTRUCTED BY TARGETING THE IGG1 GENE TO THE STABLE CHROMOSOME

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Background and novelty: Chinese hamster ovary (CHO) cells have widely been used for largescale production of biopharmaceuticals. Recombinant CHO cells are usually constructed by random integration. In random integration, the integration site is not specific and the expression level varied between cells. Recently, gene-targeting methods which enable site-specific integration of expression vectors have been developed. However, the efficient region for exogenous gene expression has not been clarified. In this study, we constructed antibody producing cells by using a gene-targeting method which focused on the stable chromosome.

Experimental approach: According to our previous results, chromosomes A and B (chromosomes are named A to T in order of decreasing length) are considered as the sole paired chromosomes in CHO cells and are considered to be stable. Three different regions on chromosomes A and B were selected on the basis of CHO genomic BAC library sequences as targeting sites. CHO-K1 cells were stably transfected with humanized IgG1 expression vectors by using CRISPR/Cas9 system. The transfection without using CRISPR/Cas9 system (random integration) was also performed.

Results and discussion: Cell growths were not affected by gene-targeting sites. All cell pools constructed by gene-targeting to chromosomes A and B showed higher specific production rates than the cell pool constructed by random integration. Particularly, one selected region showed dramatically high specific production rate, and it was 6.4 times higher than that of random integration. These results indicate that targeting to chromosomes A and B may represent an effective strategy for establishing high-producing CHO cell lines.

Bibliography, Acknowledgements:

This research is partially supported by the developing key technologies for discovering and manufacturing pharmaceuticals used for next-generation treatments and diagnoses both from METI and AMED, Japan.

Disclosure of Interest: None declared





PO326

EFFICIENT TRANSIENT EXPRESSION OF HUMAN MATURE BONE MORPHOGENETIC PROTEINS BY PRO-PEPTIDE ENGINEERING

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Background and novelty: Bone morphogenetic proteins (BMPs) are crucial for the formation of various organs including bone, cartilage, muscle, kidney and blood vessels, and are currently investigated for clinical application. However, the production of BMPs can be challenging due to the incomplete processing of the pro-peptide, which hinders the maturation and the secretion of the disulfide-linked homo-dimeric BMPs.

Experimental approach: In this study, we developed a simple method for the transient production of bioactive mature BMPs from mammalian cells. Transfected HEK-293 cells had higher recombinant protein yields compared to CHO DG44 cells. In order to improve the successful maturation rate and to promote protein secretion, we exchanged the pro-peptide domain of various BMPs including BMP2, BMP4 and BMP7 with that of Activin A, another member of the TGF- β (TGF = transforming growth factor) family, which has previously shown efficient protein processing and secretion capacity in the transient expression of Activin A.

Results and discussion: Subsequently, all three BMPs were successfully secreted by HEK-293 with final protein productivities of over 100 mg/L in a 6-day transient production process. The predominately mature isoform of each BMP secreted into the medium significantly reduced the burden of downstream processing. Our study has shown an efficient strategy for the production of recombinant mature homo-dimeric BMPs of high quality. Future work on process optimization and scale-up and bio-activity assessment are under investigation.

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The authors would like to acknowledge Dr. Mingtao Huang for the constructive advices on protein engineering.

Disclosure of Interest: None declared





PO327

DEVELOPMENT OF RETROVIRAL VECTORS CAPABLE OF SITE-SPECIFIC GENE INSERTION TOGETHER WITH PROTEIN DELIVERY

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Background and novelty: We have demonstrated retroviral insertion of transgene into a pre-determined site of the founder cells using integrase-defective retroviral vectors (IDRVs), in case that Cre expression plasmid was transfected into the cells before retroviral transduction¹. Here we generated novel hybrid IDRVs (Cre-IDRVs) incorporating viral genome together with bioactive Cre recombinase, and validated repeated site-specific gene insertion into the CHO cell genome by Cre-IDRVs harboring an scFv-Fc expression unit.

Experimental approach: We designed a fusion protein expression vector (pGPM-Cre) by inserting Cre gene in the gag-pol precursor genes (pGPM). Viral vector plasmids encoding reporter genes and/or an scFv-Fc expression unit flanked by wild-type and mutant *loxPs* were constructed for IDRV. CHO cells introducing reporter genes flanked by a compatible pair of *loxPs* were used as the founder cells.

Results and discussion: We produced Cre-IDRVs by transfecting viral vector plasmid, pGPM, pGPM-Cre and pVSV-G into 293FT cells. Western blotting revealed that Cre proteins were encapsulated into retroviral virions. When Cre-IDRV was prepared at a ratio of 1:1 (pGPM:pGPM-Cre), the maximum efficiency of G418-resistant colonies expressing GFP was observed. The site-specific gene insertion was confirmed by genomic PCR and amplicon sequencing. For the practical application, we generated Cre-IDRV harboring transgene expression unit. The scFv-Fc expression unit of Cre-IDRV could be repeatedly inserted into the targeted chromosomal locus.

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This research is partially supported by the developing key technologies for discovering and manufacturing pharmaceuticals used for next-generation treatments and diagnoses both from the Ministry of Economy, Trade and Industry, Japan (METI) and from Japan Agency for Medical Research and Development (AMED).

Disclosure of Interest: None declared





PO328

CRISPR/CAS9 BASED MIRNA ENGINEERING OF N-GLYCOSYLATION IN CHO CELLS

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Background and novelty: In order to produce Chinese hamster ovary (CHO) cell-derived recombinant therapeutic proteins with ensured safety, efficacy and cost-effectiveness developing tools that is capable of modulating protein N-glycosylation towards desired patterns is of prime importance. MicroRNAs are single-stranded small non-coding RNAs (19 to 25 nucleotides) that are found in a wide range of higher eukaryotes [1]. They are able to regulate the expressions of multiple genes at a time by completely or partially complementary targeting 3'-UTR of the mRNAs. With the ability of regulating complex gene networks, and at the same time, adding no translational burden onto the cells, microRNAs are perfect targets for CHO cell engineering in the context of optimizing cell lines used for biotechnological production of therapeutic proteins [2]. The purpose of the study is to identify and apply functional microRNAs to modulate N-glycosylation in CHO cells towards desired patterns. It will be the first study that investigates microRNAs with high relevance for optimizing N-glycosylation capacity of CHO cells used for biotechnological production of therapeutic proteins.

Experimental approach: In this project, in-silico selection of microRNA candidates is carried out using advanced bioinformatics analysis based on in-house RNA-seq datasets and on-line databases. Selected microRNA candidates that can modulate N-glycosylation are further screened using Crispr/Cas9 engineering in CHO cells.

Results and discussion: N-glycosylation profiles of the microRNA engineered CHO cells are analyzed and discussed.

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Special thanks to Prof. Nicole Borth and Dr. Gerald Klanert, Austrian Centre of industrial biotechnology and University of Natural Resources and Life Sciences, Vienna; DFF-Danish Council for independent Research; and Novo Nordisk Foundation.

Disclosure of Interest: None declared





PO329

ELIMINATING ANTIBODY HEAVY CHAIN C-TERMINAL LYSINE HETEROGENEITY BY KNOCKING OUT CARBOXYPEPTIDASE D USING CRISPR TECHNOLOGY IN CHO CELLS

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Background and novelty: The heterogeneity of C-terminal lysine levels often observed in therapeutic monoclonal antibodies is believed to result from proteolysis by endogenous carboxypeptidase(s) during cell culture production. Identifying the responsible carboxypeptidase(s) for C-terminal lysine cleavage in CHO cells would provide valuable insights for consistent antibody production.

Experimental approach: Five carboxypeptidases, CpD, CpM, CpN, CpB, and CpE, were studied for messenger RNA (mRNA) expression by RT-qPCR analysis in two CHO host cells and also in an antibody-expressing cell line derived from each host. CpD was found to have the highest mRNA expression.

Results and discussion: When CpD mRNA levels were reduced by RNAi (RNA interference) technology, C-terminal lysine levels increased, suggesting that carboxypeptidase D is the main contributor to C-terminal lysine processing. Most importantly, when CpD expression was knocked out by CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) technology, C-terminal lysine cleavage was completely abolished, based on mass spectrometry analysis, demonstrating that CpD is the only endogenous carboxypeptidase that cleaves antibody heavy chain C-terminal lysine in CHO cells. Our work is the first to show that the cleavage of antibody heavy chain C-terminal lysine is solely mediated by carboxypeptidase D in CHO cells and our finding provides one solution to eliminating C-terminal lysine heterogeneity for therapeutic antibody production, by knocking out CpD gene expression.

Bibliography, Acknowledgements: Benjamin Haley, Frank Macchi, Feng Yang, John C. Joly, Bradley R. Snedecor, and Amy Shen

Disclosure of Interest: None declared





PO329-a

CELL GENOME EDITING AND ENVELOPE GLYCOPROTEIN RE-DESIGN FOR THE ESTABLISHMENT OF NOVEL CELL LINES FOR STABLE PRODUCTION OF LENTIVIRAL VECTORS

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Background and Novelty: The use of lentiviral vectors (LV) based on the Human immunodeficiency virus 1 (HIV-1) in gene therapy has been growing due to their ability to transduce and permanently modify both dividing and non-dividing cells and safer integration pattern relatively to gammaretroviral vectors. To take advantage of the full potential of LV in gene therapy and facilitate clinical-to-market transition, stable producer cell lines are desirable. However, their development is very laborious, time-consuming and has been hampered by the cytotoxicity of some viral components, namely the protease and the commonly used high-titer envelope glycoprotein – VSV-G.

To overcome these problems, we have developed stable cell lines based on a genetically modified and less toxic HIV-1 protease. Although less cytotoxic, this modified protease also exhibits lower proteolytic activity, reducing titers of LV pseudotyped with non-toxic envelope glycoproteins typically used in stable cell line development – 4070A, RD114 and GaLV.

Experimental Approach: The cleavage sites for HIV-1 protease were engineered in the envelope glycoproteins to increase the proteolytic processing. For each envelope glycoprotein, 3 mutations were generated and evaluated in transient production of 3rd generation LV.

Results and Discussion: Engineering the cleavage site of envelope glycoproteins allowed to improve the titer of GaLV to competitive values when comparing with VSV-G. However, the re-designed cleavage site providing the highest titer was found to be cytotoxic due to syncytium formation. To enable the use of the newly developed and high-titer envelope glycoprotein derived from GaLV in stably producing cell lines, we are tackling syncytium formation using CRISPR/Cas9 genome editing.

Keywords: Lentiviral vectors, Genome editing, CRISPR/Cas9, Envelope glycoprotein engineering.





PO330

ANTI-CD19 CHIMERIC ANTIGEN RECEPTORS ARE ACTIVE IN T CELLS AFTER LENTIVIRAL TRANSDUCTION

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Background and novelty: Immunotherapy using T cells modified with chimeric antigen receptor (CAR) has been proven effective in the treatment of leukemia and lymphomas resistant to chemotherapy. CD19 protein has been shown to be an ideal target because it is expressed on most B-cell tumors and normal B cells, but not in other cells. Recent clinical studies have shown excellent responses of CAR T-cells in a variety of B-cell tumors. This study aimed to evaluate the activity of anti-CD19 CAR-T cells from healthy donors.

Experimental approach: Lentiviral vectors were transiently produced in HEK293T and the viral titer was 1.26×10^8 IU/mL, calculated by real-time PCR. First, to evaluate the functionality of our CAR, CD4⁺ cell line Jurkat was transduced with an MOI 5 with anti-CD19 CAR lentivirus, named JurCAR19. Cellular activation in response to anti-CD19 antigen was assessed using flow cytometry to measure CD69 up-regulation. To assess if the CAR would have cytotoxic activity, peripheral blood mononuclear cells were isolated from leukoreduction filter of platelet donation. CD3⁺ T lymphocytes, isolated and activated with magnetic beads, and modified with anti-CD19 CAR, named 19CAR-T.

Results and discussion: JurCAR19 were co-cultivated overnight with CD19⁺ cell line Sup-B15. 63.29% of JurCAR19 cells were CD69⁺, while only 3.39% of non-transduced Jurkat cells were CD69⁺ after incubation with Sup-B15. 19CAR-T cells were incubated with SupB15 (1:10) for 24 hours and showed high cytotoxicity against Sup-B15 (84.16% vs 8.19% from non-transduced T cells). We have successfully demonstrated the feasibility of the lentiviral transduction of primary CD3⁺ T cells and CD4⁺ T cell line Jurkat with anti-CD19 CAR. Both cell types showed that the expressed anti-CA19 CARs were active either by activation or cytotoxicity in response to co-cultivation with CD19⁺ cell line Sup-B15.

Bibliography, Acknowledgements: Grants #2016/08374-5 and #2016/19741-9, São Paulo Research Foundation (FAPESP).

Disclosure of Interest: None declared





PO331

MANUFACTURE OF CAR-T CELLS FOR ADOPTIVE CELL THERAPY

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Background and novelty: The recent years have been marked by notorious advances in the immunotherapy field. In particular, adoptive cellular therapy using chimeric antigen receptor (CAR)-T cells target to CD19 T-cell has demonstrated substantial clinical efficacy in several hematologic cancers. The highly demanding cell doses used in clinical trials require a scalable, efficient and GMP-compliant manufacturing process. In this work, different culture conditions were evaluated in order to stablish an efficient CAR-T cell expansion process.

Experimental approach: Different beads proportion for cell activation (1:1, 1:3, 1:5; beads/cells), IL-2 concentration and culture systems (static and stirred) were evaluated for primary T-lymphocytes culture. The best conditions were applied for CAR-T cells clinical-grade expansion.

Results and discussion: CD3⁺ T-cells were successfully selected with high purity (up to 99%) using EasySep™ Human CD3 Positive Selection Kit. It was demonstrated that for stirred systems (Erlenmeyer flasks and Spinner flasks), a lower proportion of beads/cells (1:3) determines better cell activation/proliferation and cell survival. In contrast, a higher proportion (1:1) was required to obtain satisfactory lymphocytic activation in static culture. Culture medium should be supplemented everyday with IL-2 in a concentration of 100U/mL to ensure prolonged cell activation. Spinner flasks demonstrated to be the best long-term culture system for T-cell expansion in a small-scale. Up to now, CAR-T cells were efficiently expanded using these culture parameters previously established. Scale-up will be performed using disposable and closed-systems, such as Xuri bioreactor, for clinical-grade CAR-T cell production for therapeutic applications.

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Disclosure of Interest: None declared





PO332

DEVELOPMENT OF A THERAPEUTIC RECOMBINANT RETROVIRUS PRODUCER CELL LINE USING THE SINGLE-STEP CLONING-SCREENING METHOD

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Background and novelty: Recombinant retrovirus (RV) are widely used vectors in gene therapy clinical trials since they provide long term expression of the therapeutic gene. The success of new immune therapies and gene therapy products reaching the market increased the demand for robust and high-quality/yield hosts for RV production.

Previously, we developed the single-step cloning-screening (SSCS) method enabling the rapid establishment of a high titer RV producer cell line [1]. However, the proof of concept was performed using a reporter gene. The aim of the work herein described was to establish a RV producer cell line with therapeutic interest by: using only transfection to insert the three expression cassettes encoding for RV into HEK 293 cells; choosing a transmembrane protein as gene of interest (GOI) to mimic a therapeutic target and using recombinase mediated cassette exchange system for a safer and flexible producer cell line.

Experimental approach: Moloney murine leukemia virus GagProPol was used. Originally, this component was expressed through the 5' long terminal repeat (LTR) sequence of the same virus. For safety concerns heterologous promoters were tested. The RV transgene was optimized with different LTR promoters, psi sequences and GOI design. The genetic cassettes were characterized for infectious particles production by the SSCS method. The molecular designs giving higher titers were used to establish a producer population.

The 3 expression cassettes encoding for RV components were stable integrated into HEK 293 cells genome only by transfection. Clones were isolated by SSCS method. Site-specific recombination was performed in a high producing clone to remove the reporter gene.

Results and discussion: A therapeutic relevant RV producer cell line was successfully established with safer expression cassettes and with a flexible system enabling the GOI removal or exchange.

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Disclosure of Interest: None declared





PO333

IVECTOR: A BRAIN AND SPINE INSTITUTE'S CORE FACILITY FOR BIOPRODUCTION OF VIRAL GENE TRANSFER VECTORS (LENTIVIRUS; AAV2, 8, 9, RH10; CAV-2 CANINE ADENOVIRUS).

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Background and novelty: The Vectorology core facility (iVector) produces batches of highly concentrated viral gene transfer vectors of controlled quality to provide the best solution for every application encompassing fundamental research, cell engineering, gene therapy, cellular therapy and vaccine. Lentiviral vectors have the advantage to deliver transgenes into dividing and non-dividing cells and the transgene will be stably expressed through DNA integration into the host genome. Non-integrative CAV-2 vectors due to their high retrograde axonal transport capacity are particularly promising tools for in vivo research on the brain and the central nervous system. We are focusing on AAV that specifically target brain cells according to the serotype used to generate AAV particles.

Experimental approach: The laboratory staff has developed an important collection of viral expression backbones (including constitutive, expression specific, inducible promoters and fluorescent proteins) ready to accept any gene of interest. Its BSL2 and BSL3 biocontained suites allow to satisfy every demand of viral production whatever the transgene type (unknown function, toxic, oncogenic ...). Full integrated services are provided by the staff of iVector : cloning, viral production, purification and titration.

Results and discussion: iVector staff has a huge expertise in recombinant viral particles production under different bench scales and is making available to researchers new viral platforms for gene transfer : CAV2 (canine adenovirus) and rAAV (serotypes targeting the brain). All productions are delivered under high viral concentrations in order to meet the needs for any in vitro and in vivo applications.

Bibliography, Acknowledgements: Viral vectors are developed in house in collaboration with different ICM/INSERM/CNRS/UPMC teams.

iVector core facility is ISO9001-2008 certified, is a partner in Horizon 2020 European projects.

Disclosure of Interest: None declared





PO335

IMPACT OF AGGREGATE CULTURE ON CARDIOMYOCYTE DIFFERENTIATION AND HYPOTHERMIC STORAGE

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Background and novelty: Three dimensional (3D) cultures of human pluripotent stem cell derived cardiomyocytes (hPSC-CMs) hold great promise for drug discovery and regenerative medicine applications. The transition of CM differentiation protocols from two dimensional (2D) to 3D cultures has been challenging typically resulting in lower CM purities and reduced reproducibility. Moreover, the applicability of hPSC-CMs in the clinic/industry is highly dependent on the development of efficient methods for worldwide shipment of these cells.

Experimental approach: To address these issues we established a differentiation protocol capable to produce highly pure 3D aggregate cultures of hPSC-CMs that relies on the aggregation of hPSC-derived cardiac progenitors. We evaluated the feasibility to cold store 2D monolayers and 3D aggregates of functional hPSC-CMs using a fully defined clinical-compatible preservation formulation and investigated the time frame that hPSC-CMs could be subjected to hypothermic storage.

Results and discussion: We showed that a 3D aggregate culture of hPSC-derived cardiac progenitors: improves CM enrichment and commitment. We demonstrated that hPSC-CMs are more resistant to prolonged hypothermic storage-induced cell injury in 3D aggregates than in 2D monolayers, showing high cell recoveries (>70%) after 7 days of storage. Importantly, hPSC-CMs maintained their typical (ultra)structure, gene and protein expression profile, electrophysiological profiles, and drug responsiveness after hypothermic storage.

Overall, this study provides novel insights on the impact of 3D culture on CM differentiation and hypothermic storage that could be valuable in improving global commercial distribution of hPSC-CMs.

Bibliography, Acknowledgements: Work was supported by FP7 European Union Project Cardio Repair European Multidisciplinary Initiative (HEALTH-2009_242038); Fundação para a Ciência e Tecnologia funded projects CardioRegen (HMSP-ICT/0039/2013) and CARDIOSTEM (MITPTB/ECE/0013/2013)

Disclosure of Interest: None declared





PO337

IMPROVEMENTS IN POST-FREEZE STABILITY OF CLINICAL GRADE HMSC TO SOLVE LOGISTICAL CHALLENGES OF CELL THERAPIES

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Background and novelty: Challenges in storage and transport of cell therapies are of increasing concern. To demonstrate how AlbIX®, a recombinant albumin solution from Albumedix™, can improve the stability of clinical grade hMSC during and post freezing without compromising viability, identity and multipotency, a comparative study was made between AlbIX® and a human derived serum albumin (control).

Experimental approach: Viability, apoptotic stage, identity and potency of hMSCs were measured pre and post thaw using qualified methods

Results and discussion: AlbIX® extended the shelf life of cryopreserved hMSC significantly with respect to viability, and the cells maintained their identity and multipotency. The apoptotic study may explain the mode of action of AlbIX® as preventing cells from progressing into late apoptosis compared to control. By increasing post-freeze stability, AlbIX® can solve challenges of storage and transport and add flexibility to clinical practitioners and patients in the use of stem cell therapies.

Bibliography, Acknowledgements: Clementine Mirabel (1), Anna del Mazo (1), Blanca Reyes (1), Eduard Puente (2), Daniel Vivas (1), David Quintanilla (2), Marta Grau-Vorster(1), Irene Oliver-Vila(1), Joaquim Vives (1), Francesc Gòdia (2)

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Disclosure of Interest: None declared





PO338

DEVELOPMENT OF EXTRACELLULAR VESICLES PRODUCTION WITH A SCALABLE SINGLE USE PLATFORM

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Background and novelty: Extracellular Vesicles (EV) are small membrane vesicles encapsulating cell-derived factors that can be used as diagnostic tools or as innovative therapeutic agents. Depending on their parent's progenitor cells, EV display tissue-repairing capacities which are gaining increasing attention in medicine. We describe here the development of a scalable process for clinical grade EV production from adipose tissue-derived stem cells (ADSC)

Experimental approach: Initially, EV were produced in T-flasks and purified by ultracentrifugation. Our objective was to design a scalable, single-use process for the production of clinical-grade EV using the iCELLis® Nano bioreactor, and a combination of TFF and membrane chromatography for their purification

Results and discussion: A seed of human ADSC collected by lipoaspiration was prepared in T-flasks and used to inoculate an iCELLis Nano 0.53 m² bioreactor. Cells were allowed to grow for 3 days in serum-containing media, then the culture was pursued in serum-free conditions and the production of EV monitored using nanoparticle tracking analysis. EV production rate appeared continuous for up to 14 days, and reached more than 10⁹ EV/cell/day. In total, a cumulated production of 10¹³ EV was achieved over 14 days.

EV were concentrated and purified by TFF and membrane chromatography with yields > 50%. Purified EV were analyzed by SDS-PAGE silver staining and electron microscopy, and their protein content by Mass Spectrometry. Functional assays are on-going.

In conclusion, our results demonstrate that EV can successfully be produced using iCELLis Nano bioreactor with productivities similar to T-flasks. Overall this process will support cGMP manufacturing of exosomes from bench scale to large scale manufacturing batches, creating a platform for manufacturing that can address anticipated large patient needs. In addition, TFF and/or membrane chromatography are excellent alternatives to ultracentrifugation

Bibliography, Acknowledgements: None

Disclosure of Interest: None declared





PO339

USING BACULOVIRUS AS A GENE SHUTTLE IN HMSC: OPTIMIZATION OF TRANSDUCTION EFFICACY

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Background and novelty: Gene therapies are a promising emerging field for the treatment of various diseases. Gene delivery is thereby ensured mostly by virus-based vectors. Amongst others, the baculovirus is known to achieve transient recombinant protein expression in human mesenchymal stromal cells (hMSC). Compared to other viral vectors, like lenti- or adenovirus the baculovirus is not able to replicate in non-insect cells making this virus a safe alternative. Despite this advantage the baculovirus has not been directly used as a therapeutically vector [1].

Experimental approach: To evaluate the potential of baculoviruses for gene therapy six different promoters were evaluated regarding GFP expression in hMSC after transduction using flow cytometry. To optimize the transduction process design of experiment was used to identify factors significantly influencing this process in two steps: A) Screening for significant factors, B) Optimization of those. Finally, to ensure that the MSC were not negatively influenced due to transduction, the expression of MSC-specific surface markers, as well as differentiation into chondrocytes, osteoblasts and adipocytes were verified according to the guide lines published by the International Society for Cellular Therapy in 2006.

Results and discussion: Using design of experiment the incubation time, virus concentration and surrounding solution were identified as significant factors influencing the transduction process. To minimize interactions of virus particles with medium components, PBS was used as a surrounding fluid and the incubation time and virus concentration was optimized. Neither the expression of MSC-specific cell surface markers, nor the typical MSC differentiation were negatively affected after baculovirus transduction.

Bibliography, Acknowledgements: [1] T. W. Kwang, X. Zeng, and S. Wang, "Manufacturing of AcMNPV baculovirus vectors to enable gene therapy trials," *Mol. Ther. — Methods Clin. Dev.*, vol. 3, p. 15050, Jan. 2016.

Disclosure of Interest: None declared





PO340

GENERATION OF A FUNCTION BLOCKING ANTIBODY AGAINST NOTCH LIGAND DELTA-LIKE-1 WITH THERAPEUTIC EFFICACY AGAINST BREAST CANCER

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Background and novelty: Notch signaling is an evolutionary conserved cell-to-cell communication pathway crucial during embryonic and breast development and tissue homeostasis. This pathway is often hyper-activated by overexpression of Notch receptors and/or its ligands in several types of cancers, including breast cancer, where it contributes to its development, progression and drug resistance. Our aim is to generate a function blocking antibody against the Notch Delta-like-1 (DLL1) ligand with therapeutic efficacy against breast cancer.

Experimental approach: DNA of human DLL1 full length extracellular domain (DLL1-ECD) and a truncated version, containing the minimal binding region to the Notch receptor (DLL1-EGF3), were cloned into pFUSE-Fc1-IgG1, and expressed in HEK293E6. Recombinant proteins were purified from culture media by Protein-A affinity and size exclusion chromatography. The human scFv phage display Tomlinson I+J library was used to select specific scFv against peptides targeting DLL1 binding regions to Notch. The binding ability and specificity of the selected scFv clones was evaluated by *scFv-on-Phage* ELISA.

Results and discussion: Our strategy allowed us to obtain 20 mg of pure (>95%) and stable DLL1-ECD-FC as confirmed by SDS-PAGE and thermofluor assay. DLL1-EGF3-FC yield was very low and buffer screenings are ongoing to optimize protein stability. Recombinant DLL1 and peptides will be used to select for monoclonal antibodies by Phage Display. After three rounds of panning with DLL1 peptides we identified 13 scFv positive clones, 2 of which presented high affinity to DLL1-ECD-FC. The best scFv candidates will be reformatted into IgGs and their ability to inhibit the Notch pathway will be evaluated. Anti-oncogenic effects of anti-DLL1 IgGs will be assessed in breast cancer cells.

Bibliography, Acknowledgements: iBET; iNOVA4Health (LISBOA-01-0145-FEDER-007344); FCT: PTDC/SAU-ONC/121670/2010, PTDC/BBB-BMD/4497/2014, PD/BD/113987/2015

Disclosure of Interest: None declared





PO341

VALIDATION OF A NEW SOURCE OF RECOMBINANT BASIC FIBROBLAST GROWTH FACTOR FOR THE CULTIVATION OF MESENCHYMAL STEM CELLS

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Background and novelty: Mesenchymal stem cells (MSC) are multipotent adult stem cells that are isolated from several tissues like bone marrow, adipose tissue or umbilical cord. MSC have the capacity to be induced to osteoblastic, chondrocytic and adipocytic differentiation. Recombinantly produced growth factors such as Insulin-like Growth Factor 1 (IGF-1), Platelet Derived Growth Factor (PDGF) and Basic Fibroblast Growth Factor (bFGF) are required to maintain the self-renewal and the differentiation capacities of MSCs, as well as embryonic human stem cells. Thus, changing the source of a recombinant growth factor like bFGF should not be undertaken lightly and thorough testing should be performed to validate the new recombinant protein.

Experimental approach: The aim of this work was to test and compare the performances of 3 lots of rec bFGF supplied by Kerry versus a gold standard rec bFGF. The bFGFs were first tested for any cumulative cytotoxic effects by performing multiple passages in MRC-5 cells and observing the population doubling level of these cells. The bioactivity of the rec-bFGF was next assessed in BALBc 3T3 fibroblast cells using fluorometric assay. The ability of each rec-bFGF to maintain and amplify two MSC cell lines in serum-free cell culture media was assessed by observing cumulative cell growth. The capacities of the cells to induce osteoblastic and adipocyte differentiation after 3 successive subcultures was also assessed.

Results and discussion: The bFGF supplied by Kerry showed no cumulative cytotoxic effects when compared to the control bFGF. The bioactivity of all 3 lots was also equivalent to the control bFGF. In both MSC cell lines cultivated using Kerry rec-bFGF, proliferation was equivalent to the control bFGF. Also, both MSC cell lines maintained their self-renewing capacities and preserved their potential to differentiate into either osteoblastic or adipocyte cells.

Bibliography, Acknowledgements: n/a

Disclosure of Interest: None declared





PO342

APTAMER-MODIFIED POLYCAPROLACTONE NANOPARTICLES FOR TARGETED DRUG DELIVERY

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Background and novelty: Conventional drug delivery systems often suffer from limitations such as nonspecific delivery resulting in undesirable side effects [3]. So there is an increasing effort to develop “intelligent” systems which allow the specific delivery of drugs to a desired site of action. Due to their small size, which allows efficient cellular uptake, nanoparticles (NPs) are in wide use for drug delivery [1,2]. To provide specificity of NPs, aptamers are promising candidates. They enable specific binding of the corresponding target cells in combination with a high modification potential and exhibit lack of immunogenicity [4].

Experimental approach: Within this study NPs composed of the biocompatible polymers methoxypolyethylene glycol polycaprolactone (mPEG-PCL) and a carboxylated PCL (HOOC-PEG-PCL) were used. Different anticancer drugs such as Paclitaxel (PTX) and Camptothecin (CPT) were encapsulated successfully. As a targeting ligand an aptamer directed against lung cancer cells was used.

Results and discussion: Particles were characterized by dynamic light scattering (DLS) and nanotracking analysis (NTA). Measured particle size was about 190 nm with a concentration of 7.48×10^8 particles/mL. Encapsulation efficiency of different anticancer drugs were characterized by HPLC and fluorimetry. Drug release was investigated under different conditions. Immobilization efficiency of the aptamer on nanoparticles' surface was measured as approx. 50 %. The binding specificity of the aptamer was investigated. Cellular uptake experiments were also performed. The presented system was demonstrated to be a promising tool for targeted drug delivery into specific cell types.

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Julia Modrejewski would like to acknowledge Hannover School for Biomolecular Drug Research (HSBDR) for financial support.

Disclosure of Interest: None declared





PO343

SOLUTIONS FOR ROBUST CELL THERAPY PRODUCTION

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Background and novelty: The long-term view of regenerative medicine therapies predicts an increased need for expansion solutions that ease scalability, utilize animal origin-free materials and are compatible with limited downstream processing steps. As more cell therapeutics progress through clinical testing, current in vitro culture methods are proving cumbersome to scale and lack robustness. Moreover, high quality animal origin-free reagents and downstream processing devices support the future implementation of production solutions that will be required following clinical success.

Experimental approach: Here, we describe the implementation of single use bioreactors and high quality media for expansion of cell therapies. We include examples from allogeneic mesenchymal stem cells, autologous T cells and induced pluripotent stem cells. The presentation reviews solutions for expansion of these different cells within the context of different upstream process development steps as well as scaling and processing with high yield, recovery, viability and with expected cell characteristics.

Results and discussion: The 3L bioreactor supported expansion of all three cell types: hMSCs were cultured on microcarriers whereas T cells were cultured as single cell suspension, iPSC were cultured as aggregates circumventing the need for microcarriers and other substrates. Animal origin-free media formulations including supplementation with human platelet lysate as well as a novel serum-free media formulation were investigated and were found to improve the performance for hMSCs.

The presented examples demonstrate how start to finish solutions for manufacturing, including high quality reagents, are key enabling technologies for success in commercializing cell therapies.

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Disclosure of Interest: None declared





PO344

OPTIMIZATION OF ADENO-ASSOCIATED VIRAL VECTOR PRODUCTION USING A NEW SCALABLE SUSPENSION PLATFORM

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Background and novelty: The emerging number of clinical trials in the gene therapy field poses the challenge to the industry to produce viral vectors in a scalable, reproducible and cost-efficient manner. To address this issue, our CAP-GT platform comprises high density, serum free suspension cell lines that enable reproducible transfection and high efficiency production of viral vectors.

An adeno-associated virus (AAV) based vector was the first approved gene therapy product in clinical applications. Attractive features of AAV as a gene therapy vector are e.g. its lack of pathogenicity and its ability to transduce dividing and non-dividing cells. Production of high amounts of AAV meanwhile still faces challenges concerning upscaling and stability.

Experimental approach: To provide an alternative to current production platforms, we established production of AAV by transient transfection of CAP-GT cells. Viral production was evaluated by qPCR and ELISA. To improve viral titers, we optimized culture conditions and tested different media to establish a protocol for scalable transient transfection without medium exchange step.

Results and discussion: Applying several approaches to optimize production of AAV resulted in an increase of viral titers analyzing both vector genomes as well as capsids. To address the need for scalable transient transfection without medium exchange step, we developed successfully a scalable protocol for transfection and viral production in spent medium.

Bibliography, Acknowledgements: -

Disclosure of Interest: None declared





PO345

TESTING APPROACHES FOR CELL BASED THERAPEUTICS: RAPID TESTING AND REGULATORY EXPECTATIONS

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Background and novelty: Ensuring the safety and quality of cell-based therapeutic products is achieved through a multi-tiered approach that examines several factors to establish product safety and manufacturing consistency. Over the past years there has been a dramatic increase in the number of clinical trials involving cellular based therapies. The nature of these novel therapeutic strategies presents testing challenges when compared to traditional therapies that may include limited shelf life and limited testing sample size as well as a need for reduced turnaround times. As a consequence, existing testing approaches are often not suitable for these products.

Experimental approach: We provide an analysis of the current state of the testing regimes undertaken to ensure the safety and quality of cellular based therapeutics.

Results and discussion: We outline testing strategies for raw materials, production intermediates and final product and report on novel technology including cell characterization, identity and stability, microbial testing and detection of adventitious viruses. The implementation and validation of rapid assay methods and suitability of new testing platforms that allow for streamlining testing and reporting are also discussed.

Bibliography, Acknowledgements: Dr. Leyla Diaz is a Principal Scientist on the Field Development Services team at BioReliance responsible for providing scientific leadership for BioReliance customers based in the United States. Leyla has 17 years research and product development experience spanning both human and animal health. Leyla's area of expertise are virology, therapeutic antibody development and the design and implementation of molecular and bioassays for large molecules and vaccines. Leyla is a graduate of the University of Maryland, College Park, MD with a PhD in Microbiology.

Disclosure of Interest: None declared





PO346

NOVEL BIOTECHNOLOGICAL STRATEGY TO OBTAIN IN VITRO ERYTHROID CELLS

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Background and novelty: Production of *in vitro*-generated erythroid cells, particularly red blood cells, from hematopoietic progenitor cells (HSPCs) was proven feasible but remains impractical for transfusion. This source of erythroid cells may have broader uses in toxicology, drug evaluation, drug delivery and disease modeling. However, these applications are hindered by scalability and costs difficulties due to costly factors required for culture, such as erythropoietin (hEPO). To address this issue, we proposed to modify HSPCs with hEPO-lentivirus in order for cells to produce the hormone, and to analyze the *in vitro* effect over expansion and erythroid differentiation (ED) of HSPCs in absence of exogenous hEPO (ehEPO).

Experimental approach: Preliminarily, we evaluated ED in colony forming unit assays for non-modified cells (NMC) and hEPO-modified cells (EMC), both cultured with ehEPO (E+) and without ehEPO (E-). We further studied expansion and ED in suspension cultures of NMC-E+ and of EMC-E-. Flow cytometry immunophenotyping and morphological analysis were carried out throughout culture time. hEPO production and its isoforms profile were assessed.

Results and discussion: EMC-E- reached higher expansion rates than NMC-E+ (953- vs. 369-fold). ED was demonstrated in both conditions: CD34 stemness marker and CD45 leukocyte marker decreased in correlation with the appearance of early erythroblasts. From day 11, polychromatic and orthochromatic erythroblasts were detected. CD235a and CD71, expressed at early erythroid and reticulocyte stages, increased. Secreted hEPO reached its maximum by day 7, and its isoforms profile was similar to that of ehEPO. Thus, EMC differentiated *in vitro* into cell populations highly enriched with erythroid cells (70% CD71⁺ and 75% CD235a⁺ cells), in absence of ehEPO. This strategy is applicable in biotechnological protocols dedicated to generate erythroid cells for various goals.

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Disclosure of Interest: None declared





PO347

APPLYING PROTEOMIC TOOLS TO UNVEIL HUMAN CARDIAC STEM CELLS ROLE IN MYOCARDIAL ISCHEMIA-REPERFUSION INJURY

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Background and novelty: After an Acute Myocardial Infarction (AMI), Ischemia-Reperfusion (I/R) injury is responsible for a critical decrease in cardiomyocytes (hCMs). Human myocardium harbors endogenous cardiac stem cells (hCSCs) that are activated upon I/R, contributing to myocardial repair through the establishment of an auto/paracrine crosstalk between hCSCs and hCMs in stress. Clinical trials involving transplantation of hCSCs into the infarcted myocardium have demonstrated the potential of these cells, regarding improved tissue contractility.

Using donor-derived hCSCs, our work aims at setting up the first *in vitro* human I/R injury model in order to better decipher the mechanisms of action of hCSCs upon AMI using proteomic tools.

Experimental approach: Mono-cultures of donor derived hCSCs, hCMs and co-cultures were established using human donor CSCs and hiPSC-CMs. Ischemia was mimicked by substituting growth media by Ischemia Mimetic Solution and placing the cells at 0% O₂ for 5 hours. In reperfusion, cells were placed back in their physiological conditions (3% O₂). The effect of I/R injury in hCSCs was accessed by total proteome analysis (LC-MS). Growth factor secretion, cells' viability, as well as hCSCs proliferation was monitored.

Results and discussion: More than 2000 proteins were identified in hCSCs exposed to injury including proteins associated with mitochondrial dysfunction and oxidative stress. Important features of I/R injury were successfully captured, namely hCSCs proliferation activation, increase in key growth factors secretion, and the protective effect of hCSCs on hiPSC-CMs. This system will allow further understanding on the molecular landscape of the myocardium during AMI, namely regarding hCSCs regenerative response.

Bibliography, Acknowledgements: The authors acknowledge the projects CARE-MI (HEALTH-2009-242038), CARDIOSTEM (MITPTB/ECE/0013/2013, FCT (PTDC/BBB-BIO/1414) and iNOVA4Health (UID/Multi/04462/2013) for financial support.

Disclosure of Interest: None declared





PO348

A NOVEL SCALABLE PRODUCTION PLATFORM FOR LENTIVIRAL VECTORS BASED ON HUMAN SUSPENSION CELL LINES

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Background and novelty: A rapid increase in the number of gene therapy trials and products has led to an equally increased need for industrial production of viral gene therapy vectors such as lentiviral, adeno-associated and adenoviral vectors. Current production systems are limited with respect to scalability and robustness.

With our CAP and CAP-T cell lines, we have developed a novel system for high density suspension culture, efficient reproducible transfection and high efficiency production of viral vectors. By upstream process optimization we have obtained a robust high density fed-batch culture system, which can be scaled to any current bioreactor format.

Experimental approach: We applied a design of experiment approach to increase the lentiviral titers achieved with the CAP-GT platform. In a Box Behnken approach, parameters as cell density, DNA and PEI amount were optimized in regard to yielding the highest transducing units. In a further step, the addition of supplements, as sodiumbutyrate, was titrated and analyzed in regard to influence on viral production.

Results and discussion: We indeed observed a significant increase of viral titers upon addition of supplements, as e.g. sodiumbutyrate. Using all the optimized conditions, we could increase the lentiviral titers about 10 fold compared to our previous standard protocol and also compared to adherent HEK293T cells. In addition, lentiviral production in shake flasks was shown to be scalable yielding comparable titers over a range of volumes.

Bibliography, Acknowledgements: -

Disclosure of Interest: None declared





PO349

AAV PRODUCTION IN SUSPENSION: EVALUATION OF DIFFERENT CELL CULTURE MEDIA AND SCALE-UP POTENTIAL

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Background and novelty: Recombinant Adeno-associated virus (rAAV) approaches gained an outstanding reputation in gene therapy due to their beneficial characteristics. These include long term gene expression, targeting of dividing and non-dividing cells and low intrinsic immunogenicity.[1] Established rAAV production utilizes a triple transfection of adherent HEK-293-cells, which hardly meets product yields required for clinical applications. Transferring production of rAAV to HEK-293-F suspension cells allows for more scalable processes and the advantageous use of serum-free media streamlines downstream procedures.

Experimental approach: For optimization of rAAV production, the HEK-FreeStyle™ 293-F cell line was used with different media (HEK TF, HEK GM or FreeStyle™ F17) before and after transient transfection as well as the application of a feeding supplement was analyzed. Pretests were performed in Tube Spin Bioreactors in 5 % CO₂ with orbital shaking at 185 rpm and 5 cm amplitude. For scale-up, batch processes were carried out in 125 ml shake flasks and in 2 l stirred bioreactors at 30 % air saturation and pH 7.1. Transfection efficiency and rAAV production were quantified by flow cytometry and qPCR of genomic copies, respectively.

Results and discussion: The batch cultivations in Tube Spin Bioreactors revealed that a majority of rAAV were produced within the first 24-72 h after transfection. Genomic titers from raw cell extracts were highest three days post-transfection, achieving up to 10⁹ copies/ml. Afterwards, the titer decreased, suggesting that rAAV particles either were degraded or re-infected the producer cells. Therefore, prolongation of the process e.g. by adding a feed supplement did not increase yield. Transfection efficiencies of up to 55 % and titers up to 5·10⁸ copies/ml in bioreactor processes have proven scale-up potential.

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Disclosure of Interest: None declared





PO350

DEVELOPMENT OF A COST-EFFICIENT SCALABLE PRODUCTION PROCESS FOR RAAV-8 BASED GENE THERAPY BY TRANSFECTION OF HEK-293 CELLS

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Background and novelty: In-vivo gene therapy offers perspectives to cure rare genetic disorders. Imperative to the success of these therapies are the ability to produce large quantities of clinical grade viral vectors in a cost-efficient manner. The production method presented here allowed for the reduction by 50% of the DNA amounts used for transfection, while maintaining productivity, hence reducing overall cost-of-goods (COGS)

Experimental approach: The process was developed using triple PEI-mediated transfection of HEK-293 cells grown in a fixed-bed bioreactor (iCELLis® bioreactor, Pall). Media and medium exchange post-transfection was evaluated to optimize AAV-8 productivity. Transfection conditions were optimized by adjusting cell density at transfection and pDNA quantity used. The process was scaled-up from a 0.8 to a 4 m² growth surface area to confirm its applicability in industrial applications

Results and discussion: Medium exchange post transfection with DMEM lead to a 7-fold AAV-8 productivity increase. The pDNA/cell ratio at transfection could be reduced by 50%, which presents a large opportunity for cost-reduction in the production of a viral vector. Over 100% recovery of the AAV from the fixed bed could be achieved, with average yields of 4×10^{13} VG/m². This process could be scaled from 0.8 to 4 m² growth surface, with similar specific productivity. This gives promising perspectives to industrialize within the larger scale iCELLis 500 offering up to 500 m² of growth surface (projected $2 \cdot 10^{16}$ total VG)

Bibliography, Acknowledgements: None

Disclosure of Interest: None declared





PO351

QUANTITATIVE MAPPING OF THE IMMUNE-REGULATORY PROPERTIES OF HUMAN MESENCHYMAL STEM CELL AGGREGATES ON A CHIP

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Background and novelty: Human mesenchymal stem cells (hMSCs) hold great promise for regenerative medicine, due their capability to secrete large amount of immune regulatory molecules (PGE-2, COX-2). The formation of hMSC spheroids enhanced their immune functions¹. In this study, high-density hMSC aggregates were formed in a droplet microfluidic platform. Large-throughput image analysis demonstrated robust quantitative correlations between aggregate structural organization and hMSC biological properties.

Experimental approach: Nanoliter-sized aqueous droplets containing liquid agarose and hMSCs were generated by flow focusing. The confined droplets were trapped in an array of 270 capillary anchors. After spheroid formation, on-chip immobilized hMSCs were cultivated for a 3 days and their biological functions were analyzed *in situ*.

Results and discussion: hMSC spheroid formation on chip relied on N-cadherin interactions, and increased production of PGE-2 and COX-2 was observed. It was found significant induction of caspase-3, independently of any diffusion limitation. We quantitatively demonstrated a higher caspase-3 in the core, while COX-2 was located at the boundary of the aggregate. However, we found no correlation between these two parameters. In contrast, COX-2 was found in areas with functional N-cadherin. NF- κ B and Myosin-II-A were critically required for the formation spheroids and for the spatial regulation of COX-2 expression and caspase-3 activation. Consequently, the unique data set obtained with our microfluidic platform demonstrated that the structural organization in hMSC spheroids dictated differential activation of specific signalings leading to the regional patterning of the cells' biological functions.

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Disclosure of Interest: None declared





PO352

IMPACT OF HYDRODYNAMIC STRESS ON CELL GROWTH AND MICROCARRIER-CELL-AGGLOMERATE FORMATION IN MICROCARRIER-BASED CULTIVATIONS OF ADIPOSE TISSUE-DERIVED STROMAL/STEM CELLS

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Background and novelty: Over the last years, cell-based therapies with human adipose tissue-derived stromal/stem cells have become increasingly important in the field of regenerative medicine. However, the required number of hASCs for clinical applications is in the region of a million cells per dose. To achieve these high cell numbers, stirred single-use bioreactors in combination with microcarriers have demonstrated themselves as a cost-effective solution. Although adherent mammalian cells on MCs have been well investigated for the production of vaccines, working with hASCs is more complex. This raises the question of how the induced fluid shear stress affects cell expansion and the formation of MC-cell agglomerates during cultivation.

Experimental approach: For this purpose, MC-based cell expansions were performed in single-use spinner flasks at different impeller speeds including the suspension criteria (N_{S1U} , N_{S1}). Cell numbers, substrates and metabolites, surface marker expression and the formation of MC-cell-agglomerates were measured during cell expansion. Computational Fluid Dynamics simulations were performed in order to predict the hydrodynamic stress.

Results and discussion: The results demonstrated that working at the suspension criteria achieves the highest cell densities (1.25×10^6 cells/mL). Impeller speeds below and above the suspension criteria resulted in significant lower number of cells (0.25×10^6 cells/mL). These lower cell densities can be ascribed to mass transport limitations at low impeller speeds and to excessive high hydrodynamic strains at high impeller speeds. The effect of the shear stress on MC-cell agglomerate formation at high impeller speeds compared to those resulting at the suspension criteria was insignificant. In contrast, impeller speeds below the suspension criteria resulted in strong MC-agglomerate formation.

Bibliography, Acknowledgements: -

Disclosure of Interest: None declared





PO353

IPSC DERIVED CARDIOMYOCYTES DEVELOPMENT FOR MULTI-ORGAN-CHIP CULTIVATION

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Background and novelty: TissUse Multi-Organ-Chip platform contributes to the ongoing development of systemic substance testing in vitro. Current in vitro and animal tests for drug development are failing to emulate the systemic organ complexity of the human body and often do not accurately predict drug toxicity¹. Especially, cardiotoxicity is one of the main reasons why new compounds are retracted from clinical trials. The aim of this study was to establish a dynamic multi-organ-device integrating cardiomyocytes for substance testing.

Experimental approach: 2D monolayer and 3D suspension iPSC derived cardiomyocytes differentiation protocols were established. Beating cardiomyocytes were first seen on day 8 in monolayer as well as in spheroids. Cardiomyocytes show up to 64% cardiac troponin T positive cells and 35% Myosin heavy chain positive cells by flow cytometry. Myosin II heavy chain, α -actinin, Myosin 9/10, Myosin 11 and caldesmon expression was shown by immunohistochemistry. Due to the exclusion of a lactate enrichment of cardiomyocytes, cardiac fibroblasts are also expressed in the spheroids shown by vimentin staining. Those cardiac fibroblasts lead to a more physiological heterologous cell population as seen in the human heart. Beating spheroids were cultivated for 7 days under steady culture conditions in the multi-organ-chip. The integrated on-chip micropump provides physiological-like pulsatile circulation at a microliter scale and leads to better nutrition and oxygen supply.

Results and discussion: The next significant step is to combine multiple autologous 3D organ equivalents in our Multi-Organ-Chip. This is only feasible by iPSC differentiation technology. Differentiating all cell types from one iPSC donor is crucial to overcome source and rejection problems. Combining our Multi-Organ-Chip platforms with this iPSC differentiation technology will result in a personalized system for drug and substance testing in future.

Bibliography, Acknowledgements: ¹ Marx et al., 2016 ALTEX

Disclosure of Interest: None declared





PO354

DEVELOPMENT OF A WOUND HEALING ASSAY USING INCLUSION BODIES

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Background and novelty: The regeneration capacity of adult human skin is limited, resulting frequently in scarring. In addition, chronic wounds are a serious issue. Therefore, improving the regeneration capacity of skin is an important goal in clinical research.

Recent studies have revealed the key role of epidermal lipoxygenases (LOX) from *Ambystoma mexicanum* (axolotl) in wound healing and epidermis regeneration. In the axolotl an epidermal lipoxygenase (AmbLOXe) could be detected during limb redevelopment. Studies have shown that human cells migrate and close wounds faster with AmbLOXe¹.

Experimental approach: AmbLOXe was produced heterologously in *Escherichia coli* to study its characteristics and to determine its biological effect in wound healing assays. The lipoxygenase could be expressed only in insoluble protein aggregates (inclusion bodies (IBs)). However, the production of IBs is not necessarily disadvantageous. Aggregated enzymes may show activity and display a greater robustness towards mechanical stress². We have tested the effect of AmbLOXe in wound healing assays using inclusion bodies for the first time.

Results and discussion: AmbLOXe inclusion bodies have been purified from bacterial culture and tested for sterility in cell culture. Afterwards, cell culture surfaces were covered with IBs. Keratinocytes (HaCaTs) were seeded on top of the AmbLOXe layer and cultured until confluence. Wound healing assays were performed using the following methods: scratch assay, electric cell-substrate impedance sensing and cell culture inserts. Impedance and barrier methods led to more reproducible wounds and a positive effect of AmbLOXe could be demonstrated.

Bibliography, Acknowledgements: 1. Menger, B. et al. Ann. Surg. 253, 410–8 (2011) 2. García-Fruitós, E. et al. Trends Biotechnol. 30, 65–70 (2012)

Disclosure of Interest: None declared





PO355

COMPUTATIONAL DESIGN OF RAW MATERIALS, PRODUCTS, EQUIPMENT AND PEOPLE FLOWS TO OPTIMIZE THE ERGONOMICS OF CELL THERAPY PRODUCTION ENVIRONMENT

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Background and novelty: Emergence of T-Cell therapies creates new requirements for the design of facilities as they are personalized. This leads manufacturers to develop more flexible biomanufacturing production environments. They aim to streamline transition from R&D to production and implement as much as possible automation. Through this study, we determined whether automation was necessary or whether human operators still belonged in a T-Cell therapy factory producing 5000 batches a year through a 12-day process. We used layout design and simulation software to design predictive models of bioproduction environments and optimize their conformation. We created two 3D representations of lean cell therapy plants, one with operators and a fully automated one, with the aim to determine which one implied less process steps and could be performed in the most simplified facility.

Experimental approach: The existing cell therapy process and the equipment were implemented into HakoBio platform. Provided the number of batches and the timing of each steps, the model was tested by SIMOGGA's planner algorithm software to establish the quantity of machines required and optimize the process flow to reach the requested yield. We used HakoBio's Virtual Reality tool to assess ergonomics of the cleanroom filled with necessary equipment and minimize its size. We utilized SIMOGGA's layout design software to design raw materials, waste, products and finally people or machines flows. The leanest configurations were validated using HakoBio's VR tool.

Results and discussion: We obtained two schematics of the main flows and two 3D models of T-cell therapy plants. One employed human operator, one used automated solutions. Our analyzing tools allowed us to compare their yield and cost. Results showed that a hybrid model was the most efficient design of a T-cell therapy plant.

Bibliography, Acknowledgements: We thank Mrs Emmanuelle Vin for her collaboration

Disclosure of Interest: None declared





PO356

STANDARDISATION OF HIGH THROUGHPUT SPHEROID ENGINEERING USING THE LABCYTE ECHO ACOUSTIC DISPENSER

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Background and novelty: As an *in vitro* model, Multi Cellular Tumor Spheroid (MCTS) help recreating tumour hallmarks through physiologically relevant networks of different cellular populations.

Robustness of MCTS model was validated on the Echo acoustic liquid handling platform for high throughput screening to address the unmet need of non-surgical treatment of Chordoma supporting progression of this project to Pilot Studies for Clinical Trials.

Experimental approach: 3 patient-derived Chordoma cell lines were set up at 8×10^6 cells/mL to be dispensed by contactless Echo dispenser into ULA 384 microplates. Co Cultures were established by transferring 12 500 Chordoma UCH1 or UM-CHOR with Fibroblasts, back filled up to 60 μ L/well of 2% ECM media.

After 3 days growth, 100nL of targeted-therapy agents in combination with EGFR inhibitors were transferred with Echo to achieve a dose response range between 10 μ M and 2nM. Following treatment, viability through growth Kinetics, ATP measurement and ImmunoFluorescence were performed to quantify compounds potency.

Results and discussion: Characterization of spheroids highlighted the presence of a necrotic core and a gradient of proliferating cells expressing the Clinical Biomarker and Phenotypic markers specific of tumour-like structures.

Accurate and gentle transfer of cells by Acoustics was validated, generating material with CV < 10% to enable improved data quality for a robust and reproducible process.

Comparison of drugs potency 2D vs 3D showed the importance of combination screening in a physiologically relevant model to target different sub clonal populations within the spheroids to investigate, understand and overcome resistance of cancer to therapy.

This innovative approach affords significant savings of precious patients cells and can have a major impact in future Personalised Medicine Initiatives such as Patient Cell Screening.

Bibliography, Acknowledgements: Acknowledgements to Pr Adrienne Flanagan, UCL, UK

Disclosure of Interest: None declared





PO357

SERUM-FREE SUSPENSION LIVER CELL LINES - THE USAGE IN (ECO-)TOXICOLOGICAL EVALUATION

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Background and novelty: The *in vitro* risk assessment of chemicals demand highly efficient, effective and sustainable alternatives to present adherent cell-based technologies. Our versatile *SeSus* technology provides multiple suspension cell lines with identical or better performance than the progenitor adherent cell lines. As an example, suspension liver cell lines grown in serum-free condition can help to enable high content analytics of various chemicals and improved metabolomics for risk or effect assessment. As a consequence and for the first time, single-cell suspension liver cell lines in serum-free medium are available and applied *in vitro* (eco-)toxicological evaluation.

Experimental approach: In this proof-of-concept study, we present the suspension liver cell lines ewoCL^H and ewoCL^R were prior adapted to a single chemically-defined, serum-free medium as well as suspension culturing conditions from originating adherent human and rat cell lines, respectively. Various bioassays (e.g. for CYP450 isoenzyme activities) were conducted and an improved protocol for spheroid formation of ewoCL^H was applied.

Results and discussion: The benefits of the new culturing conditions in *in vitro* testing are improved handling, higher throughput towards full automation and cost efficiency. Higher maximal cell densities and an improved viability for both cell lines were obtained. Furthermore, high repeatability and improved process stability due to the unique chemically defined medium were achieved. The suspension cell lines allow for multiple parallel effect-based outputs, which can be individually assessed after direct sample exposure and subsequent division into different cavities. A homogeneous suspension cell culture also improves the protocol for spheroid formation towards a high throughput drug screening.

Bibliography, Acknowledgements: EWOMIS is gratefully supported in the framework of the eXist program by the Federal Ministry for Economic Affairs and Energy and the European Social Fund

Disclosure of Interest: None declared





PO358

MODELLING TUMOR-STROMA INTERACTIONS TO ADDRESS DISEASE PROGRESSION AND DRUG RESPONSE MECHANISMS IN VITRO

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Background and novelty: The tumor microenvironment, composed of fibroblasts, endothelial and immune cells embedded in extracellular matrix, modulate tumor progression and drug response.

Experimental approach: To engineer a model system that depicts the influence of tumor microenvironment dynamics, we combined cell microencapsulation in an inert scaffold (alginate) and bioreactor technology. This strategy generates tumor microtissues composed of tumor spheroids, fibroblasts and immune cells that can be cultured up to months.

Results and discussion: The system was applied to a panel of breast and non-small cell lung carcinoma (NSCLC) cell lines. In co-cultures of MCF7, an estrogen receptor (ER)⁺ breast cancer cell line, with fibroblasts, increased deposition of collagen and secretion of pro-inflammatory cytokines (e.g. IL8, IL6, CXCL1) was observed along culture. This resulted in phenotypic alterations typical of advanced cancers, such as loss of tumor cell polarity and increased collective migration, enhanced angiogenic potential and reduced sensitivity to an ER antagonist. In co-cultures of H157, an advanced NSCLC cell line, with fibroblasts and monocytes, monocytes polarized into activated type 2 (M2), described as tumor associated macrophages, characterized by expression of CD163, increased CSF1R and secretion of CCL22. Moreover, M2 infiltrated tumor spheroids. Treatment with immunomodulatory drugs induced macrophage re-polarization into activated type 1, showing the plasticity of the model. In summary, this model system constitutes a new tool for dissecting the role of tumor-stromal crosstalk in disease progression and drug response and is currently being applied to patient-derived cells.

Bibliography, Acknowledgements: We acknowledge Dr C Brisken, Dr H van der Kuip and Dr M Oren for reporter cell lines; support from the Innovative Medicines Initiative Joint Undertaking (grant agreement n° 115188) and FCT (iNOVA4Health—UID/Multi/04462/2013 and SFRH/BD/52208/2013).

Disclosure of Interest: None declared





PO359

A METHOD TO SIMULATE DIFFUSION AND METABOLIC ACTIVITY IN A MULTI-ORGAN-CHIP

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Background and novelty: The Multi-Organ-Chip (MOC) developed by TissUse GmbH is a novel device emulating segments of human organs [1, 2]. One future application of this system is the dermal drug testing *in vitro*. To describe mass transfer process, a non-invasive technique for determination and simulation of permeability and metabolic activity at the scale of the MOC was established. In this study, the permeation of fluorescent target substances through a cell containing collagen gel into the MOC and the metabolic activity of cells was investigated in order to optimize a full skin model. COMSOL Multiphysics was used to simulate the permeation process and the metabolic activity.

Experimental approach: Transwell[®] systems (Corning, Lowell, MA) were used for the permeation experiments to determine the fluorescent tagged substance through a barrier and then transferred to the MOC. Additionally the metabolic activity of different cell types was investigated in order to simulate glucose consumption and lactate production of the cell containing collagen gel.

The simulation program COMSOL Multiphysics was used to determine the diffusion coefficient and the metabolic activity. For this purpose the Chemical Reaction Engineering, the CFD and the Optimization Module were included. In the next step the result of the simulation was applied to the MOC.

Results and discussion: It could be shown that the simulated diffusion coefficient from a simple permeation experiment in a transwell[®]-system can be transferred to the MOC simulation. Permeation experiments in MOC prove the correctness of the simulation. The metabolic activity of cells can also be implemented.

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Disclosure of Interest: None declared





PO360

DEVELOPMENT OF A PERFUSED ORGANOTYPIC KIDNEY MODEL USING DECELLULARIZED RAT KIDNEYS

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Background and novelty: Decellularized extracellular matrix (ECM) scaffolds of whole kidneys hold great potential for whole organ tissue engineering. These native scaffolds retain the organ specific ECM-composition, ultrastructure and 3D-architecture. Thereby, the microenvironment to support attachment, organization and maturation of tissue specific or stem cells is provided.

The aim of this study was to identify a decellularization protocol that preserves the kidney ECM as good as possible while efficiently removing cellular components and to re-endothelialize that scaffold using a standardized perfusion system, as a first step towards full recellularization.

Experimental approach: Rat kidneys were decellularized with the detergents sodium dodecyl sulfate/TritonX-100 (SDS/TX-100) and sodium deoxycholate (SDC) at different temperatures. The scaffolds were compared with regard to structure, cell removal and composition.

Moreover, we tested these decellularized ECM scaffolds by recellularization with human umbilical vein endothelial cells and hiPSC-derived endothelial cells in 3D-perfusion culture. Pressure, pH, O₂-level were monitored and controlled constantly. Glucose, Lactate and Lactate dehydrogenase were measured for metabolic monitoring.

Results and discussion: Notably, the cells were more viable on SDS/TX-100-decellularized scaffolds than on SDC-decellularized scaffolds. Histology and MRI revealed cells were evenly distributed in the scaffold, lining vessels throughout the kidney, including glomerular capillaries.

Our data show that decellularized kidneys provide a suitable platform for stem cell based renal tissue engineering and are the basis for further investigations of the potency of these scaffolds to promote and direct terminal differentiation of hiPSC-derived renal precursor cells.

Bibliography, Acknowledgements: -

Disclosure of Interest: None declared





PO361

INTEGRATING AN IN VITRO-BASED THERAPEUTIC INDEX INTO PHENOTYPIC DRUG DISCOVERY APPROACHES

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Background and novelty: 3D model systems can better reflect the cell composition, tissue structure, and biological characteristics of primary tumors. Hence, the use of heterotypic 3D models for efficacy testing allows to integrate an in vitro-based therapeutic to rank compounds. Here, we report on the development of heterotypic ovarian and pancreatic microtissue tumor models in co-culture with fibroblasts for a screening purposes.

Experimental approach: Up to 40 compounds selected from the NCATS Oncology library were used as a training compound set. The compounds were chosen to target different mechanisms driving tumor cell growth and survival. The biological response measured over a 10-day drug exposure period included (i) growth kinetic (microtissue size), (ii) potency (IC₅₀^{ATP_10_days}) and efficacy (max. response ATP and size). Unspecific cytotoxic effects were evaluated making use of the incorporated NIH3T3 fibroblasts by quantifying a secreted reporter over time. Reverse phase protein array (RPPA) and transcriptome analysis was applied on Torin-2 treated tumor microtissues for signaling pathway profiling.

Results and discussion: Compound classification based on single endpoints, efficacy, potency, growth kinetics and therapeutic index did not result in comparable and robust classifications. Only the integration of multiple endpoints did result in robust compound ranking which was not impacted significantly by changing the weight of different endpoints. Results of RPPA analysis revealed a clear down-regulation in the cell cycle and PI3K/Akt/mTOR signaling pathway, especially for S6 ribosomal protein phosphorylated at Ser 235, 236, 240 and 244, indicating a strong inhibitory effect on translation and cell growth control.

Bibliography, Acknowledgements: 3D cell culture systems modeling tumor growth determinants in cancer target discovery. Thoma CR, Zimmermann M, Agarkova I, Kelm JM, Krek W. Adv Drug Deliv Rev. 2014

Disclosure of Interest: None declared





PO362

VASCSKIN-ON-A-CHIP: INTEGRATION OF A PERFUSED VASCULATURE TO HUMAN SKIN-EQUIVALENTS.

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Background and novelty: The skin is the largest organ of the human body and composed of various different cell types. Human skin equivalents (SE), composed of one or two different cell types only, have been widely used for animal free tests of drugs and cosmetics. However, more complex systems are needed to accurately predict human drug toxicity. In particular, the implementation of a vasculature would be important for sufficient supply of the skin and its appendices with required nutrients and oxygen. Our multi-organ-chip (MOC) platform uses a miniaturized circulatory network with an integrated micropump to provide circulation of microliter-volume of medium to the tissues. Here, we integrated perfusable vascular channels in a full thickness skin equivalent (ftSE) and cultured both under perfusion conditions inside our two organ variant (2OC).

Experimental approach: Vascular channels were fabricated using photopatterning within a degradable fibrin-scaffold. The vascular channels were coated evenly with endothelial cells (EC). Following attachment of the cells, a continuous pulsatile flow was applied. After 1-3 days of mono-cultivation, the ftSE was added on top of the vascular bed for another 7-14 days perfused cultivation. FtSE were build up by growing keratinocytes on Matrigel® punches colonized with skin fibroblasts.

Results and discussion: Histological analysis showed that ftSE developed dermal/epidermal architecture and still showed consistency and vitality after long-term co-culture. ECs covered all walls forming a viable fluid tight layer and showed a physiological-like elongation and orientation with the direction of flow. Further, sprouting of ECs into the surrounding fibrin-scaffold was detected. This results suggests the MOC as a useful tool for long-term culture of skin-on-a-chip applications including drug development and cosmetics testing.

Bibliography, Acknowledgements: This project is funded by the German Research Foundation (DFG), LA1028/7-1

Disclosure of Interest: None declared





PO363

3D-PRINTED MICROFLUIDIC CHANNEL AND CULTIVATION MOLD FOR QUANTITATIVE EVALUATION OF CELL MIGRATION PROPERTY

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Background and novelty: Measurement of the cell migration property is quite important, for example, in the study of dedifferentiation of cancer cell to be a more motile cell, which initiates metastasis. The in-vitro scratch assay, which evaluates the cell mobility on the edge of a scratched cell layer, is a well-known technique. However, the assay has problems of reproducibility of the scratch and quantitative evaluation of the cell movement from the initial edge which becomes unclear by the migration. In this study, two different approaches of 3D-printed microfluidic channels and a cultivation mold were proposed to overcome these problems.

Experimental approach: (1) Cross-shaped microfluidic channels were fabricated utilizing a 3D-printer. The cells at the crossing of the channels can be removed by the injection of an air plug into one side of the channel. (2) A mold which works as a stopper while cultivation was also fabricated. The mold has a tip with 1 mm of diameter and is designed to fit inside a well of conventional 24-well culture plate. Cell suspension is introduced into the well with the tip contacting the bottom of the well. The cells were cultivated with the mold to form a patterned cell layer.

Results and discussion: (1) After optimization of the channel width and height, the cells in the microfluidic channel were successfully patterned to have a straight edge with good reproducibility. The shrinking of the pattern was optically evaluated in the course of cultivation. (2) The cultivation mold was also able to pattern a cell layer with smooth edge with good reproducibility. The shrinking of the pattern was evaluated in the same manner as in (1). Those results demonstrated that the 3D-printing technology could enhance the reproducibility of the in-vitro scratch assay.

Bibliography, Acknowledgements: This project is supported by Young-researcher oversea program from Tohoku university, Sendai, Japan.

Disclosure of Interest: None declared





PO364

CHARACTERIZATION OF SKOV-3 SPHEROIDS OBTAINED BY ULTRA-LOW ATTACHMENT AND HANGING-DROP METHODS FOR DRUG SCREENING ASSAYS

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Background and novelty: Compared to 2D culture (monolayer), 3D cancer models more closely mimic native tissues, since the tumor microenvironment established in 3D often plays a significant role in cancer progression and cellular responses to drugs.

Experimental approach: SKOV-3 3D spheroids was formed and maintained in ultra-low attachment (ULA) 96-wells plates and hanging drop (HD) plates using different initial concentrations: 1.25×10^3 ; 2.5×10^3 ; 5.0×10^3 ; 12.5×10^3 ; 25.0×10^3 (n=3). Throughout culture, images were obtained daily by inverted microscope, using *ImageJ* software to estimate spheroid's morphology, diameter and circularity. Resazurin sodium salt was used to evaluate the viability and growth of 3D spheroids according to the manufacture's specifications.

Results and discussion: The SKOV-3 spheroids formed in ULA plates presented non-spherical (circularity in the range 0.555-0.742) shape with diameters ranging from 230 to 751 μm after 24h. Throughout culture it was not observed an increase in cell diameter, although cell growth was detected in all cell concentrations evaluated. At end of 120 h, the spheroids presented diameter ranging from 138 to 642 μm . Non-spherical spheroids in HD plates were formed only after 72h of incubation with diameters ranging from 122 to 374 μm (circularity in the range of 0.620-0.875). At the end of 168h, we observed diameters from 139 to 434 μm . Similarly to ULA plates, there was no increase in spheroid diameter throughout culture. Cell growth was observed during the first 96 hours only for cultures seeded with the two highest cell concentrations. The next step of the work involves testing the drug Paclitaxel in the cultures that presented spheroids diameter around 300-500 μm at 96 hours after seeding (5.0×10^3 and 12.5×10^3 for ULA and 12.5×10^3 and 25.0×10^3 for HD) and the comparison with 2D cultures.

Bibliography, Acknowledgements: Supported by CAPES and CNPq 443981/2014-0.

Disclosure of Interest: None declared





PO365

LAB AS A SERVICE - AUTOMATED CELL-BASED ASSAYS

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Background and novelty: The use of cell-based assays in pharmaceutical industry and academic research is a growing trend that is a driving force to reduce costs for drug development. Academic research is gaining information about intracellular targets or functional mechanisms through the variety of different assays. These benefits can be used in preclinical studies and furthermore costly late-stage drug failures may be reduced by the use of cell-based assays. The use of automated systems is also in great demand and will change the testing of substances and research activities. Nevertheless, there are a lot of barriers at the moment limiting the successful application of automated systems in this field. By the lack of flexibility and the demand for skilled computer scientists & engineers just the two main aspects stated by experts shall be mentioned.

Experimental approach: Our strong background on automated cell culture technologies and expertise, gained in several projects, let us rethink the overall process chain and overcome established principles. A new service orientated platform for the execution of cell-based assays that are commonly used will be introduced. The main idea is to give access to automated infrastructure for academic research or spin-offs which cannot afford the special infrastructure.

Results and discussion: User have 24/7 access to automated infrastructure through a web based configurator. Standard predefined validated assays can be easily selected or individual assays can be composed with an intuitive software configurator. The platform will inform you about the next free time slot to execute your experiment. Intermediate step or end results can be discovered immediately after the process, in a secure data cloud. Through automation and closed data record results are more reproducible and comparable. This dynamic access to automation offers high flexibility for low throughput experiments and will push high quality research and drug development in early stage.

Bibliography, Acknowledgements: n/a

Disclosure of Interest: None declared





PO366

DEVELOPMENT OF ALTERNATIVE ANIMAL CELL TECHNOLOGY PLATFORMS: CHO BASED CELL-FREE PROTEIN SYNTHESIS SYSTEMS FOR THE PRODUCTION OF “DIFFICULT-TO-EXPRESS” PROTEINS

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Background and novelty: Animal cell technologies are commonly used for a broad range of medical and pharmaceutical applications. One main topic of these technologies is the production of proteins used for therapeutical purposes. These *in vivo* production processes are often time consuming and limited in production of so called “difficult-to-express” protein. To overcome these issues, novel cell-free protein synthesis platforms were developed based on CHO cells. A cell lysates provide the basis by including all components of the translational machinery and enabling protein production within a few hours. Microsomes present in CHO cell lysates enable posttranslational modification of proteins and insertion of membrane proteins into lipid bilayer.

Experimental approach: In this study a cell-free protein platform was developed based on a combination of CHO cell lysates and a continuous exchange reaction format. The system enables the synthesis of high yields of “difficult-to-express” proteins including membrane proteins, single chain fragments and ion channels. Produced proteins were analyzed by incorporation of radioactive ¹⁴C leucine during cell-free reaction followed by autoradiography and scintillation measurement. Activities of proteins were estimated by Kinase Assays, ELISA and electrophysiological measurements.

Results and discussion: Protein yields up to 980 µg/ml were determined while detecting correct molecular weights by autoradiography. Analysis of proteins revealed high activity of produced proteins. Posttranslational modifications of proteins were detected. A CHO cell-free system for high yield production of proteins was developed that provides a platform for future production of “difficult-to-express” proteins.

Bibliography, Acknowledgements: Cell-Free Systems Based on CHO Cell Lysates: Optimization Strategies, Synthesis of “Difficult-to-Express” Proteins and Future Perspectives (Sep 2016 PLoS ONE)

Disclosure of Interest: None declared





PO367

A HIGH-THROUGHPUT CELL PROLIFERATION ASSAY FOR SCREENING SINGLE-USE MATERIALS FOR CELL CULTURE COMPATIBILITY

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Background and novelty: The current trend in biopharmaceutical manufacturing is to move from stainless steel to single-use systems, including disposable containers and bioreactor bags. The materials used to manufacture these products can leach compounds which could adversely affect cell culture. Leachable & extractables analysis does not always predict potential detrimental effects. There is a need for leachables testing using methods that mimic real use upstream bioprocess conditions. Traditional leachables testing typically uses adherent cell lines in serum supplemented media, which does not reflect production in suspension cultures with serum-free media. Recent reports describing shake flask Chinese Hamster Ovary (CHO) cultures show promise, but are labor intensive especially with larger sample numbers. We have optimized a quantitative high-throughput cell proliferation assay to screen materials in lieu of shake flask cultures or traditional cytotoxicity assays.

Experimental approach: Our requirements for a predictive, useful cell culture assay were: utilize cells and media that reflect a typical mAb production process; match conclusions from CHO shake flask testing, including sensitivity to a known CHO cytotoxic agent; demonstrate good intra- and inter-assay reproducibility; and allow for more test samples per assay. Cell proliferation assays were evaluated and PrestoBlue* was chosen for its broad dynamic range, reproducibility, and tolerance to different media types.

Results and discussion: This assay requires significantly less hands-on time than shake flasks and has a considerably higher throughput. The assay has excellent dynamic range and very good intra- and inter-plate reproducibility. It also shows good dose response to bis(2,4-di-tert-butylphenyl)phosphate, and data generated correlates well with parallel shake flask results.

Bibliography, Acknowledgements: *PrestoBlue is a trademark of Life Technologies Corporation.

Disclosure of Interest: None declared





PO368

PRODUCTION OF RECOMBINANT FACTOR VII IN SK-HEP-1 HUMAN CELL LINE.

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Background and novelty: Nowadays it is known that the development of inhibitory antibodies by hemophiliac patients is closely related with immunogenic epitopes present in the coagulation factors. These proteins are produced in hamsters cells which insert a different post-translational modification profile when compared with the human profile. Patients with high-titer/high-responding inhibitors must be treated with bypassing agents that can achieve hemostasis. Activated factor VII (FVIIa) is an attractive candidate for hemostasis, independent of FVIII/FIX, making this coagulation factor an alternative for hemophilia patients with inhibitory antibodies. However recombinant factor VII is produced in BHK-21 cells (Baby hamster kidney cells) and as well as the others coagulation factors, it may contain immunogenic epitopes.

Experimental approach: In this context, becomes extremely important to produce recombinant proteins with complex post-translational modifications in a cell line not yet used. We have been using the Sk-Hep-1 human cell line for the production of recombinant FVII. To generate the recombinant cell line we have used a bicistronic lentiviral vector containing a FVII gene and the GFP selection marker gene.

Results and discussion: In static conditions Sk-Hep-1 cells showed, for a period of 6 months, a stable FVII production with an average of 8,03 IU/mL of FVII, 83% of cell viability and 77% of cells expressing the GFP gene. After purification with VIISelect column it was possible observe a recover of 65% of the purified protein with 95% degree of purity. This recombinant purified FVII is being used in *in vivo* experiments to determine the pharmacokinetics parameters and to evaluate the post-translation modifications profile.

Bibliography, Acknowledgements: The authors acknowledge São Paulo Research Foundation – FAPESP (2015/19017-6), Centro de Pesquisa, Inovação e Difusão (CEPID), and National Institute of Science and Technology in Stem Cell and Cell Therapy - INCTC for financial support.

Disclosure of Interest: None declared





PO369

FLUORESCENT CELL-BASED BIOSENSORS FOR DETECTION AND QUANTIFICATION OF LABEL-FREE VIRUS AND VIRAL VECTORS

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Background and novelty: The past decade witnessed the rise of investment in antiviral agents' development and viral vectors for gene therapy and vaccination. Therefore, accurate and reliable virus detection and quantification assays are needed. However, current methods are extremely time-consuming, lack high-throughput potential and/or require the use of reporter genes (label-containing virus), unacceptable in a clinical context [1]. A novel method capable of overcoming these drawbacks would be of great value for research, diagnostics and industry.

Experimental approach: Herein, we report the development of genetically encoded fluorescent biosensors for detection and quantification of label-free viruses: VISENSORS. Structural distortion maintains these stably expressed biosensors in a non-fluorescent state. Upon viral enzyme activity – thus label-free system – biosensors' fluorescence is triggered. Different designs of biosensors are being optimized by means of molecular biology and characterized by fluorescence microscopy and flow cytometry.

Results and discussion: VISENSORS' potential was confirmed by establishing HEK293 sensor cells for the detection of recombinant Human Adenoviruses (type 5) and HIV-based lentiviruses. Initial characterization shows virus detection as early as 24 hours post-infection and a signal to noise up to 4 – in line with previously reported mammalian cell-based conditionally-fluorescent biosensors – leaving room for further improvements. Adaptation to other viruses and viral vectors such as Hepatitis C virus is being evaluated. Validation against current standard assays and high-throughput antiviral screening potential are undergoing.

VISENSORS will deliver fast and accurate detection and quantification of clinically relevant label-free virus and viral vectors, allowing the establishment of new cellular platforms for diagnostic and clinical applications.

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Disclosure of Interest: None declared





PO370

DEVELOPMENT OF HIGH THROUGHPUT METHODS TO CHARACTERIZE RAW MATERIAL IMPACT TO PROCESS PERFORMANCE

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Background and novelty: Undefined components in complex media can be a source of process variability and efforts are often made to assess their impact prior to use. This is particularly challenging for perfusion processes, where small scale experimental platforms are often too unwieldy to enable quick and comprehensive characterization of such components. To address this issue, methods were developed to increase throughput and experimental power, without compromising the number of analytics available for characterization.

Experimental approach: Increased experimental throughput was accomplished by (1) adaptation of a high throughput cell culture platform to mimic perfusion processes, (2) development of batch purification protocols that enabled purification of small harvest volumes and (3) development of more sensitive analytics that require small sample size and minimal sample preparation. These methods were then used in conjunction to screen raw materials.

Results and discussion: The methods developed resulted in an over 20-fold increase in throughput, representing a dramatic increase in raw material screening capability. This improved test bed was used to assess as set of raw material lots to ensure consistent process performance. The development of these high throughput methods allows for rapid and comprehensive screening of raw materials under consideration for use in perfusion process production systems.

Bibliography, Acknowledgements: None

Disclosure of Interest: None declared





PO371

AUTOMATION-COMPATIBLE MICROFLUIDIC SYSTEM FOR MULTI-TISSUE INTEGRATION

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Background and novelty: Recently, three-dimensional cell cultures in microfluidic devices have been considered to address the limitations of 2D cell cultures in mimicking in vivo-like environments, especially perfused multi-organ networks. However, currently available platforms are still relatively difficult to operate, complex and incompatible with SLAS standards.

Experimental approach: Here, we present a simple and robust 96-well format-based microfluidic platform that enables multi-tissue networks using multi-cellular spheroids. The fully polystyrene-based low-absorption device includes 8 separate channels and each channel contains up to 10 spheroid compartments. Up to 10 same or different spheroids can be interconnected and cultured in 8 identical or different conditions in parallel per plate. Multiple plates can be stacked. Perfusion flow is generated through tilting the device back and forth on an automated system inside an incubator.

Results and discussion: In a liver-tumor network we were able to reproduce bio-activation of Cyclophosphamide (CP), an anti-cancer pro-drug, which originally is not active, but first has to be metabolized by liver to show an effect on tumor. 5 primary human liver spheroids (hLiMT) and 1 human colon carcinoma spheroid (HCT116) were fluidically interconnected and cultured in the platform. After 8 days, growth and viability of HCT116 decreased by about 50% with respect to controls (no CP and/or no hLiMT present in the network) proving evidence of successful bio-activation and therefore recapitulation of pro-drug scenarios in the proposed in vitro liver-tumor system.

Bibliography, Acknowledgements: This work was financially supported by CTI project 18024.1 PFLS-LS.

Disclosure of Interest: None declared





PO372

CHARACTERIZATION AND APPLICATION OF 3D MULTI-DONOR HUMAN LIVER MICROTISSUES FOR PREDICTIVE DILI TESTING AND MECHANISTIC INVESTIGATIONS

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Background and novelty: Three-dimensional (3D) human liver spheroid cultures from individual hepatocyte donors proved to be a valuable tool in liver research. Utilizing pooled hepatocytes from multiple donors offers the advantage of capturing a more diverse genetic background in a single assay to help mitigate potential bias of single-donor hepatocytes due to individual cytochrome P450 polymorphisms.

Experimental approach: Here we create and functionally characterize the novel 3D multi-donor human liver microtissues, which consist of pooled hepatocytes from 5 male and 5 female donors in co-culture with primary Kupffer cells.

Results and discussion: Histological analysis demonstrated polarized expression of bile-salt export pump, indicating presence of a bile-canalicular network over 4 weeks in culture. Functional testing for phase I enzyme metabolism showed stable cytochrome P450 activity for CYP1A2, 3A4, 2C9, 2B6, 2D6, 2C19 and 2A6 for at least 28 days in culture. Moreover, the prototypic cytochrome inducer Omeprazol induced CYP1A2 activity, whereas phenobarbital and rifampicin induced CYP3A4, CYP2B6 and 2C19 activities. The suitability for prediction of potential drug induced liver injury compounds was tested with eight reference drugs over 14 days drug exposure. Ambrisentan and Entacapone were predicted to be non-toxic, whereas Chlorpromazine, Ketoconazole, Rosiglitazone, Sitaxsentan, Tolcapone and Troglitazone exhibited lower safety margins of smaller than 30 fold over clinical concentrations. In addition, the inflammation-mediated toxicity of Trovafloxacin was shown to be independent of the used Kupffer-cell lot. The high biological relevance, long shelf life and high throughput format of the 3D liver microtissues allow its application for *in vitro* DILI detection and mechanistic investigations¹.

Bibliography, Acknowledgements: 1. Arch Toxicol. 2013 Jan;87(1):209-13.

Disclosure of Interest: None declared





PO373

DRUG TOXICITY ON LIVER SPHEROIDS IN A MICROFLUIDIC DROPLET ARRAY

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Background and novelty: 3D culture emerges as a more predictive *in vitro* model than 2D culture, notably in the context of the low success rate of drug development. In this work, we propose a highly integrated microfluidic platform for 3D cell culture that combines the advantages of multi-well plates (screening, selective recovery) and droplet microfluidics (low volumes, high-throughput, hydrogel encapsulation) for drug toxicity experiments.

Experimental approach: Rails and anchors¹ allow the guiding and trapping of microfluidic hydrogel droplets encapsulating cells in a large chamber. Single monodisperse spheroids are created in each immobilized droplet, up to an array of 500 spheroids on 2 cm², and can be kept in culture for several days. We have also designed combinatorial anchors capable of successively trap in a single location two droplets of different volumes and contents that can mix after merging.

Results and discussion: We investigated the toxicity of drugs (e.g. acetaminophen) on liver spheroids (rat, human). Cells were encapsulated in the first droplets and formed monodisperse spheroids within 1 day after trapping in the array. Then, we randomly trapped in the anchors one droplet from a library of different drug concentrations over a logarithmic scale (2 decades), each concentration corresponding to a specific fluorescent label. After merging, each spheroid is exposed to a drug dose for 24 hours. Toxicity results are acquired after fluorescent viability staining on chip and reveal a strong heterogeneity of the drug effect on the spheroids. Compared to a 384-well plate, this highly integrated microfluidic platform involves very small volumes (500-fold reduction) with easy fluid handling and provides high-throughput (array 50 times as dense) and high-content (static or dynamic analysis at the spheroid and cellular level *in situ*) data for toxicity tests on biologically relevant tissues.

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Disclosure of Interest: None declared





PO374

ESTABLISHMENT AND CHARACTERIZATION OF 3-D TUMOUR SPHEROIDS USING FORCED FLOATING AND HANGING DROP METHODS

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Background and novelty: 3-D cell culture may reflect more accurately the antitumor activity of compounds during *in vitro* screening for novel drugs once they better mimic the tumor microenvironment. The main goal was use two different methods, forced floating and hanging drop, to establish a 3D bladder tumor model.

Experimental approach: RT4 cells were cultured in Ultra-Low Attachment plates (ULA) and Hanging-drop plates (HD) in four different seeding concentrations, 5.0, 12.5, 25 and 37.5x10³ cells/ml, during seven days. We analysed: morphology (microscopy images); shape parameters (ImageJ); growth profile (cell count using trypan blue); presence of apoptotic cells (Annexin V-FITC); drug-sensitivity response (Doxorubicin, 3D CellTiter-Glo®).

Results and discussion: Microscopy showed that compacted spheroids were generated after 48 hours of culture in both methods. In ULA plates, only cell seeding of 5.0 and 12.5x10³ cells/ml generated spheroids with a diameter between 300 to 500 µm, considered the ideal diameter range for drug screening purpose. However, for HD plates the cell seeding was 25 and 37.5x10³ cells/ml. Both methods generated spheroids with a satisfactory circular outline (roundness of 0.80 to 0.90), regular surface with no salient edges (solidity higher than 0.90) and ellipsoidal and spherical shapes (sphericity index close to 0.90). Cell growth was observed in both 3D methods, but comparing to 2D cultures these numbers were lower. Number of apoptotic cells in the last day ranged between 5 to 10%. RT4 cells in 3-D spheroids presented a higher drug resistance once the EC50 for doxorubicin was greater in assays with 3-D spheroids (~1.0µg/ml) comparing to 2-D cultures (~0.35µg/ml). The data presented can contribute to advances in analytical techniques of 3-D cultures ensuring greater robustness, reliability and versatility of such models for drug screening purposes.

Bibliography, Acknowledgements: Supported by CNPq 443981/2014-0 and FAPESP 2015/05102-1

Disclosure of Interest: None declared





PO375

**ALTERNATIVES TO THE USE OF ANIMALS DURING
DEVELOPMENT OF BIOTECHNOLOGICAL MEDICINAL PRODUCTS:
REFINE, REDUCE, REPLACE**

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Background and novelty: Alternatives to the Use of Animals During Development of Biotechnological Medicinal Products: Refine, Reduce, Replace

In vivo test systems have been used for many years in the safety evaluation of biotechnological medicinal products (BMPs). From detection of extraneous agents in starting materials for the production of BMPs to potency batch release testing of final product. In Europe, the use of animals for scientific purposes is regulated under EC Directive 2010/63/EU. Underpinning these various regulations and guidance is the '3Rs'; reduction, refinement and replacement of the use of animals for scientific purposes, observed by organisations and institutions around the world.

Experimental approach: Here we describe the recent industry developments in the replacement of the use of animals in safety evaluation of BMPs. Recently experimental data has shown the effectiveness of *in vitro* methods for the detection extraneous agents.

Results and discussion: Developments include proposed replacement of *in vivo* tests with alternative methods utilising new validated technologies, such as next generation sequencing, through to elimination of compendial *in vivo* test methods based on evaluation of historic *in vivo* data without the need for replacement tests. Clear industry guidance will be provided and discussed.

Bibliography, Acknowledgements: Dr Sarah Sheridan (née Sarah Gilliam) is a Principal Scientist within the Field Development Services team at BioReliance, responsible for providing primary scientific leadership. Sarah has worked at BioReliance for 21 years and has a total of 24 years experience working within the biotech industry. Sarah's areas of expertise include the use of *in vivo* and *in vitro* test systems in the pre-clinical quality control testing of human and veterinary biopharmaceuticals. Sarah is a graduate of the University of Cambridge, UK, with a PhD in Veterinary Science.

Additional Authors: Wisher, MH and Whiteman, M

Disclosure of Interest: None declared





PO376

SOFT BIOCOMPATIBLE MICROCARRIERS FOR ADIPOSE-DERIVED MESENCHYMAL STEM CELLS EXPANSION

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Background and novelty: Emergence of stem cell-based regenerative medicine recently led to the necessity to reach a sustained production of such cells (1). Hence, new bioreactors and carriers were designed for cell expansion. However, to meet this increasing demand, improvement of both quality and quantity of stem cells remains necessary.

Experimental approach: Substrate stiffness being known to strongly influence *in vitro* stem cell fate and differentiation (2,3), we developed a novel class of soft biocompatible alginate microcarriers. Our expertise in the field of microbeads design using jetcutting technology (4) enabled us to engineer +/- 200 µm alginate beads of various M/G monomer ratio. This leads to the production of beads displaying different stiffness. A further alginate surface treatment was carried out to reach an optimal cell anchoring.

Results and discussion: We report here growth data and characteristics including stemness and viability of adipose-derived mesenchymal stem cells grown on these new carriers. Beads specifications, surface treatments as well as new bioprocess design for larger scale stem cell productions are also discussed.

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Disclosure of Interest: None declared





PO377

ASSESSMENT OF MITOCHONDRIAL ACTIVITY IN 3D HUMAN LIVER MICROTISSUES AS A TOOL FOR MECHANISTIC INVESTIGATIONS OF ADVERSE DRUG EFFECTS

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Background and novelty: Mitochondrial dysfunction is a major mechanism of drug-induced liver injury, which was shown to be induced through the parent drug or a reactive metabolite generated through cytochrome P450 activity (1). Until now, most existing cell-based models utilized 2D-cultured hepatocytes, which exhibit only little metabolic competence and allow only for short-term drug exposures. Here we investigated whether 3D human liver microtissues would be a useful tool for assessment of mitochondrial activity, since these tissues exhibit preserved metabolic functions, which enables long-term toxicity-testing.

Experimental approach: 3D Human Liver Microtissues were tested for their compatibility to analyze mitochondrial oxygen consumption rates (OCR) with the Seahorse XF96 platform. This platform allows real-time measurement of mitochondrial activity on drug-treated liver microtissues.

Results and discussion: We found that 3D human liver microtissues exhibited an increased spare respiratory capacity in comparison to 2D culture. Moreover, a panel of potential mitotoxic drugs (e.g. Amiodarone, Diclofenac, Fialuridine, Troglitazone) and non-mitotoxic drugs (e.g. Bosentan, Entacapone, Ximelagatran) was tested. The mitotoxic drugs showed a dose-dependent decrease in oxygen consumption rate, before the cellular viability was affected. In contrast, the non-mitotoxic drugs did not show decrease in OCR before cellular survival was affected. This suggests that measurement of mitochondrial activity is a valuable tool to detect drugs with potential with mitochondrial liabilities and is therefore a powerful tool for mechanistic investigations of adverse drug effects.

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Disclosure of Interest: None declared





PO378

BONE MARROW-ON-A-CHIP: LONG-TERM CULTURE OF HUMAN HEMATOPOIETIC STEM CELLS IN A 3D MICROFLUIDIC ENVIRONMENT

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Background and novelty: Multipotent hematopoietic stem cells (HSCs) give rise to all blood cell types. The bone marrow stem cell niche in which the HSCs are maintained is known to be vital for their sustainment. Unfortunately, to this date, no *in vitro* model exists that truthfully mimics the aspects of the bone marrow niche and simultaneously allows the long-term culture of HSCs.

Experimental approach: In this study, we present a novel 3D co-culture model, based on a hydroxyapatite-coated zirconium oxide scaffold mimicking the trabecular bone. Primary human mesenchymal stromal cells (MSCs) derived from the bone marrow of femoral heads were employed to build up a suitable environment within the scaffold for the culture of HSCs. Subsequently, primary human HSCs isolated from umbilical cord blood were seeded in this bone marrow mimicking environment. The whole culture was carried out within our microfluidic multi-organ-chip platform.

Results and discussion: Immunofluorescent stainings, qPCR, and scanning electron microscopy data demonstrated that the MSCs had produced an appropriate environment for HSC cultivation.

Using flow cytometry and CFU-GEMM assays, it could be shown that HSCs remained their native phenotype for at least eight weeks. Additionally, without the addition of further cytokines erythropoiesis and granulopoiesis could be observed in the system.

Moreover, cells cultured for four weeks in our bone marrow model passed the gold standard test for native multipotent HSCs by successfully engrafting in irradiated immunocompromised mice.

Here we present for the first time a novel human *in vitro* bone marrow model, capable of the successfully long-term culture of functional multipotent HSCs in a microfluidic environment. Predestining it as a model for sophisticated *in vitro* drug testing, thus, serving as an alternative to animal testing.

Bibliography, Acknowledgements: *We thank the Vivantes Hospital in Friedrichshain Berlin and the Immanuel Hospital Berlin.*

Disclosure of Interest: None declared





PO379

INFLUENCE OF TACROLIMUS TREATMENT ON ENDOTHELIAL CELLS

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Background and novelty: Tacrolimus, an immunosuppressant, is widely used to inhibit adverse reactions of the immune system after a transplantation to prevent organ rejection. But variability of drug absorption leading to different blood levels of Tacrolimus is a major problem in dose adjustment. Long time application of higher levels is limited due to undesired effects. For a successful integration of new organs an optimal vascularization is necessary, which allows a good nutrient supply in the new organism.

Experimental approach: To investigate the influence of Tacrolimus on vascularization, human umbilical vein endothelial cells (HUVECs) have been used as model system. By screening different concentrations of Tacrolimus with CellTiter-Blue® assay the IC₅₀ value and lethal dose *in vitro* was determined by dose response curve.

The monitoring of cell death dynamics was performed analyzing LDH release and Caspase 3/7 activity and using non-invasive impedance sensing. The cytotoxic effect of Tacrolimus on formation of tubular-like structures was analyzed with an angiogenesis assay. A 3D spheroid culture assay was performed to verify results achieved from 2D culture system and to examine the influence of Tacrolimus on spheroid morphology.

Results and discussion: The results show a significant effect of high Tacrolimus concentrations on cell viability and angiogenesis. There is a significant increase in LDH activity after Tacrolimus treatment. This implies the cells undergo necrosis due to loss of membrane integrity. For the IC₅₀ value HUVECs showed a lower formation of tubular-like structures that means shorter structures with a lower number of connecting points in the angiogenesis assay. The spheroids with a specific cell number and spheroid size showed a significant change in morphology due to cell death with higher Tacrolimus concentrations.

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Disclosure of Interest: None declared





PO380

IN-VITRO VASCULOGENESIS TO INTERCONNECT ORGANOIDS IN A MULTI-ORGAN-CHIP PLATFORM

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Background and novelty: The Multi-Organ-Chip platform is one of the first microphysiological systems developed to evaluate toxic effects of drugs, cosmetics and alike in a sub-systemic mode. At the scale of a microscope glass slide, it comprises several compartments for any co-cultivation of human 3D tissue constructs. The organoids are physically separated, yet, interconnected through perfused microfluidics. Minute volumes of medium enable crosstalk. The organoid cultures are, however, not sufficiently vascularised to overcome limitations in size and complexity. We summarise our efforts to recreate a continuous endothelium and link it to advances in modelling niches inside the bone marrow.

Experimental approach: Three major aspects were addressed: (1) Implement a near-physiological, pulsatile flow. It should provide an in vivo-like shear stress regime, which is required for a phenotypical behaviour of the cells. Complex fluid dynamics created by an on-chip micropump were characterised and optimised using micro particle image velocimetry. (2) Create an endothelial lining within the chip's microfluidic system. The optimised flow promoted vitality and the expression of typical markers. The cells could be cultivated for more than 106 days. (3) Establish capillary-like vessels as a direct route to the organoids. Fibrin hydrogels containing an endothelial / stromal cell co-culture enabled the self-organised formation of microcapillaries. Eventually, connecting the capillary network to an organ equivalent such as the bone marrow model showed general feasibility of the approach.

Results and discussion: Basic features of blood vessels could be emulated inside our platform. A continuous endothelium is crucial for physiological-like interactions, regulation and homeostasis within organoid (co-)cultures as well as for long-term tissue cultivation. Moreover, it is a requirement for replacing medium with a full blood surrogate and to enable immunological queries.

Bibliography, Acknowledgements: -

Disclosure of Interest: None declared





PO381

CELLULAR BIOASSAYS DEVELOPED WITH FUNCTIONALLY IMMORTALIZED CELL LINES

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Background and novelty: A major limitation of current research is the shortage of physiologically relevant cells. This shortage limits the development of relevant cellular bioassays that are robust, reproducible and scalable. We developed an immortalization regimen allowing the efficient and reproducible establishment of novel cell lines with an in vivo like physiology.

Experimental approach: For the development of cellular bioassays we used various functionally immortalized cell lines which were previously established with the CI-SCREEN technology. These include endothelial and lung epithelial cells as well as osteoblasts (CI-huVEC, CI-huAEC, CI-huOB). The key feature of these cell systems is that they combine the positive advantage of cell lines – the unlimited cell supply – with the advantage of primary cells – the physiological relevance

Results and discussion: These versatile cell systems were used either unmodified or after the integration of reporter genes. Using the CI-huVEC cells bioassays were developed that assess cytokine dependent modulation of proliferation. Tube formation as well as wound healing were analysed with fluorescent CI-huVEC cells, which allow an easy quantification. The lung epithelial cells (CI-huAEC) were used to establish a barrier assay and the osteoblasts (CI-huOB) were cultivated in spheroids for up to 50 days and to study the effect on CA2+ deposits and the alkaline phosphatase activity.

Our results demonstrate that the functional immortalization technology provides physiologically relevant cells in sufficient numbers needed e.g. for drug discovery, ADME/Tox testing and in the long term for regenerative medicine approaches.

Bibliography, Acknowledgements: .

Disclosure of Interest: None declared





PO382

THE CHOICE OF TUMOR-STROMA CELL PAIR FOR CANCER CELL MODEL DESIGN HAS AN IMPACT ON TUMOR CELL GROWTH AND DRUG RESPONSE

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Background and novelty: Tumor microenvironment has been described to strongly impact cancer development and drug response; therefore it is critical to design more predictable tumor cell models incorporating features of the tumor microenvironment.

Experimental approach: Non-Small Cell Lung Carcinoma (NSCLC) cell aggregates were microencapsulated in alginate capsules, alone or in combination with different sources of fibroblasts. The effect of fibroblasts was assessed by analysis of tumor growth and drug response, both *in vitro* and *in vivo*.

Results and discussion: Microencapsulation of H1650 and H1437 spheroids in mono- or co-cultures with fibroblasts resulted in viable cultures with tumor aggregate area increasing continuously during the 4 weeks of culture. Tumor growth in co-cultures *in vitro* was dependent on the source of fibroblast used. Co-culture of H1437 with dermal fibroblasts (hDFs) prior to implantation significantly enhanced *in vivo* tumor growth.

NSCLC co-cultures with fibroblasts presented, in general, lower sensitivity to therapy. H1437-hDFs co-cultures showed less sensitivity to 3 weeks of volasertib treatment *in vitro*, with higher DNA concentration (2-fold higher versus mono-cultures) and resazurin reduction activity (35% versus 22% in mono-cultures). H1650 co-cultures with normal lung fibroblasts (NFs) also demonstrated lower sensitivity to erlotinib and docetaxel treatment *in vitro*, with higher resazurin reduction activity (71% versus 29% in mono-cultures with 3 weeks of treatment) and higher viable area of aggregates, respectively.

Taking all together, NSCLC 3D models incorporating specific type of fibroblasts presented reduced sensitivity to SOC drugs, better reflecting the clinical observations.

Bibliography, Acknowledgements: We acknowledge support from the Innovative Medicines Initiative Joint Undertaking (IMI grant agreement n° 115188) and FCT (iNOVA4Health – UID/Multi/04462/2013, PD/BD/105768/2014 and SFRH/BD/52208/2013).

Disclosure of Interest: None declared

