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POSTER ABSTRACTS

KERRY

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P-100

CRISPR-BASED TARGETED EPIGENETIC GLYCO-ENGINEERING IN CHO CELLS

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Background and novelty: The epigenome, i.e. the genetic signature that contributes to modulation of gene expression, has not been fully explored yet. Interestingly, DNA promoter methylation has been reported to result in a loss of productivity in CHO cells. Traditional approaches for epigenetic control, e.g. addition of chemicals to the media, are feasible when genome wide epigenetic changes are desired, but inappropriate when specific loci should be modulated. Because of recent breakthroughs within the CHO field, i.e. application of genome editing tools, a new and more elegant way to study epigenetic effects in CHO is possible.

Experimental approach: To enable direct epigenetic control of individual genes, we constructed CRISPR-based epigenetic editing tools that induce site-specific DNA methylation or demethylation. gRNAs were designed against example promoters of: i) the α (2,6)-sialyltransferase (ST6GAL1) gene, which is actively transcribed in human, but silenced in CHO, ii) the α (1,6)-fucosyltransferase FUT8 gene, an interesting target for optimization of antibodies, as the absence of fucose increases antibody functionality. In all cases we aimed to both activate and silence (or vice versa, respectively) the corresponding promoter.

Results and discussion: For ST6GAL1 up to 60% of cells showed expression after treatment, which was shown to be stable for more than 80 days. Gene expression was again downregulated by subsequent targeted re-methylation of the promoter. Cell growth or recombinant protein productivity was not affected. Fut8 downregulation by targeted promoter methylation achieved 50% knockdown efficiency compared to the 150kb full gene deletion by active Cas9 KO.

These epigenetic tools do not only allow to build a new layer of cell control and enable a more sensitive investigation of gene function but – other than gene knockout or overexpression – the modulation is readily reversible.

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P-101

VECTOR OPTIMIZATION – EXPRESSION OF A NOVEL FC-FUSION PROTEIN

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Background and novelty: Efficient recombinant expression of traditional monoclonal antibodies is achieved by expressing an optimal ratio of light chain (LC) to heavy chain (HC). Since the LC acts as a chaperone, an optimal higher LC:HC expressed protein ratio will yield the highest titers. Cell line development platforms will utilize vector configurations to optimize this LC:HC ratio (e.g., chain orientation, IRES). However, this may not be the best approach to produce some of the newer novel and potentially difficult to express Biotherapeutic formats.

Experimental approach: Dual tandem expression vectors were evaluated for the generation of cell lines producing an Fc-fusion protein consisting of an LC and an HC fusion protein. The LC was placed either in the first cassette (the traditional location for LC being the stronger cassette likely due to transcriptional interference and the influence of chromatin opening elements) or in the second cassette. In addition single expression vectors were co-transfected at multiple LC:HC ratios. Following the CHOZN® GS cell line development protocol we evaluated minipools and subsequently clones for titer.

Results and discussion: In minipools higher titers were observed when two single expression vectors were co-transfected at LC:HC ratios of 1:1 or 1:2 as compared to a ratio of 2:1. Both of the dual expression vector formats yielded minipools with even higher titers, however the highest titers were observed when the HC was placed in the first (stronger) cassette. Subsequent subcloning yielded clones producing >2.5g/L when the HC was in the first cassette while all clones derived from placing the LC in the first cassette were discarded following the initial evaluation due to low titers. This demonstrates that expression of this novel Fc-fusion protein (and possibly others) can be significantly improved by simple vector modifications without making additional changes to the cell line development process.

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PROTEOMIC ANALYSIS OF CHO CELL CLONES PRONE TO PROTEOLYTIC CLIPPING.

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Background and novelty: Product degradation issues such as clipping represent a common quality issue faced in the production of Fc-fusion proteins in Chinese hamster Ovary (CHO) cells. This form of product degradation is mainly due to the actions of either intracellular or extracellular host cell proteases. The aim of this study was to gain improved understanding of the intracellular events which play a role in determining why cell lines from the same cell line development project can have varying levels of Fc-fusion protein clipping.

Experimental approach: Differential quantitative label-free LC-MS/MS proteomic analysis was used to group clonally-derived cell lines (CDCLs) based on the level of clipping of the Fc-fusion protein. The analysis was carried out over two times points in culture and clones were labelled as either 'high' or 'low' clipping phenotypes.

Results and discussion: 200 differentially expressed (DE) proteins were identified between the two experimental using quantitative label-free LC-MS/MS analysis. DE proteins were assessed functionally using Gene Ontology analysis which showed a significant enrichment of biological processes and molecular functions related to protein folding, response to unfolded protein and protein translation. The levels of several proteases were also increased. This study identified protein targets that could be modified using cell line engineering approaches to improve the quality of recombinant Fc-fusion protein production in the biopharmaceutical industry.

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MANIPULATION OF ORGANELLE CONTENT FOR INCREASED PRODUCTIVITY

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Background and novelty: Biopharmaceutical technology development is tailored towards the production of recombinant proteins with desired product quality at commercial quantities. Regulatory agencies require production cell lines to originate from a single cell. Different methods can be used to achieve clonality, such as single cell sorting via flow cytometry. Following cloning, hundreds of clones are screened to identify high producers. Therefore, the ability to enrich cells with high expression levels during the cloning process could reduce timelines and reduce the number of clones screened.

Experimental approach: Characterisation of a panel of cell lines expressing a model antibody (mAb) identified the content of an organelle as a cell attribute that correlated with productivity. Gating based on high organelle content during cell sorting generated pools/cell lines with increased productivity compared to unsorted cells. Moreover, chemical directed evolution of the host cell was performed with a chemical known to increase the content of that organelle. The heterogeneous host, and evolved cells, were then transfected to generate stable pools expressing an easy to express (ETE) and difficult to express (DTE) mAb.

Results and discussion: The evolved host outperformed the standard host, with at least a 2-fold increase in productivity and higher culture viability throughout the fed-batch. Transcriptomic analysis by RNAseq was then undertaken to investigate differences between the two hosts to understand the mechanism(s) and pathways involved in the underlying increased performance.

In summary, the content of a specific organelle has been identified as a marker for screening during cloning to increase the proportion of high producers isolated. Using the outputs of the subsequent RNAseq analysis, we are investigating targets to engineer a novel host cell line with increased potential for higher expression of ETE and DTE molecules.

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TRIPLE BENEFIT OF BHRF1-MODIFIED HYBRIDOMA CELLS

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Background and novelty: BHRF1 protein with homology with the anti-apoptotic protein Bcl-2, was transfected in hybridoma cells. Besides blocking the cytochrome C secretion, somehow promotes the influx of pyruvate into the mitochondria. Thus, lactate generation/secretion was reduced and higher amounts of ATP produced, resulting in higher cell growth rates and more efficient nutrient consumption.

Experimental approach: KB26.5 hybridoma and its derivative cell line transfected with BHRF1 were used.

Protection against apoptosis was corroborated by means of glutamine starvation. The effect of shear stress was assessed in shake flasks and in perfusion cultures in bioreactors.

Differences in cell growth, specific glucose consumption and lactate production rates were assessed in 2L-Bioreactors. Eventually, metabolic Flux Balance Analysis (FBA) was conducted using a reduced genome-scale metabolic model (*Mus musculus*) to quantify the effects of BHRF1 on cell's growth and metabolism.

Results and discussion: BHRF1-KB26.5 cells were more robust against nutrient depletion stress. They recovered after 72h of glutamine starvation whereas its parental cell line died of apoptosis only after 24h.

Significant effects on cell growth rate (0.040 vs 0.027 h^{-1}) and reduction in the specific rates of glucose and main amino acid consumption, and a decrease in the specific lactate production rate (1084 vs 2355 [nmol/mg·h^{-1}]) were observed. Overall, final cell density was doubled using identical media.

Robustness against shear stress was tested with Pluronic-free media in shake flasks. Also, lower ratio of cell death during perfusion cultures in bioreactor was observed.

Metabolic FBA revealed that BHRF-1 transfected cells doubled the influx of pyruvate into mitochondria, reduced the cytoplasmic lactate generation and increased malate-aspartate shunt flux. Thus, TCA fluxes and ATP generation increased, resulting in a more efficient cell line in terms of cell growth and substrate-biomass yield.

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HOST CELL ENGINEERING STRATEGY FOR ADVANCED RECYCLING ANTIBODIES

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Background and novelty: As described elsewhere, our DXB11 (dhfr⁻) host cell engineering strategy enhanced monoclonal antibody (mAb) productivity by nutritional control. Introduction of taurine transporter (TAUT) into DXB11 parent cells increased glutamine uptake and accelerated glutathione metabolism. By forcing the overexpression of TAUT, we were able to control DXB11 host cell functions on the complexity of advanced recycling antibodies (rcAbs) and thereby increase the mAb titer. rcAbs offer significant advantages for efficacy by dissociating the antigens in endosomes and recycling free antibodies back to plasma. We can reduce the instability in an rcAb-producing CHO cell line whereby mAb titer during the cell line development (CLD) was decreased.

Experimental approach: In this study, we used DXB11/TAUT host cells and a chemically defined medium (CDM) for the development of cell lines producing recycling antibodies (rcAbs). These not only have pH-dependent antigen-binding but also a specific mechanism of mAb uptake into cells. TAUT overexpression improved DXB11 cell performance. Rapid-growth DXB11/TAUT cells were further developed with a CDM, and these enabled the establishment of strains that produced 2.0-fold higher yields of rcAb than did DXB11 parent cells.

Results and discussion: Yields of these DXB11/TAUT/rcAb strains up to 7.0 g/L/17 days under 1-L bioreactor fed-batch conditions. In contrast, the mAb yields of DXB11/rcAb were up to 3.5 g/L/17 days. In addition, the mAb properties of DXB11/TAUT/rcAb were comparable to those of DXB11/rcAb. These results suggest that our TAUT overexpression strategy has a unique potential for improvement of DXB11 host cells and is useful for the CLD of advanced antibodies with increased complexity. Nevertheless titer of advanced rcAbs is decreased during the early stage of CLD. Only recently our updated strategy with mutant TAUT overexpression shows significant promise.

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FULLY AUTOMATED AND ENHANCED CLONE SCREENING AND EVALUATION

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Background and novelty: We will present how we accelerated a clone selection process by 4 weeks while increasing the information density obtained for each clone. This was achieved by increasing the throughput and integrating new powerful analytical technologies as gene expression and glycosylation analysis at a very early stage.

Experimental approach: Using our in-house developed MTP-based cell culture system we enable a fully automated fed-batch process at an early stage in clone selection. Due to the high throughput of more than 600 cultivations at the same time the MTP-based system considerably increases the number of clones that can be evaluated and speeds up processing. The potential to integrate real-time monitoring of metabolites and other secondary seletion criteria, e.g. glycosylation and gene expression level analysis, further supports reliable clone selection.

Results and discussion: We demonstrated the technical feasibility to couple the MTP-based cell culture system with glycosylation analysis as well as the LightCycler® technology to perform automated RT-qPCR gene expression analysis for a large number of cell culture samples. The results of the RT-qPCR analysis showed that the identified top clone displayed the highest mRNA expression level for the HC of the examined mAb which correlated with the highest specific productivity recorded at the protein level. Furthermore, RT-qPCR analysis may also be beneficial to monitor the expression of stress markers or it can be applied to improve prediction of clone stability attributes related to promoter methylation or transgene copy number.

The early availability of product quality data will also improve the chances to select the most suitable clone and to reduce risks and required effort during later development stages. This is of particular importance when expressing complex molecule formats such as bispecific antibodies, glycoengineered antibodies or antibody cytokine fusion proteins.

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CHO PROMOTERS IDENTIFIED BY RNA-SEQ FOR RECOMBINANT PROTEIN EXPRESSION

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Background and novelty: CHO cells are the main platform used for the production of biotherapeutics. Optimal heterologous protein expression needs tuning up many parameters among which the expression vector design plays a key role. Today, due to the limited genomic information of CHO, viral promoters are a typical choice but significant drawbacks are linked with the use of these sequences.

We performed RNA-seq of suspensionadapted CHO-K1 cells, during a typical industrial bioprocess, in order to compare gene expression profiles between growth stages and select genes with high levels of expression to use their promoters for generating new expression vectors, as an alternative to viral sequences to improve production process efficiency.

Experimental approach: RNA-seq was carried out from 3 biological replicates of CHO-K1 cells, cultured in perfusion mode and with serum-free media and RNA was purified during exponential and stationary growth phases. We selected 10 transcripts with the highest levels of expression, in both culture stages, for using their promoter regions (named P1-10). For each one 1 kb was amplified by PCR and inserted into pZsGreen11 expression vector for transient screening in CHOK1 and HEK293T cells.

Results and discussion: The expression of ZsGreen protein under CMV and endogenous promoters was analyzed by flow cytometry. Out of the 10 promoters examined, 4 showed high levels of transcriptional activity. The results are highly promising since we established a rational workflow for the identification of promoters from RNA-seq, the isolation of their sequences and the generation of new expression vectors. The screening revealed that the promoter activity of P3 was greater than the other endogenous sequences. In fact, it was equivalent to CMV activity. Our results showed that P3 constitutes an alternative to the use of viral promoters, leveraging the endogenous transcriptional regulatory machinery available during mammalian cell cultures.

P-108

CRISPR/CAS9 MEDIATED KO OF MICRORNAS FOR PRECISE CELL LINE ENGINEERING

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Background and novelty: The CRISPR/Cas9 system represents a rapid and straightforward gene editing tool allowing for precise modification of host genomes. In addition, microRNAs (miRNAs) have proven to serve as versatile tools to improve production cells. These small non-coding RNAs are regulators of global gene expression. As an individual miRNA can regulate up to several hundred target genes, miRNAs might offer great potential to serve as targets for CRISPR/Cas9-mediated editing. While overexpression of miRNAs to improve the performance of biopharmaceutical production hosts has received major interest in the past years, effects of precise knockout of unfavorable miRNAs in CHO production cells have rarely been addressed.

Experimental approach: The current study aimed at applying a novel strategy to increase product yields of CHO cells using CRISPR/Cas9-mediated deletion of a miRNA causing adverse effects. In a previous miRNA screen we were able to identify miR-744 as being associated with reduced titers. Thus, the genomic precursor sequence of miR-744 was deleted by two sgRNAs flanking the miR-744 gene in the CHO genome. A plasmid encoding both required sgRNAs in a tandem array and Cas9-T2A-GFP were used to allow for simultaneous delivery of all required components, thus reducing screening effort for potential knockout (KO) cell lines.

Results and discussion: After FACS, seven putative, clonal miR-744-KO cell lines could be recovered. From these, three were identified and confirmed as miR-744-KOs by sequencing. Subsequent characterization of obtained KO clones on genetic, transcript and phenotypic level proved functionality of miR-744 KO. mAb titers were significantly improved during batch cultivation of miR-744 KO cell lines compared to a non-targeting sgRNA control. In summary, the present study elucidates the role of miRNAs, which bear adverse effects on production cells, as novel targets for CRISPR/Cas9 genome editing in the context of cell line engineering.

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EVALUATION OF AN LNCRNA DELETION SCREEN IN CHO CELLS

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Background and novelty: An innovative approach to improve productivity and growth of Chinese hamster ovary (CHO) cells includes engineering of non-coding genes. Long-non-coding RNAs (lncRNAs) are abundantly transcribed in CHO cells and are differentially regulated, implying an important role in the regulation of cellular processes. Their coding gene targets can be identified based on triplex forming sites primarily in the promoter of target genes[1].

Experimental approach: A CRISPR-AsCpf1[2] deletion screen using paired sgRNA was performed with 500 lncRNA found to be differentially expressed between different batch phases. After stable integration of AsCpf1 sgRNA pairs, the corresponding lncRNAs were deleted by introduction of the AsCpf1 enzyme (where Cas9 transfection was used as control) and the resulting cell pool was expanded in two sequential batch cultures. The sgRNA pairs present in the pool were amplified by PCR and sequenced at high depth by NGS.

Results and discussion: Firstly, the representation of the library was thoroughly evaluated in the plasmid pool, showing an over 95% representation of sgRNA pairs, but also revealing many potential caveats in preparation of such screens, including mutations in the synthetized sgRNA sequence with potential effect on activity. A comparison between the sgRNAs integrated and those present after deletion and expansion was used to identify target lncRNAs which were depleted. The underlying assumption was that lncRNAs targeted by the depleted pair were essential as cells that deleted this specific lncRNA died. For validation of identified targets, they were knocked out from the genome individually and the effect on growth and productivity analyzed. In addition, the potential target genes for triplex formation were analyzed to understand the growth reducing mechanism. Based on such deletion screens, we can learn more about the biology of lncRNAs in CHO cells, so as to be able to use them to improve phenotypes for recombinant protein production.

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INCREASING PROTEIN PRODUCTION UPON TARGETED INTEGRATION IN CHO CELLS

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Background and novelty: A conventional way to produce therapeutic proteins in CHO cells is to randomly integrate a gene encoding the recombinant protein into the genome. This approach generates a set of clones with diverse and unpredictable phenotypes, which requires tedious screening efforts in order to find a high-producing clone. In a previous study, we developed a method for targeted gene integration in CHO cells using CRISPR/Cas9 and recombinase-mediated cassette exchange (RMCE), allowing fast generation of isogenic CHO cell lines with uniform and stable phenotypes1. Although targeted integration brings speed and predictability to cell line development, it currently cannot outperform random integration in terms of protein expression levels. Therefore, we focus on increasing protein production in isogenic CHO cell lines using targeted multi-copy gene integration.

Experimental approach: To increase protein production, we designed a set of RMCE donor plasmids carrying multiple copies of a gene of interest (GOI) and different genetic elements (promoter, Kozak sequence, poly(A) tail, insulator). These plasmids were integrated by RMCE into a master cell line with a "landing pad". Generated isogenic populations were cultivated in batch, and protein titer was measured. To further increase GOI copy number, a master cell line with multiple "landing pads" were developed using CRISPR-mediated targeted gene integration.

Results and discussion: Using the comparable isogenic RMCE system we reveal the effects of different multi-copy vector elements on protein expression. Initial experiments show that increasing gene copy numbers may or may not lead to increased protein expression, depending on the gene expression cassette design. Further development of the targeted multi-copy integration approach will provide a platform to study the effects of increased protein production on CHO cells.

Acknowledgements & Funding: Novo Nordisk Foundation

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TRANSCRIPTIONAL REGULATION BY TRIPLEX MEDIATED LNCRNA INTERACTIONS

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Background and novelty: Stage-specific long non-coding RNAs (lncRNAs) regulate expression of protein coding genes by functioning as signals, decoys, guides and scaffolds for chromatin modifiers. However, the mechanism of their mode of action to gain locus specificity is not yet well established. Understanding the cross-talk of lncRNAs with other epigenetic regulators of gene expression in Chinese Hamster Ovary (CHO) cell lines can provide a wealth of information to control gene expression for more reliable therapeutic protein production. This study reveals a plausible mechanism of rapid, strong and possibly transient regulation of gene expression across a 9-day batch culture through RNA-DNA-DNA triplex mediated lncRNA interactions with protein coding genes.

Experimental approach: Triplex forming oligos in lncRNA transcripts and corresponding Triplex Target Sites (TTSs) within and around (1.5kb upstream to 1.5 kb downstream of the gene body) the coding genes were identified. Changes in expression levels of lncRNA and plausibly interacting coding genes with TTSs around the gene body were analyzed at 24-hour intervals. Localization of these TTSs was also evaluated to investigate enrichment of TTSs within different chromatin states.

Results and discussion: Almost all the pairs of differentially expressed (DE) lncRNAs and their triplex forming coding gene targets were found to have significant positive or negative correlation in their expression levels. Indications for a specific role of lncRNA-triplexes in enabling rapid and large changes in transcription of coding genes was also observed. As such TTSs were predominantly found in the promoter or enhancer region of the targeted coding gene. Further functional characterization of this interactome with CRISPR based deletions or overexpression and follow-up of changes in the expression of their triplex forming targets can provide a deeper understanding of cellular regulatory mechanisms that control behavior and phenotypes.

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USING CRISPR TO INCREASE HEK293 CAPABILITIES FOR BIOPROCESS INDUSTRY

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Background and novelty: Genomic editing through CRISPR systems has transformed research in all cell biology related fields. CRISPR can be applied to biopharmaceutical manufacturing industry for the improvement of producer cell lines. We propose a novel application of CRISPR focused on increasing HEK293 cell line capabilities for bioprocess industry. Firstly, a glutamine deficient was generated by KO of wild-type Glutamine Synthetase (GS). Otherwise, CRISPR was used to target a gene of interest into a defined chromosome location using micro-homologous End Joining in order to improve genomic context and raise up product titters

Experimental approach: Glutamine auxotrophic cell line was constructed using a single sgRNA against GS followed by single cell isolation by cell sorting. Clone 4C3 was identified through sub-culture in Glutamine-deprived media. IFNg gene coupled to GS marker in a bicistronic plasmid was then randomly transfected and productivity was tested

For targeted integration approach, an eGFP overproducer HEK293 line was developed transfecting eGFP gene. Resultant cell pools were subjected to single cell cloning through cell sorting and N1D8 cell line with improved fluorescence and single eGFP insertion in chromosome location 6q24.3 was obtained. Integration vector with IFNg gene and MMEJ homology arms was then co-transfected along with Cas9

Results and discussion: GS auxotrophic cells lines were successfully obtained using CRISPR enabling positive transfected cell selection using of glutamine free media. On the other hand, derived IFNg producing cells lines reached 2,6 higher specific productivity after MSX amplification, when compared to control cell lines

CRISPR was also successfully used to target the integration of IFNg genes into a specially developed cell line with a single insertion and great accuracy. Producer cell lines obtained exhibit a two-fold increase in specific productivity compared with control cells obtained using random integration

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A TOOL FOR COORDINATED OVEREXPRESSION OF MULTIPLE GENES IN CHO CELLS

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Background and novelty: Engineering of multiple genes in Chinese hamster ovary (CHO) cells has recently become popular. Yet, identification of beneficial combinations and the effects of the engineered genes on the phenotype is a time consuming and elaborate task. Here, we present the proof-of-concept of a new toolbox for coordinated expression of multiple transgenes in CHO cells by **m**ultiplexable **a**ctivation of **a**rtificially **r**epressed **ge**nes (MAARGE).

Experimental approach: MAARGE comprises four genes of interest (fluorescent proteins in the here shown proof-of-concept) that can all be stably integrated into the genome in a single integration event. Three genes are repressed by repressor elements (RE) that are integrated between the promoter and translation start site of the respective genes. GuideRNA (gRNA) targets flanking the REs then allow to specifically delete the RE with Cas9 and thus to activate the expression of the corresponding gene(s). Activation of the individual fluorescent proteins was evaluated by flow cytometry and microscopy. Fluorescent activated cell sorting was used to isolate cells with all possible combinations of the activated genes.

Results and discussion: The transgenes are efficiently repressed by an extended 5'-UTR including a polyA signal. Upon transfection of cells with Cas9 and the respective gRNA(s) we observe that both individual and multiplex activation of the initially repressed genes is possible and specific. By transfection of a stable cell line with all three gRNAs and subsequent sorting, it was possible to isolate cells that stably express all potential gene combinations. This tool provides a novel and fast way for coordinated overexpression of multiple genes in mammalian cells.

For cell engineering studies, the selected genes could be expressed linked to fluorescent genes and cells with the desired co-expression pattern sorted, thus obviating the necessity to subclone for a phenotypic characterization of the engineered cell pools.

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CHO GENOME MINING FOR SYNTHETIC PROMOTER DESIGN

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Background and novelty: Synthetic promoters are an attractive alternative for use in mammalian hosts as they can be designed *de novo* with user-defined functionalities. In this study, we describe and validate a method for bioprocess-directed design of synthetic promoters utilizing CHO genomic sequence information. We designed promoters with two objective features, (i) constitutive high-level recombinant gene transcription, and (ii) upregulated transcription under mild hypothermia or late-stage culture.

Experimental approach: CHO genes varying in transcriptional activity were selected based on a comparative analysis of RNA-Seq transcript levels in normal and biphasic cultures in combination with estimates of mRNA half-life from published genome scale datasets. Discrete transcription factor regulatory elements (TFREs) upstream of these genes were informatically identified and functionally screened *in vitro* to identify a subset of TFREs with the potential to support high activity recombinant gene transcription during biphasic cell culture processes. Two libraries of heterotypic synthetic promoters with varying TFRE combinations were then designed *in silico.*

Results and discussion: Synthetic promoters exhibited a maximal 2.5-fold increase in transcriptional strength over the CMV promoter after transient transfection into host CHO cells. Further, stably transfected cells utilizing a subset of synthetic promoters exhibited increased reporter production — up to 1.6-fold that of cells employing CMV, both in the presence or absence of intron A. The increased productivity of stably transfected cells harboring synthetic promoters was maintained during fed-batch culture, with or without a transition to mild hypothermia. Our data exemplify that it is important to consider both host cell and intended bioprocess contexts as design criteria in the *de novo* construction of synthetic genetic parts for mammalian cell engineering.

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KDELR1 GENE DYNAMICS AND OVER-EXPRESSION IN RECOMBINANT CHO CELLS

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Background and novelty: Over-expression of endoplasmic reticulum (ER) chaperones is a popular cell engineering approach to enhance the folding and assembly of recombinant proteins in CHO cells. However, whether CHO cells are capable of efficiently retaining ER chaperones during either exogenous overexpression or during natural upregulation during unfolded protein response (UPR) has not been investigated yet in recombinant CHO cells. These soluble ER chaperones have a C-terminus KDEL (Lys-Asp-Glu-Leu) motif which is recognized by KDEL receptors that ensures their retrieval from post ER-compartments. We hypothesize that overexpression of KDELR1 will increase the rate of retention of chaperones to the ER; therefore, increase the abundance of chaperones in ER and enhance protein folding and assembly.

Experimental approach: *Kdelr1* and KDEL chaperones gene expression during either 6-day batch culture or during ER stress induction by tunicamycin were analyzed using Real-Time-PCR (RT-PCR). The target genes were KDEL receptor 1 (*Kdelr1*), the four KDEL chaperones [Binding immunoglobulin protein (BiP), Calreticulin, endoplasmin (GRP94), and Protein disulfide isomerase (PDI A1)]. In addition, KDELR1 was stably overexpressed in recombinant IgG1 cell line, and the effect of over-expression on IgG1 productivity were investigated, by ELISA.

Results and discussion: *Kdelr1* showed less than 2 fold-increase in expression level during both batch culture and ER stress induction. On the other hand, KDEL chaperones showed several fold increase in both cases. This uncorrelated upregulation proposes a possibility of ER retention saturation or at least hindered retention of ER chaperones under stress conditions. The stable over-expression of *Kdelr1* in recombinant IgG1 CHO cell improved the productivity by 1.23 folds.

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TRANSLATIONAL ENGINEERING THROUGH THE NON-CODING GENOME IN CHO CELLS

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Background and novelty: LncRNAs are non-coding transcripts >200 nucleotides and have recently emerged as key regulators of epigenetics, splicing, microRNAs and translation. mRNA translation is a central regulatory step for cell growth, and yield and quality of recombinant proteins. Although recombinant genes are often codon optimized, we do not have all the information required around tRNA abundance, modifications and charging to fully harness codon usage in recombinant sequences. Here we present the first lncRNA and tRNA expression landscape in CHO cells under a variety of conditions and discuss the implications for recombinant protein production.

Experimental approach: A CHO-S cell line grown in (fed)-batch was sampled at day 4 and 7 of culture while six IgG1 producing CHO cell lines cultivated in an ambr15 system with different feds were sampled before inoculation, at day 4, 7 and 12. The whole transcriptomes were investigated using a mouse microarray providing the surveillance of 24,881 mRNAs and 35,923 lncRNAs for CHO-S samples and RNA-Seq for the Symphogen samples, while tRNA abundances were quantified using a previously optimized ARM-Seq protocol.

Results and discussion: Thousands of differentially expressed lncRNAs were filtered by counting the occurrences of each transcript, assessing sequence conservation, secondary structure and RT-qPCR validation. The behaviour of a group of lncRNAs is described for the first time in CHO cells and we discuss application for cell engineering. The ARM-seq protocol allowed the identification of 4-5 fold more tRNAs compared to standard sequencing. Each tRNA was quantified and compared to gene copy numbers and codon usage. Ultimately, tRNA quantifications were used in a translation elongation model to calculate the decoding speed of model recombinant proteins and to generate codon optimized versions based on tRNA abundances.

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SUBCLONABILITY ENHANCEMENT OF CHO LEADS TO A BETTER GROWTH PHENOTYPE

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Background and novelty: Generating new Chinese Hamster Ovary (CHO) production cell lines is a tedious and expensive process. One of the major bottlenecks is the poor and slow subclonability of CHO that currently is an unsurpassable bottleneck taking 2-4 weeks and yielding a low number of colonies. This can be enhanced using growth enhancing, but typically expensive additives. Here we show an easy way to enhance subclonability using subcloning by Single Cell Sorting (SCS) itself as the selection pressure.

Experimental approach: CHO cells were subjected to SCS into 50 96-well plates and the 10 largest colonies picked after 21 days, pooled to circumvent clonal variation and again subcloned by SCS. As before colonies were picked after 21 days, pooled, expanded and evaluated in a new round of SCS, where the number of microscopically and macroscopically visible colonies were counted, and a batch culture, all in comparison to the starting cell line.

Results and discussion: Subjecting three different CHO cell lines to this approach resulted in enhanced subclonability without the use of any growth enhancer. The resulting cell lines showed i) a higher number of colonies (6 to 25% more colonies in total); ii) faster outgrowth of colonies (14 days after SCS, 14 to 24% more colonies were visible with the naked eye; iii) better growth characteristics in a standard batch culture (~20% increased growth rate in two cell lines, ~ 25% higher cell density at the end of exponential phase in the third).

Thus, training cells for better subclonability by subcloning itself leads to a general improvement of the growth phenotype.

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REDUCED CLONE VARIATION OF CHO CELLS BY SINGLE TARGETED INTEGRATION

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Background and novelty: TurboCell™ is a CHO cell-based production cell system with a single targeted-integration site. For this cell system, stable expression has been shown over long-term cultivation of the Master Cell Bank (MCB) as well as of different ProducerTurboCells (PTCs). Due to the same integration loci all generated PTCs show high similarity for produced IgGs of the same subfamily. Nevertheless, based on a natural clonal variance product-dependent varieties in PTC process performance can be observed.

Experimental approach: In a quantitative proteomic approach using Tandem Mass Tag (TMT) labelling, we compared the TurboCell™ to the parental CHO-K1 host cell line and four different PTCs during routine sub-cultivation and three long-term-cultured cell lines. We identified differentially regulated host cell proteins during routine and long-term cultivation. Proteins identified by bioinformatics analysis were validated using qRT-PCR and Western Blot. Expression of these proteins was studied during progress of a fed-batch process.

Results and discussion: By TMT analysis we could identify up to 4600 proteins. Principal component analysis of these data revealed that all cell lines harbored a nearly identical expression profile except a very small number of individual proteins, emphasizing the advantage in clonality and robustness of a single targeted integration site. PCA identified 32 host cell proteins significantly differentiating the cell lines. Expression of four of these host cell proteins was confirmed by mRNA and protein analysis. Two of those proteins were chosen for studying their expression during a real industrial standard fed-batch process. It could be shown that the expression of both proteins increased during progress of the cultivation. Expression of one protein varied between the individual PTCs, whereas the expression of the other protein differed during long- and also short-term cultivation of the clones.

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COMBINED EFFECT OF C-MYC AND XBP1S ON CHO CELLS RH-EPO PRODUCER.

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Background and novelty: CHO cells are widely used in pharmaceutics industry for r-proteins production. However, there are some limitations, as the deficient use of Glc & limited secretory capacity/protein processing. It has been seen that overexpression of c-Myc (C) allows improving cell growth & Glc metabolism efficiency in CHO cell. Likewise, it has been suggested that the overexpression of xbp1s (X) has a positive effect on qp. The combined effect of C & X on CHO cells has not been evaluated.

Experimental approach: To obtain rh-EPO-CHO (CC) cell line the RMCE technique was used. Then C (pCDH-puro-Myc) & X (pCMV5-Flag-XBP1s modified), were transfected using lipofectamine. Three high producer clones were isolated (XC-3, XC-4 & XC-5). They were batch cultivated using serum free medium at 37°C, 20mM Glc in spinner flask. Then the higher qp clone & the CC clone were cultured at 37°C & 33°C; 20mM & 40mM of Glc.

Results and discussion: At 37°C & 20mM Glc, the co-transfected clones showed an increase in viable cells respect to the control. Just for XC-4 & XC-5 the specific cell growth rate increased. XC-3 clone there wasn't change. For XC-4 the qp & Qp increased. While XC-3 showed only an increased on Qp. However, XC-5 showed a decrease on qp, but there wasn't changes on Qp. There was an increase in specific consumption of glutamine, while the $Y_{amm/gln}$ decrease for XC-4 & XC-5. While for XC-3 there weren't changes in those parameters. On the other h&, $Y_{lac/glc}$ increased in all XC clones.

When the best clone, XC-4, was culture at higher Glc concentration (40mM & 37º or 33ºC), the $Y_{lac/glc}$ decreased. However, there weren't changes on qp or Qp when the cells were cultured to 33°C at any Glc concentration evaluated.

There is a positive effect of C & X on culture of CC, increasing the growth & productivity. C helps decrease $Y_{lac/glc}$ when Glc concentration increases, while X increases qp at 37 ° C. Mild hypothermia doesn't show a positive effects on qp or Qp in the clones evaluated.

P-120

FRUCTOSE METABOLISM IN HEK293: STUDY OF INTRACELLULAR FLUXES

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Background and novelty: HEK293 cells displays an unbalanced metabolism, characterized for high glycolytic fluxes in regard to tricarboxylic acid cycle (TCA) fluxes. Thus, large amounts of lactate are secreted and accumulated in the culture broth.

A simple change in the carbon source, glucose replacement by fructose, completely eliminates lactate generation and secretion.

The objective of this work is the study of the different physiologic dynamics in HEK293 batch cultures using fructose instead of glucose as a carbon source, due to possibility of elimination of lactate generation and to analyse the metabolic behaviour of this situation by means of flux balance analysis.

Experimental approach: Experiments were performed in 2L-batch bioreactor using glucose and fructose-based media to study the different metabolic behaviours.

Time profile of main metabolites concentration and oxygen up-take rate were analysed.

A reduced model from the human genome-scale metabolic model (Recon 2.2) was used to perform the FBA.

Results and discussion: Substitution fructose for glucose in HEK293 cell cultures eliminates lactate generation without a decrease in specific productivity, althought there is a slight reduction in cell growth.

The analysis of metabolic flux balances performed indicated that the lack of lactate production is a consequence of a restructuration of the core metabolic pathways (carbon metabolism), mainly glycolysis, that is highly reduced. The glycolytic fluxes decrease allows reaching a balance with the TCA-related fluxes, so NADH produced into the cytoplasm can be fully regenerated into the mitochondria. Such metabolic restructuration supresses the need of regeneration NADH into the cytoplasm and therefore no lactates is produced.

These findings could be used for rethink the bioprocessing strategies based on animal cell lines, since the main drawback (lactate generation and accumulation) is fully supress.

P-121

ANALYSIS OF TRANSLATION INITIATION IN CHINESE HAMSTER OVARY CELLS

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Background and novelty: New next generation sequencing methods are enabling new aspects of Chinese Hamster Ovary (CHO) cell biology to be precisely monitored. One such technique, ribosome footprint profiling (RiboSeq), can capture a snapshot of active translation across the genome and provide a single nucleotide resolution analysis of protein synthesis. RiboSeq can also highlight differential usage of translation initiation sites (TIS) and reveal new upstream open reading frames (uORFs). In this study, we focus on utilising RiboSeq for the study of global translation initiation in CHO cells for the first time.

Experimental approach: 16 CHOK1 cell samples were cultured at 37°C for 48 hours. Half of the replicates were kept at 37°C for extra 24 hours, whereas the other half were grown at 31°C. All samples were harvested at 72 hours. Four replicates grown at 31°C and four fully grown at 37°C were treated with harringtonine, a drug that selectively immobilizes initiating ribosomes on their template mRNA, accomplishing an effective ribosome enrichment at TIS. The control replicates were flash frozen in DMSO, a procedure that captures all translating ribosomes (both initiating and elongating). The ribosome-free segments of mRNA were degraded by using a nuclease. The ribosome protected mRNA fragments (RPF) were separated from the ribosomes, purified and converted to a deep sequencing library. The RiboSeq bioinformatics analysis pipeline encompassed the mapping of the RPF against Ensembl CHOK1 genome, the analysis of triplet periodicity and RPF length distribution, and the application of a peak calling algorithm to identify differentially enriched sites between samples at 31°C and 37°C

Results and discussion: *RiboSeq* has improved the characterization of translation in CHO cells by allowing the identification of uORF and TIS, showing great potential in the prediction of protein translation.

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ANALYSIS OF CHROMATIN ACCESSIBILITY IN CHO CELLS USING ATAC-SEQ

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Background and novelty: DNA of eukaryotic cells is tightly packaged in nucleosomes with accessibility of genomic elements such as promoters, enhancers, and repressors regulating transcription. Targeted engineering of regulatory elements can be used to modulate gene expression in order to genetically engineer Chinese hamster ovary (CHO) cells with industrially beneficial traits such as improved growth rate or productivity. The Assay for Transposase-Accessible Chromatin sequencing (ATAC-Seq) protocol enables profiling of open and closed chromatin states. We aim to identify changes in chromatin states in cells that underwent a reduction in culture temperature and correlate accessibility to differential gene expression.

Experimental approach: ATAC-Seq library preparation was performed on DNA extracted from 50,000 cells in 4 biological replicates of CHO cells cultivated at 37°C and 31°C. Following library preparation, > 50 million, 75bp paired-end reads were acquired for each sample. Reads were aligned to the CHO genome and mtDNA reads removed. Peaks were called by comparing enriched coverage to background coverage. Differentially accessible peaks were identified within the genome and correlated to regulatory elements and expression data.

Results and discussion: This study has identified differentially accessible chromatin states after a reduction in culture temperature. We have identified regulatory elements such as repressors and enhancers that correlate with significant differential expression of genes after temperature shift. We have identified a cohort of genes that exhibited on/off expression with increased accessibility of enhancer elements. We have also found evidence of differentially accessible chromatin states correlating to expression of long non-coding RNAs.

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EVALUATION OF NEW TECHNOLOGIES FOR IMPROVED CLONE SELECTION

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Background and novelty: The most fastidious step of cell line development is to ensure monoclonality of the selected cell line. Recently new devices allowing to assess cell productivity on a single cell level, sorting, imaging and dispensing in plates at the same time have been developed. We decided to evaluate side by side three different platforms: (1) the current Merck platform which combines a cell sorter and a plate imager (2) the Beacon platform from Berkeley Lights, combining cell culture on chips an epifluorescent microscope and light detection technology export cells on culture plates and (3) the Cyto-Mine Single cell analysis system that uses picodroplet technology combined with flow cytometry and microfluidics to dispense cells into plates.

Experimental approach: For this comparative experiment, we used a pool of CHOK1-SV cells expressing a mAb of interest and made it go through all the different clone selection platforms. For each workflow, approximately 30 clones were selected and upscaled, and fed-batch analyses in shake tubes or deep well plates were performed to select the top clones. The best clones from each platform were then cultivated in an AMBR 15 system to assess viability, viable cell density, productivity and quality attributes of the mAb.

Results and discussion: Compared to the current Merck platform (1), the two other workflows (2 and 3) were more straightforward and give a stronger monoclonality assessment namely because of the possibility to visualize cells during cloning experiments. The first fed-batch experiment results showed that all the clones obtained through the three different platforms were approximately at the same range of viability, viable cell density and productivity. Altogether these tests will help us modernize our current workflow mainly by reducing clone development timelines and by increasing selection robustness.

P-124

AVOIDING UNSTABLE TRANSGENE INTEGRATION IN HORIZON CHO CELL LINES

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Background and novelty: Issues related to expression instability in Chinese Hamster Ovary (CHO) cell lines remain unresolved, despite them being considered as the preferred platform for production of biotherapeutics. Knowledge of well characterized genomic loci for reliable large-scale production is of prime importance to the industry. Here, we suggest factors to consider for avoiding instability and report genome wide landing pads for favorable transgene integration into CHO cell lines.

Experimental approach: Horizon Discovery CHO-K1 (HD-BIOP3) derived biotherapeutic-expressing cell lines, sampled for 6 phenotypes with low, medium or high copy number and with stable as well as unstable transgene expression, were sequenced for genomic and transcriptomic profiles. Exact sites were identified for 22 random integration events in various cell lines and based on genomic, epigenetic and transcriptomic patterns around the site, factors differentiating low copy number stable phenotype from medium or high copy number unstable phenotypes were identified. Transcriptomic differences were also checked to detect genes that are upregulated in favorable or unfavorable phenotypes.

Results and discussion: Analysis of rearrangements in transgene integration along-with genomic and transcriptomic characterization of the integration site could suggest that expression stability can be controlled at 3 levels: 1) presence of an active chromatin state, low genomic variability and high transcription level flanking around the integration site, 2) organization of the transgenic locus, with low transgene fusions and no structural variation upon integration, and 3) avoiding enrichment of stress related cellular processes resulting in apoptosis which can thereby cause upregulation of ECM regulators and cell clumping due to release of DNA following cell death. Genome wide favorable and unfavorable genomic loci have also been deduced based on the identified criteria to allow reliable transgene expression.

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RNAI BASED SELECTION TOOLS FOR CHO CELL LINE DEVELOPMENT

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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 have to designate a considerable part of their protein synthesis machinery to the translation of the antibiotic resistance [1] and the selectivity of the system is based on the translation of the resistance gene, not on the product gene. We're developing various tools for cell line selection that work on the transcriptional level, thus avoiding burdening the translational resources of producer cells by selection markers.

Experimental approach: The tools under development utilize RNAi to select for cells that transcribe the product gene at high levels. siRNAs that are spliced out from the product gene's mRNA protect the cells from a suicide gene which is transiently transfected as a mRNA and which causes non-protected cells to die rapidly. Several potential killer genes and protective siRNAs were tested for their killing potential and specificity.

Results and discussion: The combination of thymidine kinase (TK) and the pro-drug ganciclovir showed promising results in transient experiments. Cells expressing the GoI and splicing out a siRNA targeting the TK, showed increased growth rates and viability. Non-producing cells were killed by the transfection of TK mRNA, as they lacked siRNAs to inhibit the translation of TK. The development, optimization and comparison of stable selection tools is currently in progress. 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 subsequent steps.

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P-126

EXPEDITING UPSTREAM STAGES OF PROTEIN BIOMANUFACTURER WITH 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®) possess a dominant chromatin opening capability and thus confer stable transgene expression. UCOE®-viral promoter (e.g. CMV) based plasmid vectors markedly 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 thoroughly evaluated in Chinese hamster ovary (CHO) cells. Further to this, improvements in plasmid vector design through the addition of transcription termination elements and introns may improve the efficiency of transcription and thus increase levels of protein production.

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. Experiments were replicated with different transcription termination elements. After vector optimisation, the GFP reporter gene was replaced with the Enbrel antibody gene and Fed-batch analyses were conducted.

Results and discussion: The results at 21 days post-transfection clearly indicate that the Rps3 UCOE®-gpCMV and -hCMV combinations give the highest transgene expression. The SFFV promoter linked with either of the two UCOEs® was the least effective with expression levels 17-fold lower. The Rps3 UCOE®-hCMV construct has been further modified to include transcription termination elements, both Pause-type and Co-transcriptional -type elements. Results indicate that the Pause-type terminator improves levels of gene expression. This is being tested to see if the results gathered from the GFP experiments are reciprocated with protein expression.

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DISPENCELL, A NEW SOLUTION FOR ISOLATION OF SINGLE CELLS

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Background and novelty: Development of manufacturing cell lines is a tightly controlled field where one of the most challenging steps is to deliver proof of clonality to ensure consistent supply and quality of the protein of interest.

Historically, the first method used was limiting dilution based on Poisson's law. However, this is very time consuming and inefficient. With growing technology development, devices such as single cell sorters, printers or microfluidics systems (cell traps, oil droplet) allowed to increase throughput and efficiency. All these methods have their advantages and drawbacks in terms of ease to use, rapidity, efficiency and impact on cell health.

Here, we tested a new solution for gentle and traceable single cell isolation called DispenCell. The solution consists of an impedance-based pipetting robot for automatic single cell dispensing, a disposable sensing tip to prevent contamination, and a laboratory information management system (LIMS) for post-processing single cell quality control.

Experimental approach: FACS (reference method of the platform) and Dispencell sorting were compared in a two days-study. Two different CHOK1 pools were sorted into proprietary cloning media in 6 plates for each technique. Plates were then imaged 4 hours post-sorting and number of cells/well were counted. A two-week follow-up picture allowed to calculate the number of wells recovered. In addition, ease of use, as well as handling time for each technique, were documented.

Results and discussion: Pictures from one FACS plate and one DispenCell plate were visually inspected at day 0, and day 12. Regarding plating efficiency and recovery, the two methods gave similar results. For the DispenCell sorted plate, impedance-based and image-based cell identification correlated well (89% similarity), making the impedance signal a robust way to assess monoclonality. Further performance improvements will enable the DispenCell to become a valuable technology for cell plating

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MATHEMATICAL OPTIMIZATION OF A CHO CELL GENOME-SCALE METABOLIC MODEL

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Background and novelty: Chinese hamster ovary cells (CHO) are the dominating expression platform for recombinant glycoproteins, however, the complex production framework in combination with the trade-off between growth and recombinant protein production makes the process expensive. We propose a mathematical approach to combat this issue by viewing the metabolic network of the cell as a linear programming optimization problem. The optimization method, OptForce[1], aims in detecting the genes the knock-out or overexpression of which will result in a higher yield for our product of interest. It acts upon the genome-scale model of the CHO cell [2], comprised of the already annotated genes, allowing a holistic approach with more options i.e. genes, to perform a genetic intervention.

Experimental approach: Two optimization problems are solved; one for the growth and one for the stationary phase of the culture, where the main differences are the cellular uptake and the objective function of the cell. The permissible upper and lower bounds for all intracellular reactions are first curated using a recently developed algorithm by the Kiparissides lab, named carbon constrained FBA (ccFBA), augmented with additional nitrogen based constraints.

Results and discussion: The ccFBA algorithm resulted in stricter bounds for the reactions leading to a significant reduction of the solution space. The optimization algorithm suggested genetic interventions to improve the yield of the antibody while maintaining a viable growth rate for both phases. The genes' interventions for the two models are then compared and concluded to a final result. In the future we will apply thermodynamic bounds to fix the directionality of the intracellular reactions, improving the fidelity of the results.

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P-129

ELIMINATION OF THE WARBURG EFFECT IN CHINESE HAMSTER OVARY CELLS

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Background and novelty: Toxic bioproducts such as lactate have posed considerable challenges in bioprocessing since they limit growth and impact critical quality attributes by altering the regulation of biosynthetic enzymes. To mitigate the negative effects of lactate accumulation and control the culture pH, base is added to the media during the course of a bioprocess. However, base addition increases osmolarity, which also negatively impacts the bioprocess by inhibiting growth and shortening the time in which the cells can produce the recombinant protein. We report the first elimination of lactate production in CHO cells via genetic engineering.

Experimental approach: Gene knockout was via CRISPR/Cas9 and verified at multiple levels. The knockouts were introduced into parental and biotherapeutic producing CHO cell lines. Parental lines were further used to generate additional biotherapeutic producing cell lines following a typical industrial workflow.

Batch and fedbatch were carried out for both producing and nonproducing cell lines to characterize the phenotypic impact of the knockouts on important cellular attributes.

Results and discussion: We eliminate the Warburg effect in a CHO cell line by using CRISPR/Cas9-based engineering to simultaneously knockout enzymes responsible for lactate production and ancillary regulators. The resulting cells remain proliferative while consuming significantly less glucose without requiring additional oxygen, and can be used to generate protein producing lines using standard industrial processes. In a pH-controlled fedbatch process, the Warburg null cells require minimal base addition to maintain a stable pH, allowing an extended growth phase. The knockout strategy was also applied to a CHO cell line producing Rituximab, again resulting in a prolonged growth phase. Additionally, protein production was maintained or improved. Thus, Warburg null CHO cells may be useful for engineering production cell lines with enhanced bioproduction traits.

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ENGINEERING OF THE SECRETORY PATHWAY OF CHINESE HAMSTER OVARY CELLS

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Background and novelty: The secretory pathway, process necessary for the export of recombinant proteins into the external environment, is thought to be a limiting step in cells for the production of recombinant proteins. In this study, the secretory pathway of the Chinese hamster ovary (CHO) cells, a widely used expression system, has been manipulated to investigate whether its engineering can improve the secretory capacity of two different CHO host cell lines under batch and fed-batch conditions.

Experimental approach: Two cell lines were engineered to ectopically over-express members of the SNARE family, a family of proteins involved in the fusion machinery of vesicles, and the effect(s) of the overexpression on growth, culture viability and recombinant protein productivity of the CHO hosts determined

Results and discussion: In a CHO-S cell line, overexpression of specific levels of SNAREs increased culture longevity and late culture viability, possibly through an impact on the autophagy pathway, and during transient expression of model proteins under batch culture conditions, IgG1 Adalimumab and the fusion protein Etanercept, showed an increase in titre. Effects were only observed with specific levels of SNARE expression. Transferability of this approach to a second CHO host, an industrial host CHO-DG44 derived cell line, was then determined. No effect was observed in the CHO-DG44 cell line engineered with the different SNARE proteins. Differences in the approaches and environment, as well as the intrinsic differences in the CHO host cell lines, might explain the divergence of observed effects between the cell lines. Nonetheless, a positive impact upon the overexpression of target SNAREs at specific levels was observed with regard to growth and productivity in CHO-S host cells suggesting SNARE manipulation was successful for the engineering of CHO-S cells.

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MINIMIZING CLONAL VARIATION DURING CHO CELL LINE ENGINEERING

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Background and novelty: Current methods for generating engineered CHO cell lines are based on random integration, which introduces high genomic and phenotypic diversity. The large variation between randomly engineered clones hampers studies of transgene effects, gene functions and discovery of novel cellular mechanisms. In this study we present a cell line development platform based on site-specific targeted gene integration, where clonal variation is minimized enabling robust comparative studies of genome engineered CHO cells.

Experimental approach: We minimized clonal variation using a two-staged approach to develop isogenic CHO cell lines. As a first stage we created master cell lines by integrating a landing pad for recombinase-mediated cassette exchange (RMCE) site-specifically into a pre-defined stable locus of the CHO genome using CRISPR/Cas9. To show that we can use the master cell lines to generate highly comparable cell lines, we generated twelve RMCE derived subclones expressing four different recombinant proteins. These cell lines were cultivated in controlled environmental culture conditions and analysed in terms of growth, stability, transgene transcript levels and global transcriptional response.

Results and discussion: The results show an extraordinary comparability of the twelve subclones: They all display high consistency in growth, transcript levels and global transcriptional response to recombinant protein expression, enabling improved studies of the impact of transgenes on the host transcriptome. We further observe high consistency in subclone phenotypes and transcriptomes over three months in culture. The platform facilitates robust comparative studies such as differential gene expression analysis of genome engineered CHO cell lines. Altogether, this cell line development platform has huge potential to accelerate the discovery and verification process of potential cell line engineering targets.

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ENHANCEMENT BY REDUCTION – PUSHING N-GLYCOSYLATION IN CHO CELLS

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Background and novelty: The ability to produce human like N-glycosylation patterns has greatly contributed to the success story of CHO cells. Nevertheless, various reports show that N-glycan maturation is decreased when the recombinant protein load is high, a problem of increasing relevance as the boundaries of productivity of CHO cells are being pushed further and further. In this project we aim to identify the dominant key player enzymes in N-glycosylation for various model proteins and push the most relevant reactions.

Experimental approach: Out of the four galactosyltransferases involved in N-glycosylation, B4GALT1 is the dominant isoform, but the contributions of the other isoenzymes have not been fully evaluated. Therefore, we first studied the activity of each individually by removing the respective other three isoenzymes by CRISPR/Cas9 and a paired sgRNA approach. Three different glycoproteins were produced transiently and analyzed by mass spectrometry at the glycopeptid level. Furthermore we established a short interval mRNA transfection protocol, with which high productivities can be achieved that correlate to a drop in N-glycan processing.

Results and discussion: The results show that the contributions of B4GALT2 and B4GALT3 vary from model to model and that the enzymes only yield low levels of galactosylation when acting alone. B4GALT4 hardly produced any galactosylated N-glycans. To further enhance N-glycosylation B4GALT1 and ST3GAL4 will be overexpressed in a cell line with a "cleaned-up" Golgi, referring to the lack of the other galactosyl- and sialyltransferases. The superiority of this system will be assessed with the mRNA transfection set-up, which allow the detection of bottlenecks in galactosylation and sialylation as well as branching.

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LACTATE REDUCTION IN CHO CELL CULTURES THROUGH THE METABOLIC ANALYSIS

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Background and novelty: Chinese Hamster Ovary cells (CHO) display Warburg metabolism characterized by high glucose consumption and high lactate production under aerobic conditions. Lactate is a by-product widely reported to inhibit cell growth in culture, imposing an important burden to industrial processes. In order to reduce lactate secretion and thus its inhibitory effects, two different approaches have been applied: i) Bioprocess Engineering, controlling extracellular conditions in the bioreactor to trigger concomitant glucose/lactate consumption, and ii) Synthetic Biology, to generate non-lactate producer mutant cells knocking out lactate dehydrogenase and the regulators responsible for inhibiting pyruvate conversion to acetyl-CoA.

Experimental approach: The metabolic profile of CHO cells was investigated in batch bioreactor cultures performed under three conditions: normal WT, controlled WT, and zero-lactate CHO (CHO ZeLa). Exometabolomic data was integrated into a reduced genome-scale metabolic model using Dynamic Flux Balance Analysis (DFBA) to capture the dynamic changes occurring over time in CHO cell metabolism. Furthermore, labelling experiments with ^{13}C-glucose were performed in order to reduce the degrees of freedom of the model (i.e. NADPH generated by PPP versus cytosolic malic enzyme).

Results and discussion: FBA showed that in wild-type cell line, lactate is produced to fulfill the NADH regeneration requirements in the cytoplasm and only a small amount of pyruvate is introduced into TCA through Acetyl-CoA. When concomitant glucose and lactate consumption was triggered, as well as in CHO ZeLa, glucose uptake was significantly reduced and a balance between glycolysis and TCA cycle fluxes was reached, yielding a more efficient substrate consumption. Moreover, DFBA illuminated the metabolic mechanisms by which wild-type CHO switches from a Warburg (glucose consumption/lactate production) phenotype to a glucose/lactate co-consumption phenotype.

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CELL LINE IMPACT ON ANTIGEN BINDING OF A THERAPEUTIC MAB

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Background and novelty: Monoclonal antibodies (mAbs) constitute a large and growing subset of the marketed-biotherapeutics glycoproteins. Although mAbs are bifunctional molecules since variable (V) and constant (C) regions are considered independent domains, and that glycosylation has no effect on antigen binding, there are several studies suggesting the influence of C regions of different mAbs with identical V regions on the antigen binding activity.

We developed a novel humanized single-chain variable fragment (scFv) against human interferon-α2b (hIFN-α2b) fused to the human Fcγ1 region (hz.scFvFc). The engineered antibody was subject to a UNL-CONICET patent application to be used as a therapeutic candidate for autoimmune diseases. In this work, we study the impact of the producer cell line on the affinity constant and antigen-neutralizing ability of this novel molecule.

Experimental approach: The hz.scFv-Fc antibody was produced in CHO-K1, HEK293 and NS0 cells. Affinity constants were measured by competitive ELISA and antigen-neutralizing ability was evaluated by three independent bioassays. N-glycosylation structures were analyzed by hydrophilic chromatography.

Results and discussion: Results showed significant differences both in affinity and antigen-neutralization ability between these molecules, where hz.scFv-Fc produced in HEK293 cells presented the lowest affinity constant and antigen-neutralizing ability. After analyzing the N-glycosylation pattern, we found that both, the percentage of occupied N-glycan sites and the structure of the main oligosaccharides differ depending on the host cells. These results evidence that the producer cell line influences the mAb affinity and neutralizing action, leading to the conclusion that the host cells should be carefully taken into account in order to develop a new therapeutic antibody.

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EFFECTS OF MITOCHONDRIA RELATED GENES PGC1A AND DRP1 IN CHO METABOLISM

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Background and novelty: Biopharmaceutical products have become an important asset for pharmaceutical industry during the last decades. Mammalian cell lines are the preferred choice for large scale production, with CHO cells alone representing 70% of recombinant protein production for biopharmaceutical purposes.

This study aims to improve cell metabolism, and therefore recombinant protein yield, of CHO cells by targeting mitochondria. PGC1α and DRP1, genes related to mitochondria biogenesis and mitochondria fission process respectively, have been shown to cause increase on mitochondrial numbers when overexpressed in cancer cells, enhancing oxidative metabolism. Overexpression of ectopic PGC1α and DRP1 will be performed on CHO cells to enhance their oxidative metabolism, and therefore their protein production.

Experimental approach: CHO-K1 cells (EPO producers) transfected with the genes of interest a subjected to a clone selection process to create stable CHO-K1 clones overexpressing PGC1α, DRP1 or both. They were then assessed for gene and protein expression, relative mitochondrial content and recombinant protein yield.

Results and discussion: Differences in cell growth and mitochondrial content between transiently transfected cells expressing PGC1α or DRP1 were observed, with the overexpression of these genes leading to an increase on cell growth after 96 hours without significant differences in cell viability. Interestingly mitochondrial content was not affected.

These results represent a promising first step, as they suggest the overexpression of mitochondria-related genes can increase cell growth without compromising the viability of the cells, which constitutes a desirable trait in recombinant protein producer cells. Results from the generation of stable CHO-K1-EPO-PGC1α, CHO-K1-EPO-DRP1 and CHO-K1-EPO-PGC1α-DRP1 cells will also be presented.

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RECOMBINANT HUMAN BMP-4 PRODUCTION IN BMP RECEPTOR KNOCKOUT CHO CELLS

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Background and novelty: Bone morphogenetic protein-4 (BMP-4) has been studied as a potential therapeutic agent for long bone fractures, cartilage regeneration, and osteoarthritis. A recombinant CHO cell line producing hBMP-4, which express essential components of BMP signal transduction, is capable of autocrine BMP-4 signaling and hence has the unexpected cellular functions. We suggested that rhBMP-4 induced signaling in CHO cells can be a critical factor in limiting rhBMP-4 production and should be eliminated to improve rhBMP-4 production in recombinant CHO (rCHO) cells.

Experimental approach: The treatment of a selective inhibitor of the BMP type I receptors, LDN-193189, increased the mRNA expression and production of rhBMP-4 in rCHO cells. To establish BMP signaling-free host cells, we knocked out a BMP receptor, the *BMPRIA or BMPRII* gene in DG44 cells using CRISPR/Cas9 gene-editing technology. Using three different knockout (KO) host cell lines to avoid clonal variation, as well as a DG44 wild-type (wt) host cell line, we established rCHO cell clones producing rhBMP-4 by a stepwise selection with increasing methotrexate concentrations.

Results and discussion: KO-derived clones showed a significantly higher rhBMP-4 concentration than wt-derived clones in both batch and fed-batch cultures. Unlike wt-derived clones, KO-derived cell clones were able to express higher quantities of *hBMP-4* transcripts and proteins in the stationary phase of growth and did not experience growth inhibition. Both improved cell growth and increased levels of rhBMP-4 mRNA contributed to significant improvement of the maximum rhBMP-4 concentrations in the fed-batch cultures of KO-derived clones. Taken together, BMP signaling disruption in CHO cells by knocking out the BMP receptor significantly improved rhBMP-4 production.

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ENHANCING THE GALACTOSYLATION CAPACITY OF CHO CELLS

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Background and novelty: N-linked galactosylation increases the therapeutic efficacy of oncological mAbs[1]. The work presented herein has developed a facile and rapid cell engineering strategy that maximises mAb N-galactosylation while avoiding cell culture supplementation strategies that have been reported to negatively impact cell growth and product yield[2]. Our strategy is based on (i) knocking out the COSMC gene to eliminate cellular O-galactosylation and (ii) on overexpressing 4GalT, the enzyme that catalyses N-galactosylation.

Experimental approach: The COSMC gene was knocked out of mAb-producing CHO cells using CRISPR/Cas9 gene editing coupled with Cas9-GFP fluorescent enrichment[3]. Cells were also engineered to express human 4GalT1. Cell line characterisation was performed in 30mL shake flasks operated in batch mode, and data for viable and dead cell density, nutrient and metabolite concentrations, product titre, intracellular UDP-Gal concentration[4], cell surface N-galactosylation (lectin-aided flow cytometry) and product N- glycosylation was collected.

Results and discussion: Only moderate differences in cell culture dynamics were observed for all engineered cell lines. The $COSMC^-$ cells presented a shift towards higher cell surface and mAb N-galactosylation. The $COSMC^-$ cells also presented increased intracellular pools of UDP-Gal, thus confirming our initial hypothesis surrounding the redistribution of this nucleotide sugar from O-linked to N-linked galactosylation[5]. The greatest change in cellular and mAb N-galactosylation was observed upon 4GalT1 overexpression, regardless of COSMC expression. The $COSMC^-/GalT^+$ transfectants achieved similar levels of mAb and cellular N-galactosylation as the $GalT^+$ ones, indicating that the process is mainly controlled by enzyme availability in our cell lines. This work demonstrates that the distinct relationship between cellular and product glycosylation can be exploited to yield mAbs with enhanced therapeutic efficacy.

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MRNA TRANSFECTION OF CHO-K1 CELLS REVEALS PRODUCTION BOTTLENECKS

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Background and novelty: During development of highly efficient stable production cell lines, bottlenecks in post-translational modifications of the therapeutic protein arise frequently. These can be masked by clonal variation, obscuring a direct connection to specific productivity. Evaluating cell lines for their ability to produce a specific product is difficult, as transient transfection protocols do not attain the specific productivities of stable producer cell lines. Here, we show specific productivities approaching those of industrial high productivity clones by direct transfection of product gene-mRNA.

Experimental approach: mRNA was in-vitro transcribed by a T7-promoter protocol, m^7G-capped, poly-adenylated, and highly concentrated. Cell-specific uptake of mRNA was measured by reverse transcription and qPCR, cell-specific productivity by flow cytometry for eGFP and by biolayer interferometry for EpoFC.

Results and discussion: For non-secreted eGFP, a saturation in fluorescence was observed, even though mRNA-uptake was not saturated. For secreted EpoFc the molar uptake of the larger mRNA was lower, and no saturation in specific productivity was reached. The maximum specific qP attained for EpoFc was 18pg/c/d, which is in the lower range of standard industrial productivities. A clear correlation of increasing productivitiy and decreased glycan quality was observed, mainly with respect to the degree of terminal sialylation and antennarity. This method allows for the rapid elucidation of potential bottlenecks, independent of clonal variation, which would otherwise become visibly only in late stages of CLD. As the method can easily be adapted to different products, and can be performed with several parental cell lines in parallel, it can serve multiple purposes: assessing the inherent ability of possible parental candidates to handle the specific product, elucidating bottlenecks to assist in choosing a metabolic engineering strategy for host cell line optimization.

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CELL LINE DEVELOPMENT ¬- FROM EMPIRICAL DEVELOPMENT TO RATIONAL DESIGN

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Background and novelty: Cell lines suitable for therapeutic protein production require excellent growth, stability and productivity characteristics. The development of such cell lines has been empirical thus time-consuming and resource-intensive. More importantly, there is little fundamental understanding of cell line behavior in a given bioprocess environment as such manipulating them to obtain desirable cell line performance is a huge challenge. The powerful 'omics' technologies have opened new avenues towards host cell development, identification of biologically important genes and pathways associated with cell growth, cellular metabolism and productivity.

Experimental approach: In this study, we first applied the whole-genome sequencing and transcriptome techniques for the identification of chromosome loci that support high and stable monoclonal antibody expression. We then applied CRISPR technology to engineer two independent landing pads, each to a well-characterized locus in a CHOK1 host cell line.

Results and discussion: The resulting host (namely dual landing pad host, dLP) allows the integration of transgene(s) to one, or both loci via Recombination-mediated cassette exchange in an efficient and user-controlled manner. Furthermore, recombinant cell lines derived from the dLP host gave arise to predictable performance in productivity and stability. We applied transcriptome and metabolomics analysis to study the behavior of recombinant cell lines derived from the dLP host. We have identified pathways deregulated in some cell lines that exhibited phenotype drift after long term culture. We revealed molecular targets that may be useful for early detection of such cell lines in cell line development and management of the cell line behavior through tailored bioprocess condition.

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SMALL RNA TOOLBOX PROMOTES ADVANCED CHO CELL ENGINEERING

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Background and novelty: Due to increasing complexity of therapeutic molecules, some next generation biologics can be difficult-to-express and thus challenge cell line and bioprocess development. At the same time the secretory pathway still remains a bottleneck in cellular productivity. To overcome potential intracellular limitations especially in terms of more complex pipeline drugs, we applied different cell engineering strategies, including microRNAs (miRNAs), a mitochondrial genome-encoded small RNA (mitosRNA) and short hairpin RNAs (shRNAs).

Experimental approach: With the goal of improving productivity, CHO host cells were engineered by the introduction of miRNA-557 or mitosRNA-1978 followed by cell line development campaigns with different recombinant products. An alternative approach comprised super-transfection of an existing manufacturing clone with mitosRNA-1978 in combination with directed modulations of the cell culture process. Furthermore, we applied next generation sequencing to identify mitosRNA-1978 target genes and generated respective shRNA-mediated knockdown cells. Performance and product quality attributes of all engineered cells were analyzed in fed-batch cultures.

Results and discussion: Introduction of pro-productive small RNAs both into host cells and into manufacturing clones proved to be effective to enhance product yield without compromising product quality. Expression of miRNA-557 substantially improved product titer and cell density [1], whereas mitosRNA-1978 engineered cells revealed a vast increase in specific productivity, albeit accompanied by reduced growth characteristics. Co-engineering of two mitosRNA-1978 target genes involved in the secretory pathway resulted in increased productivity and enhanced cell growth [2]. Notably, in order to leverage the gain in specific productivity and yield maximal product titer, we applied mitosRNA-1978 in combination with directed process adaptions, ultimately improving final product concentration [3].

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NOVEL PROMOTERS DERIVED FROM CHO VIA IN SILICO AND IN VITRO ANALYSIS

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Background and novelty: As for recombinant protein production in mammalian cell lines a high rate of gene expression is desired, strong viral promoters are commonly used. However, these have several drawbacks in that they override cellular responses, are not integrated into the cellular network and thus induce stress and potentially epigenetic silencing. Endogenous promoters might have the advantage of better response to cellular signaling networks and thus cause a lower level of stress by uncontrolled overexpression of the transgene.

Experimental approach: Here we describe the identification of promoters and their enhancers from CHO cells based on histone marks extracted from the CHO-epigenome database. Potential promoter candidates were selected based on the top expression genes and tested for normalized promoter strength in comparison to viral promoters using a dual luciferase kit. Additional in silico tools were applied for promoter prediction. Successive truncation of the best promoters at the 5' and 3'-site of the initial fragments further enhanced promoter activity. Performance in stable recombinant cell lines was tested at a targeted integration site for better comparability using RMCE.

Results and discussion: Several promoters also included promoters of lncRNAs and cold inducible promoters expressed at levels comparable to or better than the CMV promoter plus enhancer in transient transfection. Different enhancers were cloned upstream of endogenous and viral promoters and observed to further enhance promoter activity up to 3 fold. Endogenous promoters seem to be more stable than viral promoters in long-term culture. Despite the high diversity of mammalian promoters, common features of all selected promoters include a high GC content, putative TFBSs and high H3K27ac marks. Our study has found a panel of endogenous promoters from low to high promoter activity that can be used both for protein production and cell engineering.

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TURBOCELL – A FAST WAY TO STABLE PRODUCTION CELL LINES

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Background and novelty: One of the most important criteria for the successful production of biopharmaceuticals is the availability of a stable production cell line expressing the target product in a suitable quantity and quality. In general, the generation of a suitable production cell clone by the traditional random gene integration is a costly process and requires a time-consuming clone stability study.

Experimental approach: Targeted integration is an innovative, fast tool in cell line development. Based on CHO-K1 cells, Rentschler Biopharma developed its Master TurboCell™ for a single copy site-directed integration of genes of interest (GoI) into a stable, pre-tagged and characterized hotspot of the CHO-K1 genome by recombinase-mediated cassette exchange (RMCE). Due to the integration of GoI into a known locus, the resulting clones show low variations concerning genetic stability, growth and protein quality characteristics, which minimalizes the total screening effort. Using the TurboCell™ line, producer clones with different target molecules can be developed in parallel within 10 weeks from DNA to the final RCBs.

Results and discussion: Stability studies showed reproducible and stable producer clone performance confirmed by the mRNA level, the productivity, the cell growth and the protein quality. Based on the stable growth and stable GFP expression of the Master TurboCell™ demonstrated for more than 120 generations, the producer TurboCell™ clones have a high inherent genetic stability, eliminating the need for extensive clone stability studies prior to GMP cell banking.

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FIND CHO-CELLS OF CHOICE: SELECTIVE CLONING IN CELL LINE DEVELOPMENT

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Background and novelty: One of the greatest challenges faced during Cell Line Development is being able to efficiently screen large cell populations for productivity & isolate high producers of interest, while ensuring monoclonality. Current methods create a bottleneck in the development of novel biotherapeutic drugs as many devices are required to isolate single cells, analyze, sort, image & dispense those 'hit' cells – resulting in resource-intensive, time-consuming & expensive workflows. This study demonstrates how Cyto-Mine® provides a robust & efficient solution for high-throughput selective screening of single CHO-cells.

Experimental approach: A large population of proprietary CHO cells, stably expressing human IgG was mixed with Cyto-Mine® human IgG-specific animal origin free detection reagent. The Cyto-Mine® (Sphere Fluidics) platform was used to encapsulate single cells into picodroplets, where they were incubated for 2 hours. The cells were analysed using Cyto-Mine® IgG secretion assay & high producers selected. Prior to dispensing the high producing cells were imaged for clonality & measured again for productivity. The data was analysed using Cyto-Mine® Software Studio Suite (Sphere Fluidics).

Results and discussion: Here we present data on the simultaneous method for the identification of high producing clones & monoclonality verification. Cyto-Mine® was used to selectively clone single CHO cells based on their antibody secretion rate. Cyto-Mine® was able to identify high producing cells from a large (approx. 100,000) starting population. The fully integrated system streamlines the cell development workflow & significantly improves the time from host cell transfection to cell bank generation. This powerful technology is poised to revolutionize cell line development with the integration of high-throughput selective screening, cell isolation & provide assurance of monoclonality in one platform.

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GENOMIC FINGERPRINTING OF CHO MANUFACTURING CLONES BY TARGETED NGS

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Background and novelty: Genetically modified CHO cell lines are used for the production of biopharmaceuticals. However, an in-depth molecular understanding of the mechanism of transgene integration into the CHO host cell genome and its influence on performance and productivity is largely missing. Next Generation Sequencing (NGS) holds great promise for strongly facilitating the understanding of CHO cell factories, as it has matured to a powerful and affordable technology for genotype analysis. The NGS technique Targeted Locus Amplification (TLA; de Vree et al. 2014) allows for robust detection of transgene integration sites, transgene integrity and structural genomic changes occurring upon integration.

Experimental approach: TLA was used to generate comparative genomic fingerprints by transgene integration site mapping of CHO production clones according to the method described by de Vree et al. Bioinformatics analysis was performed in-house. Results were corroborated using Fluorescence in situ Hybridization (FISH) and linked to upstream cultivation data, gene expression profiles generated by RNAseq and copy number measurements by digital droplet PCR (ddPCR).

Results and discussion: TLA analysis was performed for several high producing CHO cell clones, which were developed using different transfection strategies and selection markers. Transgene integration sites were precisely identified and found to co-localize with large genomic rearrangements and deletions affecting the copy numbers of protein-coding genes. Transgene integrity was validated with regard to nucleotide variants and transgene-transgene fusions within concatemers. The combination with upstream, RNAseq and ddPCR data allowed for a deeper insight into the mechanisms of transgene integration and its correlation with productivity. Taken together, TLA provides substantially more accurate genetic characterization compared to conventional methods and can be applied to several stages of bioprocess development.

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REGULATING EXPRESSION DURING SELECTION ENHANCES CHO POOL PRODUCTIVITY

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Background and novelty: Isolation of high-producing cell lines from a heterogeneous pool population represents a bottleneck in the process of manufacturing a novel biologic and requires an extensive screening of multiple hundreds of clones. This is in part due to the low integration frequency of the expression cassette into transcriptionally active regions of the host genome. Moreover, clones expressing high-level of the recombinant protein (r-protein) may experience metabolic burden and endoplasmic reticulum (ER)-associated stress and are likely to fail surviving the stringent selection process. Using an inducible expression system may alleviate some of these limitation and may result in improved pools with higher productivity.

Experimental approach: CHO cell are transfected with a cumate-inducible expression plasmid using polyethylenimine and pools selected in the presence of MSX. After two weeks of selection, pools are expanded and induced to produce r-proteins in the presence of cumate.

Results and discussion: Using the cumate-gene switch, we generated inducible CHO$^{BRI/rcTA}$ pools with volumetric productivity of up to 2 g/L achieved for a bispecific Fc fusion protein after 14 days post-induction in a platform fed-batch culture process. Reducing expression level ("off-state") during pool selection process dramatically enhances frequency of high-producers compared to a pool in which expression was induced to high level ("on-state"), mimicking a constitutive expression system. Moreover, following induction, overexpression of the r-protein during the pool selection process negatively affects pool recovery and ostensibly amplifies ER-stress and cell death compared to pool selection in the "off-state". Our data strongly suggests that using inducible expression system is a valuable approach for stable pools and clones generation.

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GENOMIC UNDERSTANDING OF VARIATION IN TRANSGENE EXPRESSION IN CHO

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Background and novelty: Recombinant CHO (rCHO) cell line generation is a critical step in the current manufacturing paradigm for large-scale production of therapeutic proteins. However, the high degree of phenotypic heterogeneity makes the rCHO cell line development process laborious and time-consuming. In this study, we investigated the major genomic causes of this phenomenon, referred to as *clonal variation,* particularly in differential transgene expression using targeted genome editing.

Experimental approach: We investigated clonal variation with emphasis on transgene integration sites and configuration of integrated transgenes. Specifically, we focused on how it impacts protein production and phenotypic responses to hypothermia. We constructed GFP expressing rCHO cell clones using random transgene integration. Application of targeted genome sequencing allowed a mapping of integrated transgenes, while identifying their genomic integration sites in representative rCHO cell clones showing different responses to hypothermia. CRISPR/Cas9 was applied to assess both transgene integration sites and transgene regulatory elements, partiicularly promoter regions.

Results and discussion: We found: (1) altered DNA sequence of randomly integrated cassettes, which occurred during the integration process, affecting the transgene expression level; (2) contrary to random integration, targeted integration of the same expression cassette, without any DNA alteration, into identified integration sites showed the similar transgene expression patterns, irrespective of integration site; (3) transgene expression pattern was affected by promoter and its elements. Thus, we have revealed the effects of integration methods and cassette design on transgene expression levels, which offers new insight to the cell line development to minimize clonal variation.

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TRACEABLE SINGLE CELL CLONING USING A NEW PIPETTING ROBOT

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Background and novelty: To ensure drug homogeneity, biotech companies should demonstrate that each new cell line has been cloned from a single progenitor cell. Because methods do not provide fully traceable cells as yet, companies may waste up to 50 weeks in validation. Here, we tested DispenCell, a new impedance-based pipetting robot allowing for traceable and gentle cloning of single cells. It is used with a disposable tip to avoid contamination and a software for quality control.

Experimental approach: First, we tested the capability of DispenCell to dispense single GFP-expressing CHO cells. Then, CHO cells secreting a therapeutic antibody were cloned using DispenCell as well as using limiting dilution as reference. Fifteen growing clones from each group were then seeded at equal density and further expanded in culture. Each clone was characterized in term of cell density, viability and protein titer.

Results and discussion: The quality control of the 192 impedance traces revealed that 68% of the wells matched the one peak signature corresponding to the signal of a single cell. Each positive well was then examined under the fluorescent microscope for the presence of a GFP cell. Of these wells, 84.5% contained a single cell, 15.5% were empty and most importantly none had more than one cell. This experiment provided a first demonstration of the practicality of the solution and of the robustness of the impedance profile for quality control.

All of the 15 growing clones randomly selected after DispenCell isolation showed high cell density and viability, and they produced the antibody within a similar range as the cells seeded by limiting dilution. These experiments showed that the robot may provide an immediate proof of single cell isolation that can be used to clone cells used for biologics production in one single round.

Acknowledgements & Funding: GM and PR are cofounders of SEED Biosciences SA, a company commercializing DispenCell.

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IN VIVO EFFICACY OF RECOMBINANT FACTOR VII PRODUCED IN HUMAN CELL LINE

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Background and novelty: Approximately 20 to 30% of patients with severe hemophilia A and 5% of patients with hemophilia B, who use factor VIII (FVIII) and factor IX (FIX) replacement therapy, respectively, develop antibodies that inhibit the activity of the infused factor. Patients with high-titer/high-responding inhibitors must be treated with bypassing agents that can achieve hemostasis. Many studies have identified activated factor VII (FVIIa) as an attractive candidate for hemostasis, independent of FVIII/FIX, making this coagulation factor an alternative for hemophilia patients with inhibitory antibodies. Over the years, studies in Blood Center of Ribeirao Preto, Brazil, have been using human cell lines for production of recombinant coagulation factors. Sk-Hep-1 cells were chosen for rFVIIa production and showed, for a period of 6 months, an average of 8,03 IU/mL of rFVII yield.

Experimental approach: To confirm the promising *in vitro* results, the efficacy of rFVIIa produced in Sk-Hep-1 cells were evaluated in the severe tail-bleed model in hemophilia A mice. In this model, after the infusion of 8 mg kg^{-1} rFVII or NovoSeven, the mouse tail was amputated at a diameter of 3 mm under general anesthesia. Immediately upon lesion, the tail tip was submerged in isotonic saline solution (0.9%), which was kept at the physiological body temperature of the mice using a water bath, until hemostasis occurred. The volume of total blood loss was calculated over an observation period of 15 min. The hemoglobin lost present in the isotonic saline was measured.

Results and discussion: Results from severe tail-bleed model show that our rFVII and NovoSeven both reduced blood loss. However, the rFVII produced in SK-Hep-1 cells stopped the bleeding 2-fold faster than NovoSeven, showing the efficacy of our coagulation factor. The pharmacokinetics analyses are in progress.

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TRANSCRIPTOMIC PROFILING OF CHO PARENTAL AND AB-PRODUCING CELL LINES

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Background and novelty: Chinese hamster ovary (CHO) cells are well-established hosts for producing recombinant protein therapeutics. The growth and production characteristics of different CHO cell lines depend upon cell-intrinsic properties, gene transfection process, culture media and bioprocess conditions. To understand how differences in key cellular pathways between CHO cell lines may impact cell viability and productivity, we compared the transcriptomes of CHO-K1 and DG44 parental and IgG-producing cells.

Experimental approach: CHO-K1 and DG44 parental cells and IgG1 mAb-producing cell pools were grown in chemically defined protein-free media within shake flasks and harvested at mid-exponential growth stage. RNA-seq was carried out to facilitate comparisons of the transcriptomes of these cell lines, followed by differential gene expression and gene set enrichment analyses.

Results and discussion: Firstly, gene set enrichment analysis of expression profiles in parental DG44 vs CHO-K1 revealed that DG44 exhibited cell cycle arrest, ER stress and ER stress-mediated apoptosis which were significantly rescued by ectopic expression of *Dhfr* gene in DG44 producer. Secondly, the adenylate cyclase-modulating GPCR signaling pathway was significantly upregulated, suggesting enhanced cyclic AMP signaling, in DG44 parental and producer cell lines vs CHO-K1 counterparts. In contrast, glycosphingolipid metabolic process was downregulated in DG44 parental and producer. Finally, the oxidative phosphorylation pathway was upregulated in CHO-K1 and DG44 producers vs respective parental cells driven by higher cellular energy requirements during protein production, while enzymes related to protein deglycosylation were downregulated. The differences identified between parental and producer CHO-K1 and DG44 may be exploited to guide cell line engineering strategies.

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AUTOMATING CELL LINE DEVELOPMENT: IDENTIFYING CLONES MORE EFFICIENTLY

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Background and novelty: The identification and subsequent expansion of stably expressing, high producing cell lines through traditional cell line development (CLD) workflows is both challenging and labor intensive. Furthermore, the biopharmaceutical industry's focus on fast-to-clinic timelines demands new tools and methods that help accelerate the accurate identification of lead candidate clones. In an effort to meet these needs, an automated CLD 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 robotic liquid handler integrated with both incubation and cell imaging functionalities for plate barcoding, storage/incubation, imaging, screening, hit-picking, feeding, passaging, and early titer assessment. Additionally, this automation platform was paired with a software-based data handling tool to enable complete system data integration and more efficient identification/screening of lead candidate clones. Pilot runs were performed in which parallel CLD project arms were run to compare the automated CLD workflow with the manual CLD process.

Results and discussion: Results of pilot CLD projects demonstrated comparable candidate clone screening and scale-up between manual and automated methodologies as determined by key metrics; confluence, number of selected/expanded clones, and time from single cell to research cell bank. In addition, the automated platform demonstrated benefits for operators (ergonomics; manual time requirements), data handling and storage, and increased throughput capacity in support of the CLD process and subsequent generation of stably expressing cell lines.

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A HEK293 EXPRESSION SYSTEM FOR THE STABLE PRODUCTION OF GLYCOPROTEINS

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Background and novelty: Popular mammalian host systems used for biologic therapeutics production are animal derived cell lines. These cell lines can potentially produce glycoproteins with non-human glycans that may illicit immunogenic responses. Here, we developed a fully human expression system based on HEK293 cells for the stable and high titer production of recombinant proteins.

Experimental approach: HEK293 cells were first engineered by knocking out *GLUL* (glutamine synthetase) using CRISPR-Cas9 system. Expression vectors using human *GLUL* as selection marker were then generated, with recombinant human erythropoietin (EPO) as our model protein. Selection was performed using methionine sulfoximine (MSX) to select for stable and high EPO production cells. The expression of exogenous genes in a selected cell pool was then characterized. 2 L stirred-tank fed batch bioreactor cultures were performed, followed by glycosylation analysis.

Results and discussion: Four HEK293 clones with *GLUL* knockout were obtained and characterized. EPO production cell pools were then generated and demonstrated to have stable EPO expression. In the fed-batch bioreactor culture, EPO production of up to 92700 U/mL by ELISA or 696 mg/L by densitometry was demonstrated. N-glycosylation of the produced EPO was similar to endogenous human proteins and non-human glycan epitopes were not detected. We thus demonstrated the use of the HEK293 system for the stable, high titer and xenogeneic-free production of EPO and possibly other complex recombinant proteins.

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A NEW CHO EXPRESSION SYSTEM (CHO-C) FOR HIGH YIELD MAB PRODUCTION

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Background and novelty: Monoclonal anitbodies are the fast growing class in biopharmaceitics. During 2015~2017, about 70% FDA approved mabs are produced in chinese hamster ovary cells (CHO). Efficient production and secretion of mAbs in CHO cells depends on combination of genetic, metabolic and culture condition optimization. A major aim of our study is to develop a noval CHO expression system which includes a super vector, an engineered CHO host and customized bioprocess for fast generating high-yielding therapeutic antibodies.

Experimental approach: We've developed a super vector which has optimized HC/LC ratio and noval signal peptide, named J2.0. We have also applied the CHO-specific microarray transcriptomics technology to identify genes that contribute to high productivity. Through compared 6 different high and low producer clones which has 7 fold difference in productivities, we chose one target gene "C" to engineer in CHO-DXB11 cells.

Results and discussion: The main feature of the engineered CHO (CHO-C) includes lower lactate productivityand longer life span during the fed-batch culture (CHO-C: 90% vs DXB11:62%). Moreover, CHO-C is stable over a continuous period of approximately 100 generation. Four mAbs were chosen for examine CHO-C platform.The expression titer of four different mAb expressing stable clones is ranged from 330~410 mg/L (6 day batch culture). Note that those high titer clones with low gene copy numbers, indicated they are potentially more stable. Through fed-batch process development, 2-5 g/L mAb productivity can be achieved using CHO-C expression system. In conclusion, the CHO-C platform provides an attractive system for generating good quantity and quality mAbs.

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BOOSTING PRODUCTIVITY BY MITOSRNA ENGINEERING AND PROCESS ADAPTATION

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Background and novelty: Despite continuous progress in cell line and bioprocess development resulting in high-yielding production processes, novel molecule formats are of increasing complexity and can be challenging in terms of manufacturability. The present study focuses on the improvement of an existing manufacturing clone that had been selected in comprehensive cell line development before. However, associated with the complexity and size of the respective molecule format, the titer was rather low when compared to standard IgGs. We thus aimed at increasing product yield by combining cell line engineering with directed modulations of the cell culture process.

Experimental approach: An existing manufacturing clone secreting a complex bispecific, tetravalent antibody was stably super-transfected with a pro-productive human mitochondrial genome-encoded small RNA (mitosRNA-1978), which has already been shown to increase the specific productivity of IgG expressing CHO cells. Subsequently, we aimed at enhancing the total product concentration by adapting cell culture parameters. Engineered cells were evaluated in fed-batch cultures.

Results and discussion: When analyzed in fed-batch cultures, the mitosRNA-1978 engineered clones revealed an increase in specific productivity, whereas maximum viable cell density was reduced. To further boost the yield from mitosRNA-1978 expressing cells, we aspired to optimize the upstream cell culture process for enhanced cell growth. Notably, by applying the respective mitosRNA, increased seeding cell densities and enhanced initial culture temperature we successfully improved the final product concentration of the production clone [1]. Taken together, this integrated approach of cell line engineering and appropriate process modulation constitutes a successful strategy to maximize product yields, especially for the manufacturing of therapeutic proteins that are complex and thus difficult-to-express.

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IMPROVED CELL LINE DEVELOPMENT WITH LEAP-IN TRANSPOSASE & VIPS IMAGING

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Background and novelty: There is significant pressure in the biopharmaceutical industry to reduce timelines from DNA to IND, and a large portion of the timeline on the critical path is the cell line development (CLD) process. This months-long process typically requires two especially time and resource-intensive steps: 1) single-cell imaging to ensure the regulatory requirements for monoclonality are met, and 2) screening 1000s of clones to find the highest producers. The Janssen R&D CLD group has been working to identify solutions to improve the efficiency and reduce the bottlenecks associated with these two key steps.

Experimental approach: First, we have partnered with ATUM to evaluate their Leap-In transposase-mediated integration system with the intent of increasing transgene copy number and generating more consistent and higher-producing transfection pools to reduce the extensive clone screening required during CLD. Second, we have partnered with Solentim to pilot their Verified In-Situ Plate Seeding (VIPS) system and develop a process for seeding and imaging of single cells in microtiter plates to ensure monoclonality in a single 1-day step.

Results and discussion: We have incorporated transposase-mediated transgene integration and a VIPS monoclonality imaging step into our CLD process for mAb and non-mAb projects and found that we can generate cell lines with high productivity, high viability, and a high assurance of monoclonality after a single round of limited clone screening. In addition, we have found that the transfection pools can reproducibly reach 3-5 g/L and generate material that is analytically representative of the clones later derived from the pools. In summary, these emerging technologies have allowed our group to reduce clone screening 4-fold, shorten our overall CLD timeline by 25%, and hold potential for accelerating development efforts all without compromising the productivity or product quality of our production cell lines or processes.

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BIOGENIC MAGNETIC PARTICLES FOR MEMBRANE-BASED LIGAND PRESENTATION

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Background and novelty: Several biological reactions require interactions between a receptor and a membrane-bound ligand presented as a multimeric protein. In order to investigate such reaction *ex vitro,* the ligand must be presented as surface protein on genetically engineered animal cells or produced as engineered multimers, both linked to drawbacks (contamination of target cells by presenting cells, cost). Here, we investigate a new cell-free presentation system based on biologically produced magnetic nanoparticles (magnetosomes) with a biological membrane containing a set of specific proteins used for ligand immobilization. For proof of feasibility, CD40L was chosen as a ligand because an immobilized trimeric state is necessary for mimicking CD40L natural biological activity.

Experimental approach: Functionality of decorated magnetosomes, produced in engineered *M. gryphiswaldense* strains, was tested in sensor cells stably expressing a membrane-bound human CD40 receptor (hCD40) and a NF-kB-inducible secreted embryonic alkaline phosphatase (SEAP). Binding of the hCD40L to its receptor triggers a signaling cascade leading to the activation of NF-kB and the subsequent production of SEAP.

Results and discussion: Utilizing magnetosome membrane proteins as anchors, particles with varying CD40L densities (5 to 165 ligand) were generated. Their ability to stimulate sensor cells upon interaction between the CD40L and its receptor was investigated. For CD40L-functionalized magnetosomes but not for mock-decorated particles, SEAP was expressed in range corresponding to 1 ng/mL rhCD40L, hence confirming the functionality of the magnetosomes. Moreover, magnetic cell sorting upon ligand binding to the sensor cells was investigated.

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HEK293 ALLOW RESCUE OF PROTEINS THAT ARE DIFFICULT TO PRODUCE IN CHO

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Background and novelty: CHO cells are the workhorse of the biopharmaceutical industry for expressing recombinant human proteins and monoclonal antibodies as high quality and yield. However, some products cannot be produced in CHO cells at meaningful quantities or qualities, where a cell line of human origin might be beneficial.

Experimental approach: 25 recombinant human secreted proteins that are difficult to express in CHO cells were investigated based on their expression in 3 different CHO and HEK293 cell lines and monitored by transcriptomics analysis to reveal features correlating with performance as secretional host.

Results and discussion: Ten of the 25 proteins showed improved expression in all HEK293 systems compared to CHO in both a transient and a stable episomal expression system. In particular, proteins with propeptides and/or disulfide-linked homo-oligomeric forms benefited from expression in HEK293. Transcriptomics showed an overall high transcription of the target genes in both cell lines, indicating other bottlenecks for efficient secreted production. CHO cells showed an overall higher expression of their secretion machinery compared to HEK293, even though they secreted smaller amounts of the target proteins. Particular differences in expression of specific genes of the secretion machinery included higher levels of propeptide convertases in HEK293 over CHO. Our results suggest that HEK293 may be a valuable fallback strategy for proteins that are difficult to express in CHO, especially for proteins with complex post-translational modifications or requiring oligomerization. Moreover, the findings suggest target-proteins in the translational and secretion machinery in CHO or HEK that may be engineered for improved expression of human recombinant proteins.

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IMPLEMENTATION OF THE BEACON PLATFORM FOR CELL LINE DEVELOPMENT

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Background and novelty: Generating a highly productive manufacturing cell line typically involves long timelines and are resource intensive, due to the need to screen large numbers of candidates in protein production studies. As a result, miniaturization and automation strategies are often employed to allow for reductions in resources and higher throughput. However, current automation approaches rely on the use of standard cell culture vessels and bulky liquid handling equipment.

Experimental approach: The Beacon platform from Berkeley Lights offers to eliminate these bottlenecks through growing cells on nanofluidic chips. Berkeley Lights' OptoElectro Positioning (OEP) technology projects light patterns to activate photoconductors that gently repel cells to manipulate single cells on nanofluidic culturing chips. Integrated fluorescence imaging capabilities allow for analytics of cells in real time. Using this integrated technology platform, common cell culture tasks can be programmed through software, allowing maintenance and analysis of thousands of cell lines in parallel on a single chip.

Results and discussion: We describe the ability to perform key cell line development work on the Beacon. We demonstrate that CHO cell lines can be isolated, cultured, screened, and exported at high efficiency. We then compare this head to head with a FACS-enabled plate-based workflow across four active biologics programs. Resulting subclones from both Beacon and FACS processes were evaluated in small scale production experiments and bench-scale bioreactors comparing growth, productivity, and product quality. We demonstrate the ability to generate a diverse and comparable set of candidate clonal cell lines with reduced resources. Additionally, we performed validation experiments to demonstrate that the Beacon process provides high assurances of clonal derivation and zero cross-contamination.

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ENHANCING CHO CELL LINE DEVELOPMENT BY RATIONAL IN SILICO OPTIMIZATION

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Background and novelty: Novel bioinformatics tools hold great potential for enhancing monoclonal antibody (mAb) development. Early identification of developability risk factors is key to predictable timelines and success during mAb development. We have developed new predictive *in silico* tools reliably assessing and significantly minimizing developability risks of mAb candidates. The *in silico* optimization was shown not only to improve product quality properties of the mAb but also to enhance product titers during CHO cell line development by several-fold.

Experimental approach: Difficult-to-express mAb candiates were assessed using two different *in silico* prediction and optimization tools. The first bioinformatics tool compared the mAb sequence to an internal database of functionally folded mAbs, while the other algorithm was applied to identify and modify intrinsic aggregation prone regions of the mAb. Based on the analytical results, various *in silico* optimized mAb variants were generated. Wildtype and optimized mAbs were used for stable cell line generation to assess differences with regards to developability. Finally, intracellular analysis of stable cell lines was performed by high-resolution fluorescence microscopy.

Results and discussion: Besides improved drug product properties it was found that mAb product titers were significantly enhanced by the *in silico* optimization. While the wildtype mAbs could only be produced at product titers of 1 g/L, cells producing the *in silico* optimized variants achieved titers of up to 6.7 g/L. Intracellular analyses revealed that wildtype mAbs accumulated within the ER, which presumably suppressed the secretion process. Cells producing the *in silico* optimized mAb variants, however, did not show mAb accumulation in the ER and regained the phenotype of a high-producing clone. Taken together, our case study highlights that an *in silico* optimization toolbox represents a powerful instrument to advance antibody development.

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IMPACT OF NABU AND RA ON HISTONES AND NUCLEAR SIGNALING IN CHO CELLS

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Background and novelty: Recently retinoic acid (RA) was found to increase CHO cell productivity, an effect long known for sodium butyrate and other short chain fatty acids [1]. Here we elucidate, by western blotting and a triple SILAC nLC Orbitrap experiment, the overlapping and diverging CHO signaling events correlated to histone modifications affecting (product) gene transcription.

Experimental approach: Effects of 150 nM RA (found to be optimal) compared to a 2 mM NaBu treatment of two CHO lines were investigated. Protein samples were taken at six timepoints after agent addition. Histone fractions and nucleus proteins were extracted, separated by SDS- or acid urea (AU)-PAGE and analysed by western blotting with antibodies against total Rb-protein, two phosphorylated variants of it and histone H3/H4 modifications. For a global view of the nucleus proteome and signaling, a triple SILAC cultivation with similar conditions followed by nLC Orbitrap analysis with quantification on proteome and phoshoproteome level was done.

Results and discussion: Addition of NaBu resulted in increased productivity linked to decreased growth as observed with other CHO lines before [2,3]. In our hands, contrary effects for RA were found. For both agents changes in modification abundance of histone H4K5 and H3K4, correlated with elevated transcriptional activity, were detected. For NaBu no impact on amount or phosphorylation pattern of the Rbprotein (central cell cycle regulator) was detected, while for RA an increase in amount of Rb and Rb phosphorylated at pS608 was detected, but no impact on Rb pS807/811 was found. In the SILAC experiment differences between RA and NaBu in short term (60 min) influence on phosphoproteome and long term (24 h) changes of nucleus proteome were identified (presented in detail on the poster). Phosphoproteome analysis revealed signaling events related to epigenetic processes such as modulation of HDAC modification complexes.

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BREAKTHROUGH CLD PLATFORM FOR BISPECIFIC ANTIBODIES EXPRESSION

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Background and novelty: Bispecific antibodies (bsAb) represent a fast-growing class of molecules offering new therapeutic perspectives. Their development is hampered by the difficulty to produce these complex molecules composed of multiple polypeptide chains. Currently, bsAbs are produced in vitro after assembling half antibodies produced separately or in vivo from co-culture of two cell lines producing each half antibody. A production system supporting bsAb formation in one cell is a most attractive strategy.

Experimental approach: Co-transfection of independent proprietary vectors, each harboring a different polypeptide, was used to optimize the chain-to-chain ratio. Early screening of stable pools by microcapillary electrophoresis allowed to rapidly distinguish the different hetero- versus homodimeric populations. Isolated heterodimer high-producing cells were then evaluated for their productivity in fed-batch cultures. The purified product was characterized by SEC HPLC and cIEF to assess the correct product was formed.

Results and discussion: Modulating the relative expression of the polypeptide chains was found to be the key determinant. Early characterization screening combined with a fast process development platform lead to an efficient production of bsAb meeting both quantitative and qualitative criteria. Top clones were shown to maintain stable titer and heterodimeric composition for >60 generations, a pre-requisite for commercial manufacture of bsAb. This approach was not specific to one bsAb format but enabled the successful expression of over 10 different bsAb formats reaching as high as 6.5g/L in bioreactor with >90% purity of heterodimers. Selexis platform offers high flexibility and was successfully applied to a large panel of multi-polypeptide molecules (IgG, Ig- or Fc-fusion, new scaffold, virus) with a gene to GMP service in 9 months.

P-161

MONITORING THE FUNCTIONAL IMPACT OF CHO ENGINEERING USING CHEMSTRESS®

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Background and novelty: CRISPR/Cas9 enables rapid, efficient and precise engineering of CHO cells. Unlike previous methods, the precision enabled by CRISPR/Cas9 should minimise undesirable disruption of cell function. One issue with demonstrating this is that functional impact can only be measured indirectly. Valitacell®'s ChemStress® function profiling array is designed to probe the various cellular functions that drive bioprocess performance. ChemStress® is a simple, affordable multiwell plate carrying a battery of chemicals chosen to mimic real bioprocess stresses. Clone growth and titer are monitored in response to each chemical. Valita®PROFILE data analytics can be used to process ChemStress® profiles to compare clone responses. This is used here to compare the functional impact of untargeted and targeted engineering efforts.

Experimental approach: CHO cell backgrounds were modified in an untargeted manner and also in a targeted manner with CRISPR/Cas9 to introduce an empty "landing pad", which was later populated with various protein products. ChemStress® data were collected to monitor the functional impact of these variant clones with the expectation that background and empty landing pads should show little difference in their Valita®PROFILEs, indicating no major perturbation of bioprocess-relevant function.

Results and discussion: Measuring the functional impact of engineering is difficult because a production clone has many components that impact upon overall performance. While high throughput omics technologies give an insight into these multiple components, ultimately omics data lie beneath function and therefore require interpretation. ChemStress® and Valita®PROFILE are designed to measure cell functions directly. Here ChemStress® has been used to demonstrate the benefits of targeted CRISPR/Cas9-based engineering, which does not perturb cell function, as shown via the high similarity of Valita®PROFILEs from the background and empty landing pads.

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CHARACTERISATION AND OPTIMISATION OF LV PRODUCER CELL LINES

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Background and novelty: Large-scale production of lentiviral vectors (LV) for therapeutic applications in gene therapy is challenging with current transient transfection processes. The development of producer cell lines (PCL) enabling the generation of large quantities of LV will not only reduce costs but improve consistency between batches. To generate PCLs, isolation of stably transfected cells by limiting dilution cloning (LDC) in antibiotic selective media is required. Oxford BioMedica has developed a bespoke Automated Cell Screening System (ACSS) which uses state of the art automation to isolate, culture and screen up to 3000 clones.

Experimental approach: Inducible HIV-1-GFP PCLs were developed by stable transfection of an adherent cell line constitutively expressing the Tet Repressor protein with HIV-1 inducible packaging components and a GFP genome. The ACSS was used to LDC the stably transfected cells in 96-well plates and over 400 clones were isolated. The clones were monitored to ensure monoclonality and a high-throughput screen was performed to assess LV productivity. The best 100 clones were selected, expanded and after several rounds of screening for LV-GFP production the 2 best PCL clones were chosen. These clones were further adapted to a suspension growth mode and LV production evaluated. Various PCL characteristics and LV production parameters were investigated from both the adherent and the suspension PCLs.

Results and discussion: These studies allowed the identification of key parameters and processes necessary for the production of high titre LV from adherent and suspension PCLs. In addition, the best suspension adapted PCL yielded LV of equivalent titre and quality to LV produced using the standard suspension transient transfection process. Evaluation of the copy number of the LV components at the beginning and end of the PCL suspension adaption process indicated that the PCLs were stable and had retained the LV components with no evidence of gene loss.

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TARGETED KNOCK-IN INTO CHO CELL GENOME USING GENOME EDITING TOOLS

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Background and novelty: Genome editing technology based on programmable nucleases such as TALEN and CRISPR/Cas9 has recently emerged as a powerful technique for precise modification of target locus in the cell genome. Previously, we have attempted targeted knock-in of transgenes into the *hprt* locus of CHO cells using TALEN and CRISPR/Cas9 [1,2]. Here, we generated antibody producer CHO cells by CRISPR-mediated knock-in of a *loxP* site followed by Cre-mediated re-targeting for antibody gene integration.

Experimental approach: Founder CHO cells (CHO/[DsRed]HR) were established using CRISPR-based systems for pre-introducing a *loxP* site into the *hprt* locus of CHO genome [2]. The founder cells were transfected with a donor plasmid (R2/scFv-Fc) [3] encoding *Blar* gene and an *scFv-Fc* expression unit flanked by compatible mutated *loxPs* and a Cre expression vector.

Results and discussion: After transfection of the donor plasmid into founder cells carrying a *DsRed* expression unit, the clones losing red fluorescence and resistant to blasticidin were obtained, indicating that Cre-mediated re-targeting of the transgene was successfully achieved. The cell growth and recombinant scFv-Fc productivity were evaluated for the cells established. During the culture, no obvious differences were observed in both cell growth and scFv-Fc productivity of the re-targeted cells (CHO/[scFv-Fc] RT) compared with the cells generated using CRIS-PITCh and TAL-PITCh systems [1,2].

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P-164

MOLECULAR MECHANISM OF RECOMBINANT PROTEIN PRODUCTION INSTABILITY

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Background and novelty: CHO cells are the predominant system for producing protein therapeutics. Significant progress has been made to increase the product yield through cell engineering and culture processes. However, one of the major challenges for commercial production is the unpredictable decrease in protein expression observed during long-term culture (LTC). Such instability generates problems for process yields, time-lines, cost and regulatory approval. The underlying causes and the precise molecular mechanisms resulting in cell line instability have yet to be fully elucidated.

Experimental approach: Growth characteristics, recombinant (r-) protein expression (ELISA, Western blot), r-gene copy numbers (qPCR), mRNA expression (qRTPCR) and r-gene localisation (FISH) of two CHO cell lines, producing Herceptin-like monoclonal antibody, were assessed throughout the period of LTC. Finally, transcriptomic profiling was performed to identify differentially expressed genes in stable and unstable cell lines over LTC.

Results and discussion: Two CHO cell lines were characterised to gain an understanding of the molecular mechanisms underpinning production instability over LTC. Our results showed that one cell line was stable in terms of r-protein production whilst production by the other cell line decreased by 42%. Both stable and unstable cell lines retained r-gene copies over LTC. In contrast, recombinant H and L chain mRNA expression decreased, with the decrease being more pronounced in the unstable cell line. FISH analysis revealed two different integration sites in the stable cell line whereas for the unstable cell line, the r-gene was localised to a single chromosomal site. Transcriptome profile of cell lines across 85 days showed a number of mRNA species that were expressed differentially between early and late generations. The number and identity of differentially expressed mRNAs provides molecular indicators of cell line phenotypic profiles of instability of r-CHO cells.

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ACCUMULATIVE TRANSGENE INTEGRATION SYSTEM USING CRE-LOXP

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Background and novelty: The establishment of recombinant animal cell lines with stable and high expression of transgene is an ongoing requirement for biopharmaceutical protein production. We have previously reported an accumulative site-specific gene integration system (AGIS) using Cre-recombinase and mutated loxP sites, in which repeated transgene integration into a predetermined chromosomal site of mammalian cells is possible[1-3]. However, the process of establishing cells with multiple copies of transgene integration is still time-consuming. In this study, we improved AGIS to facilitate and accelerate the establishment of high-producer Chinese hamster ovary (CHO) cells.

Experimental approach: The founder cells used for transgene integration were CHO/R1 cells, in which a mutated *loxP* site *(loxP1)* and an expression cassette encoding the red fluorescent protein, DsRed, were introduced into the *hprt* locus. For the transgene integration using Cre-mediated AGIS, cells were transfected with donor plasmids and Cre-expression vector by lipofection or electroporation. The target cells were screened using drugs and/or FACS.

Results and discussion: AGIS was used to introduce antibody genes into a predetermined locus of CHO cells. A new strategy for AGIS to facilitate and accelerate the integration and screening processes was designed. Simultaneous transfection of two donor vectors for integration and screening of targeted cells by color change allowed us to efficiently establish producer cells. Furthermore, the use of a minicircle DNA vector as a donor improved the efficiency of targeted cell generation. Incorporating this integration and screening strategy into AGIS greatly reduced the time required to establish cell lines with multiple copies of the transgene.

Acknowledgements & Funding: This work was supported in part by grants for developing key technologies for discovering and manufacturing pharmaceuticals used for next-generation treatment and diagnoses, both from METI and AMED under Grant Number JP17ae0101003.

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P-166

OPTIMIZED COMBINATION OF GENETIC ELEMENTS ENHANCING MAB PRODUCTION

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Background and novelty: To satisfy the continuous demand for high-titer bio-pharmaceutical cell lines, enhancing the level of mRNA transcript coding for a protein of interest, is one possible engineering strategy. In mammalian cells, efficient translation and corresponding protein yield also highly depend on mRNA stability and localization, and on the efficient initiation of the entry of the protein into the endoplasmic reticulum. The presence of specific genetic elements, e.g. appropriate signal peptides and 5′ and 3′ untranslated regions (UTRs), play an important role in this respect.

Experimental approach: The influence of individual 3´and 5´UTRs and different UTR®Betatech expression cassettes (supplied by UniTargetingResearch AS) for heavy (HC) and light chain (LC) in a double-gene vector expressing a mAb was investigated in fed-batches using a CHO DG44 host cell line. After combining the most promising elements, the optimized expression vector was tested with three different mAbs in our MTX-free CLD 3.0 platform. For this purpose, pools and clones were generated and fed-batch performances were compared to the standard approach.

Results and discussion: Initially, the screening of different element combinations showed a high impact on specific productivity and yield of mAb in fed-batch. The results revealed a significant 1.7-fold increase in average fed-batch titer employing the best UTR®Betatech expression cassette (std. = 0.6 g/L; UTR®Betatech = 1.0 g/L). In particular, different element combinations for HC and LC proved to be beneficial. In a following experimental setup, an even higher increase ranging from 2.0 fold to 5.3 fold in average final pool fed-batch titers was achieved for the different mAbs. In summary, use of UniTargetingResearch's vector technology significantly increased the final protein concentrations and therefore contributes to the continuous improvement of the standard expression system for mAb in the Sartorius-Stedim Cellca process.

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THE CELLCA CHO EXPRESSION PLATFORM FOR DEVELOPMENT OF BIOSIMILARS

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Background and novelty: The market for therapeutic proteins including biosimilars is steadily growing, resulting in an increased demand for fast and efficient cell line and process development platforms. Additionally, new challenges arise due to strict requirements for biosimilars regarding quality and productivity.

Experimental approach: The Cellca CHO expression platform is suitable for high-titer production of biosimilars with variation of glycan structures. We have established a biosimilar cell line development approach including a first selection based on glycan profile already on pool level. Following, 48 clones are screened in fed-batch for productivity and protein quality using the ambr15 system. Moreover, individual media and process modifications using the ambr15 and ambr250 system are available to achieve similarity to the originator. Additionally, genetic engineering was established to specifically modulate protein quality e.g. to increase sialylation in order to achieve the desired sialylation profile of the originator. The fed-batch performance of the technology is directly transferable from small scale systems to large scale bioreactors not only in terms of process performance but also achieving robust protein quality.

Results and discussion: Sartorius has an excellent track record in the generation of biosimilar cell lines. Several high producing (> 3 g/L in a standard fed-batch process) biosimilars with the target protein quality profile were successfully generated for customers using the Cellca CHO expression platform. During cell line development not only the physicochemical protein characteristics were tested, but also binding assays and functional assays such as ADCC were performed to support clone selection. In this line, we also offer in depth innovator analytics and bioassay development for biosimilars.

P-168

METABOLIC ENGINEERING TO REDUCE GROWTH INHIBITORY BYPRODUCTS FORMATION

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Background and novelty: CHO cells in fed-batch cultures produce metabolic byproducts, mainly derived from amino acid catabolism, some of which are growth inhibitory and limit the peak cell densities achievable. In earlier work in our lab, nutrient feeding strategies that control residual concentrations of certain amino acids in a low range yielded reduced inhibitory byproduct formation in fed-batch cultures, resulting in higher growth and productivities.

Experimental approach: In this study, genetic engineering approaches were undertaken to reduce biosynthesis of inhibitory byproducts and to confer prototrophy towards the hard to supplement amino acid, tyrosine. In the branched chain amino acid (BCAA) pathway, the first enzyme, branched chain aminotransferase 1 (BCAT1), which catalyzes first degradation step for all the three BCAAs was knocked out of CHO cells. In phenylalanine-tyrosine (phe-tyr) catabolic pathway, four enzymes, intrinsically expressed at negligible or very low levels in CHO cells, were overexpressed ectopically.

Results and discussion: Clones with the BCAT1 KO event produced negligible amounts of BCAA catabolism linked inhibitory byproducts in fed-batch cultures, resulting in significantly enhanced peak cell densities and titers. These clones also exhibited high viabilities (>95%) for extended length of fed-batch cultures (upto 20 days). Cells overexpressing phe-tyr enzymes produced lower levels of corresponding byproducts and were able to grow and proliferate in tyrosine-free medium (tyrosine prototrophy). Additionally, data on use of tyrosine prototrophy as an expression/selection system analogous to glutamine synthatase (GS) system will be discussed as part of this presentation. These engineered attributes, when combined together, can yield highly productive, efficient and robust CHO cell factories.

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BISPECIFIC MOLECULE FORMATS AND CONCEPTS FOR CELL LINE DEVELOPMENT

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Background and novelty: Bispecific antibodies (BsAbs) combine two distinct binding specificities within a single biologic. More than 50 different BsAbs are currently in various stages of clinical development across a number of therapeutic indications (1). These engineered, often antibody-derived molecule formats entering into biopharmaceutical development pose significant challenges on the generation of high-yielding CHO cell factories. This talk will highlight the most recent advances at Boehringer Ingelheim to improve cell line development of asymmetric bispecific difficult-to-express proteins.

Experimental approach: To ensure high productivities and reliable product qualities we extended our cell line development workflow by the following approaches: (i)we applied several expression vector setups during transfection, (ii) used an HTRF (Homogeneous Time Resolved Fluorescence) screening assay to determine clones with favorable heterodimer content, (iii) implemented a gene regulating microRNA which led to a titer boost and (iv) employed a combination of in-depth mass spectrometric and genetic analyses via Next Generation Sequencing to identify and deselect clones with inferior product qualities.. Moreover, *in vivo* product- and organelle-staining delivered insights into the cellular processing of asymmetric, bispecific molecules in CHO cells.

Results and discussion: Combining a rationally designed novel host cell line with innovative genetic elements and screening tools enabled the development and selection of high-performing CHO production cell lines with reliable product quality.

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CO-AMPLIFICATION OF EBNA-1 AND PYLT FOR IMPROVING PROTEIN PRODUCTION

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Background and novelty:

<< Co-amplification of EBNA-1 and PyLT through dhfr-mediated gene amplification for improving foreign protein production in transient gene expression in CHO cells >>

Despite the relatively low transfection efficiency and low specific foreign protein productivity (qp) of CHO cell-based transient gene expression (TGE) systems. To improve TGE in CHO cells, Epstein-Barr virus nuclear antigen-1 (EBNA-1)/polyoma virus large T antigen (PyLT)-co-amplified recombinant CHO (rCHO) cells stably expressing EBNA-1 and PyLT were established using dihydrofolate reductase/methotrexate-mediated gene amplification. The level of transiently expressed Fc-fusion protein was significantly higher in the EBNA-1/PyLT-co-amplified pools compared to control cultures. Increased Fc-fusion protein production by EBNA-1/PyLT-co-amplification resulted from a higher qp attributable to EBNA-1 but not PyLT expression. The qp for TGE-based production with EBNA-1/PyLT-co-amplified rCHO cells (EP-amp-20) was approximately 22.9-fold that of the control culture with CHO-DG44 cells.

Experimental approach:

1. Construction of EBNA-1- or PyLT-amplifying vectors
2. Development of EBNA-1- and/or PyLT-amplified rCHO cells
3. Fc-fusion protein-expressing vectors
4. CHO cell-based TGE systems
5. Cell concentration, viability, Fc-fusion protein assay, and metabolite assay
6. qRT-PCR analysis

Results and discussion: Fig. 1 Western blot analysis and Fc-fusion protein production of the EBNA-1-amplified pools or PyLT-amplified pools at various MTX concentrations.
Fig. 2 Western blot analysis, Fc-fusion protein production, and qp of the EBNA-1/PyLT-co-amplified pools at various MTX concentrations.

Fig. 3 Profiles of cell growth, viability, and Fc-fusion protein concentration of EBNA-1/PyLT-co-amplified clones during TGE-based culture.

Fig. 4 The qp, relative mRNA expression, relative amount of replicated DNA, and transfection efficiency of EBNA-1/PyLT-co-amplified clones during TGE-based culture.

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IMPROVING ENERGY HOMEOSTASIS TO IMPROVE RECOMBINANT PROTEIN PRODUCTION

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Background and novelty:

· Vitamins are essential micronutrients required to support the growth and propagation of any living cell. Mammalian cells cannot synthesize them, and the lack of vitamins in the diet is directly linked to severe cellular defects [1-3].

· The **sodium-transporter SLC5A6** participates in the uptake of **vitamin B5** in mammalian cells and thereby in promoting an efficient energetic metabolism [4, 5].

· The **peroxisome proliferator-activated receptors (PPARs)** are ligand-activated transcription factors regulating the expression of genes involved in lipid homeostasis, anti-inflammatory response, cell proliferation and differentiation [6, 7].

Experimental approach:

– We designed an **improved selection method** based on the co-expression of SLC5A6, relying on mammalian cell dependence on vitamin B5 for energy production.

–We deciphered genetic changes in lipid metabolism occurring in the cells during B5 selection.

Results and discussion:

RESULTS

– The vitamin B5 metabolic selection yields cell lines producing difficult-to-express recombinant proteins at homogeneous and high level, using the selective advantage of improved cell metabolism, growth and viability, unlike state-of-the-art methods.

– We highlighted the role of **PPAR activation** in cell **improved cell fitness and production of difficult-to-express** therapeutic proteins.

DISCUSSION

– The **vitamin B5 selection** is a new and powerful metabolic selection method to recover mammalian cells stably expressing a gene of interest at high levels.

– B5 deprivation probably leads to a **state of fasting** in CHO cell, where an unknow PPAR ligand is produced.

– The effect of **activating PPAR**, collectively improves the lipid homeotasis and decreases byproducts such as lactate, **improving the cell's metabolic fitness**, which in turn may further **increase the production of the protein of interest.**

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PRODUCTION OF RECOMBINANT FACTOR VII IN NOVEL HUMAN CELL LINES

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Background and novelty: The coagulation factor VII (FVII), a vitamin K-dependent glycoprotein, is the main therapeutic choice for hemophilia patients who have developed inhibitory antibodies against conventional treatments (FVIII and FIX). The FVII-based commercial recombinant products available are produced in non-human mammalian cell lines (CHO, BHK). Although they have similar structures compared to human native protein, they can exhibit non-human glycosylation profile that can lead to immunogenic reactions. Here we evaluate the expression of rFVII in promising novel human cells lines, SK-HEP-1, HUH-7, HKB-11, cultured under serum-free suspension conditions.

Experimental approach: SK-HEP-1 and HKB-11 were cultured in CDM4CHO and HUH-7 in CD 293 medium. The cells were maintained in a 5% CO_2 37°C environment under 150 rpm orbital stirring. The recombinant cells were generated by two lentiviral transduction cycles (MOI=1-2) and then submitted to a sorting process.

Results and discussion: As FVII requires vitamin K for biological activity, 5 µg/mL was initially used for medium supplementation. This concentration, however, reported as efficient for serum-containing cultures, was cytotoxic for the cells cultured under serum-free suspension conditions. It was possible the supplementation of only 0,1 µg/mL for HUH-7 and 1 µg/mL for HKB-11 and SK-Hep-1. Molecular characterization showed that SK-HEP-1, HUH-7, HKB-11 presented 5.8; 0.9 and 0.8 integrated viral vectors per cell. ELISA quantification of the 48-hour culture supernatant detected 241.0, 217,4 and 78,5 ng/mL total FVII in the cultures of HKB-11, SK-HEP-1 and HUH-7 cells. The levels obtained are lower than those for HKB-11 and SK-HEP-1 cells generated and cultured under attached serum-containing conditions using the same lentiviral vector construction (Freitas et al., 2017). Strategies to enhance FVII expression in serum-free suspension conditions are being studied.

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LEAP-IN TRANSPOSASE MEDIATED STABLE CELL LINE DEVELOPMENT

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Background and novelty: ATUM has discovered a pair of novel transposon/transposases. These Leap-In® transposases have been engineered to increase their activity in mammalian cells. We have developed a set of cognate transposons that are highly effective for mammalian cell engineering, including for therapeutic protein production.

Experimental approach: Cells were co-transfected with transposons & transposase mRNA, then subjected to selection. Productivity and product quality were assessed for the resultant stable pools. Clonal lines were obtained using the Solentim VIPS system to deposit single cells and verify monoclonality. Product quality and yields were determined for clones, which were also characterized for molecular properties such as integrant structure, insertion site, copy number and integrant stability.

Results and discussion: Leap-In transposases generate stable transgene integrants with an array of unique characteristics: 1) Single copy integrations occur at multiple genomic loci, primarily in open chromatin segments. Leap-In mediated stable pools, with clonal distributions biased toward high producers, exhibit productivities rivaling/exceeding clonal productivities achieved by random integration. Fewer than 100 clones typically need to be screened. 2) There is no size limit, and integrated transgenes maintain their structural integrity. Multiple independent transcriptional units may therefore be combined into a single construct for integration. This ensures control of expression of each open reading frame for multi-subunit proteins, thereby guaranteeing the correct chain ratio in every recombinant cell. 3) Leap-In mediated integrations are extremely stable.

These properties combine to yield stable pools whose titer and product quality are indistinguishable from their derivative stable clones. This offers the opportunity to initiate process development from stable pools, & to generate representative material for POC and toxicology studies in parallel with cloning.

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BIOLOGICALISATION: CELL AND NATURE-BASED DIGITAL MANUFACTURING

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Background and novelty: Biologicalisation is an exciting development employing Industry 4.0 principles in concert with biological and bio-inspired materials, chemistries, and functions to support more efficient and sustainable manufacturing. From product design to development and manufacturing, biomimetic product designs and bio-integrated manufacturing systems describe this *biological transformation of manufacturing* (1).

Experimental approach: Many processes, chemistries, systems, and supply chains are enhanced by harmonizing digital manufacturing principles with biological structures and chemistries. This allows efficient and robust production to support a global circular economy.

Progress in understanding biological elements, phenomena, materials, and chemistries enables this revolution. In fact, a 2018 Nobel Prize winner in Chemistry, Frances H. Arnold, illustrates this point. She invented systems directing the evolution of enzymes now routinely used to develop tools such as manufacturing catalysts. This technology also supports other 4.0 goals of more sustainable manufacturing of pharmaceuticals and renewable fuels (2).

Results and discussion: An example of this development in pharmaceutical manufacturing is in the production of the drug Lipitor. The environmentally stressful waste produced was first greatly reduced through a palladium-catalyzed cross-coupling reaction. But the remaining methanol and tetrahydrofuran in the waste stream were still significant. Recent developments with a bio-inspired structuring of the reaction supports these cross-couplings reactions to occur in water, at room temperature, and with only PPM of transition metal catalysts. Basically, the palladium-driven catalysis takes place within micelles formed by a self-assembling surfactant-- thereby avoiding the use of organic solvents. This biomimetic super-structuring of the reaction provides many benefits, including the reduction or elimination of environmentally stressful materials (3).

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DEVELOPMENT OF A PRODUCTION CELL LINE FOR PASYLATED HUMAN DNASE I

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Background and novelty: Recombinant human deoxyribonuclease (rhDNase I) is a pancreatic nuclease currently used to treat cystic fibrosis (CF) patients. Treatment is performed by inhalation of DNase I into the airways, which results in the degradation of double-stranded DNA to low molecular weight forms, thus reducing the viscoelasticity of CF sputum and improving lung function [1][2][3]. However, short half-life of rhDNase I constitutes a current handicap of the treatment, hence provoking the quest for a novel, long-acting rhDNase I with more durable enzymatic activity [4]. Therefore, we modified the rhDNase I by genetic fusion with a highly soluble disordered polypeptide chain made of Pro, Ala and/or Ser (PAS) using XL-protein's PASylation® technology. PASylation has been shown to increase the plasma half-life by enlarging the hydrodynamic volume of therapeutic proteins [5][6].

Experimental approach: A PAS amino acid sequence of several hundred residues was attached to the C- or N-terminus of rhDNase I. Chinese hamster ovary (CHO) cells were transfected by a targeted integration approach using Rentschler Biopharma´s recombinase-mediated cassette exchange (RMCE)-based TurboCell™ cell line development system. This system is based on CHO-K1 cells tagged with the gene for green fluorescent protein flanked by recombinase recognition sites that enable site-specific integration of the gene of interest. Using the RMCE technique we generated cell line pools stably expressing PASylated rhDNase I as well as, for comparison, wild-type rhDNase I [7]. Additionally, a fast kinetic assay to compare and verify the DNA-degrading activity of PASylated rhDNase from cell culture supernatants was established for in-process control [8].

Results and discussion: Assay development and pool-stage cell-culture data will be presented for several versions of PASylated rhDNase using TurboCell™ CHO cell lines. Moreover, data from downstream processing and protein characterization will be presented.

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AN ALTERNATIVE FLUX BALANCE APPROACH FOR MAMMALIAN MODELS

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Background and novelty: Constraint-based modeling has been applied to analyze metabolism of numerous organisms via flux balance analysis (FBA) and genome-scale models (GeM) including mammalian cells. Previously a community-wide genome-scale model for Chinese Hamster Ovary (CHO) cells has been published which improves our understanding of their metabolism. This study introduces a non-traditional approach for FBA that is particularly suited for mammalian cell lines such as CHO and may provide enhanced model sensitivity. This modeling approach can also yield additional insights into metabolism and cell line characterization.

Experimental approach: A previously published constraint-based GeM for mammalian cell lines was adapted to incorporate an alternative objective function. Next metabolomics datasets from published literature and experiments were used to evaluate the feasibility of this alternative FBA approach for CHO cells.

Results and discussion: Growth and metabolomics data of 7 replicates of CHO cell cultures were applied to evaluate the impact of exchange flux measurement inputs on flux balance analysis, and showed that some particular inputs can alter the optimization results. An estimation approach was used to directly predict and streamline these exchange flux inputs. Subsequently, these modifications were incorporated into an alternative objective function viable to describe CHO cell performance. Resulting predictions using cell-line specific models were compared with measured values and indicated specific characteristics of cell lines. Furthermore, sensitivity data calculated based on these solutions yields a visualization of the metabolic capabilities and efficiencies of cell lines, providing useful insights into metabolism that can be used to enhance CHO cell performance in bioreactors.

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FINDING STABLE GENOMIC LOCI IN CHO CELLS USING ATAC SEQUENCING

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Background and novelty: Genetic instability in continuous cell lines, particularly Chinese hamster ovary (CHO) cells, can result in product loss over time and has contributed to the complex cell line development (CLD) campaigns within the biopharmaceutical industry. Openly accessible/stable genomic loci are desirable for transgene residency resulting the long-term maintenance of gene expression. Random integration is the most commonly implemented method for stable CLD and as a result the probability of a transgene integrating into a stable, open and highly transcriptionally active location is low. With the recent advancements in genomic engineering tools such as CRISPR-Cas9, the targeting of a genetic payload to a pre-determined locus is now possible – the next task is deciding which genomic site.

Experimental approach: Widespread profiling of the chromosomal landscape of CHO cells was carried out using the ATAC-seq method. Four biological replicates were cultivated at 37°C and temperature shifted to 31°C and library preparation was performed on DNA extracted from 50,000 cells. Pooled library preparations were sequenced with > 50 million, 75bp paired-end reads being acquired for each sample. Reads were aligned to the CHO-K1 genome and mtDNA reads removed. Peaks were assigned by identifying regions of enriched coverage compared to background coverage.

Results and discussion: We identified constant, highly accessible regions that do not respond to a reduction in culture temperature. These regions were either genic, found within or nearby genes, or intergenic regions located distally from transcribed regions. We leveraged RNA transcript expression data to identify highly accessible/expressed loci for downstream validation. These sites, identified based on transposase accessibility represent prime loci for transgene targeting using CRISPR-Cas9 for recombinant protein production.

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SPEEDING UP THE CELL LINE DEVELOPMENT PROCESS USING NEW TECHNOLOGIES

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Background and novelty: State of the art CHO platforms allow generation of high yielding production cell lines with short cycle times. Our technology development strategy combines efforts for further optimizing speed and yield of our CHO platform with continous expansion of our technology toolbox to deal with challenging molecules and special requirements. By integrating vector and cell line technologies we are aiming for further reducing cycle times and screening efforts of cell line development. The presentation will cover the generation of novel host cell lines using cell line engineering techniques as well as the design and results of our acceleration strategy.

Experimental approach: The experimental approaches include the usage of transcriptomics and genome engineering technologies to develop novel host cell lines, which are characterized in the Novartis production process. Also, the combination with FACS vector technology enables a significant acceleration from DNA to clone titer information as well as a fast process from transfection to single cell cloning. The presentation shows the high-throughput clone ranking strategies based on flow cytometry driving the acceleration scenario.

Results and discussion: The novel cell lines lead to the generation of high-producing pools and clones as well as to an increased clonal stability. Also, the FACS vector approach allows to pick out high-producing clones during the single cell cloning step and enables a straightforward and fast clone ranking strategy for the acceleration scenario. This approach shortens the CLD timelines from DNA to CHO clone titer to only 8 weeks.

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HOST CELL FROM SCRATCH

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Background and novelty: In the past different cell lines with the DHFR selection system were used. It is well known that DG44 cell lines have a genetic background deriving from several rounds of undirected mutation and might thus be more prone to genetic instability then CHO-K1 derived cell lines. We created a new host cell starting from scratch with the goal of enabling fast and lean process development by using the GS selection system. The work was supported by Omics (MFA and NGS).

Experimental approach: Here, we want to present two aspects of how we approached this challenge: First, by using a combination of directed evolution to our proprietary platform and sequential targeted KO of the GS gene via ZFN several host candidates were created and selected via comprehensive omics-data analysis, in-depth characterization and benchmarking under process conditions and second by a RNAseq-driven analysis that reveals distinct differential expression of genes contributing to mAb glycosylation.

Results and discussion: We created several host cell candidates by directed evolution approach an characterized them via omics. The improved host enabled faster and leaner process development. The new BI CHO-K1 GS Platform was shaped and achieves titers of up to 8 g/L in 80L bioreactors and up to approx. 6 g/L after scale up to 2000L scale.

To compare the DG44 host with the new CHO-K1 a global analysis was performed on glycopattern and transcript level. Six mAb projects with >550 analyses were reviewed concerning their glycopattern distribution. Additionally, nearly 200 RNAseq data were used for a pathway-oriented analysis of the glycosylation-associated transcripts. Gene expression was compared between the hosts as well as for host vs. producing cell lines. Expression patterns were host cell specific and depended on whether a mAb was expressed or not. The data supports host selection and CQA assessment. The outcome is an intensive knowledge database and a porfolio of tools for targeted PQ modulation.

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METABOLOMICS INSIGHTS INTO KEY FACTORS INFLUENCING CHO GLYCOSYLATION

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Background and novelty: Glycosylation is a critical quality attribute in biologics production, impacting product stability, efficacy and immunogenicity. The effects of different culture conditions on glycosylation are relatively well documented; however, the underlying biological mechanisms behind these glycosylation variations are still poorly defined. There is also limited knowledge on how culture factors potentially influence or predict these changes, which restricts the development of glycosylation-specific control strategies. This study uses a metabolomics approach for the in-depth characterisation of antibody-producing CHO cultures operated under different conditions, prior to identifying key metabolites having strong associations with observed glycosylation pattern changes.

Experimental approach: Fed-batch CHO cultures producing Adalimumab were performed under different operating conditions, including changes in pH, dissolved oxygen levels and a temperature shift strategy. Culture supernatant and cell samples were obtained at multiple time points for metabolomics analysis. N-glycosylation profiles of the product were also obtained at the same time points. Key metabolites strongly associated with glycosylation pattern changes were identified using univariate and multivariate analysis tools.

Results and discussion: Different operating conditions had the largest impact on galactosylation; particularly, lower pH reduced galactosylation levels, in agreement with earlier studies. The galactosylation level was also highly correlated with levels of nucleotide sugars, selected amino acids and compounds linked to energy generation and redox reactions. Importantly, these correlations were maintained under different operating conditions, suggesting their possible utility as indicators of product glycosylation status, potentially contributing towards the development of glycosylation-specific control strategies.

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FEASIBILITY STUDIES FOR USING THE BEACON IN CLD WORKFLOWS

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Background and novelty: The Beacon leverages a unique technology using Opto-Electro-Positioning (OEP) to import single cells into thousands of nanopens on individual OptoSelect chips. Cells can be cultured and assayed at this nanoscale to identify both growth and production properties within days of loading the chip. These properties can be analyzed for thousands of single-cell derived clones which would otherwise be impractical at any other scale. Top producing clones can then be exported using OEP which moves small packets of cells into 96-well plates for further expansion. This single technology has the potential to fundamentally improve the Cell Line Development (CLD) process.

Experimental approach: To evaluate the potential benefit the Beacon offers for CLD, a series of on-site feasibility studies were performed. The Beacon was first used to identify rank order of antibody productivity for previously established CHO clones. Next, a Beacon CLD workflow was directly compared to the standard Sanofi CLD workflow to evaluate clone productivity as well as the resources utilized in generating clones. Finally, monoclonality assurance of the Beacon export process was assessed using GFP and RFP expressing clones.

Results and discussion: The Beacon feasibility studies demonstrated the potential this technology may have to improve CLD workflows for clone generation. These results demonstrated 1) a high level of monoclonality assurance of exported clones, 2) increased clone screening throughput, 3) accurate clone ranking from the on-chip productivity assay, and 4) a short workflow to identify top expressing clones. Since the Beacon CLD workflow can identify top producing clones early in the timeline, fewer clones need to be assessed from the 96-well plate stage onward. This reduces both consumables and FTEs per project allowing for greater capacity to handle more CLD projects per year.

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INNOVATIVE BIOENGINEERING SOLUTIONS TO THERAPEUTICS PRODUCTION.

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Background and novelty: Novel biotherapeutics formats and non-IgG complex molecules are constantly emerging. Whilst the SUREtechnology platform of Selexis increases the transcriptional activity of a given transgene and hence the number of high producing clones, post-transcriptional cellular machineries represent the next limitations for the establishment of recombinant cell lines suitable for manufacturing.

Experimental approach: CHO-M cell lines expressing various recombinant Easy to Express Proteins (ETEPs) and Difficult to Express Proteins (DTEPs) were used in an 'omics analysis. Differentially modulated genes were found to belong to protein folding, vesicular trafficking and the energy metabolisms pathways. We developed molecular tools and engineering strategies to stably overexpress these candidate genes as potential Metabolic-Improving-Proteins (MIPs) to optimize cell fitness to a given product. We created a collection of genetically engineered CHO-M cells (so called CHO-Mplus Libraries™) targeting general cellular metabolisms and specific cellular machineries.

Results and discussion: We found that a combinatorial engineering approach provided consistent solutions to restore and improve many cellular functions overloaded by the recombinant proteins. We show cases with customized engineering solutions to generate CHO cell lines expressing DTEs with high productivities while maintaining product quality and attributes. By providing a global engineering strategy, the Selexis' SURE CHO-Mplus Libraries™ platform offers a comprehensive and innovative solution for successful therapeutics cell lines generation.

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EVALUATION OF THE HT-NIC TECHNOLOGY TO GENERATE CLONAL CELL LINES

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Background and novelty: It is required that cell lines used in pharmaceutical production must be derived from a single cell referred as "clonality". Together with ALS Automated Lab Solutions we have developed a novel cloning technology: High-throughput Nanowell-based Image-verified Cloning (HT-NIC). It reliably images individual cells seeded into nanowells and automatically isolates selected clones from liquid medium using the ALS CellCelector picking platform. We have evaluated the performance and potential of the technology for routine application in cell line development.

Experimental approach: Experiments were designed to validate the technology with respect to reliability of imaging, efficiency of picking, colony growth and absence of cross-contamination. In addition, site-by-site cell line development campaigns were performed using either the classical semi-solid procedure of our CHO.RiGHT platform or the novel HT-NIC technology. Production cell lines derived from both cloning methods were compared in fed batch processes and best producing cell lines were taken forward to long-term expression analysis.

Results and discussion: The HT-NIC technology allowed highly efficient detection and isolation of monoclones with in-process validation. Fed batch performance was comparable between producer cell lines generated with HT-NIC and semi-solid cloning which is considered one of the most gentle approaches to isolate cell clones.

In conclusion, the HT-NIC technology allows generating clonal high producer cell lines with a single cloning round.

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SYSTEMATIC IDENTIFICATION OF KEY METABOLIC REGULATORS OF CELL FATE

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Background and novelty: Metabolism generates regulatory signals that affect all other molecular levels, from epigenome to proteome, and which are powerful enough to modulate key cell decisions such as proliferation, differentiation and death. Metabolic-driven regulation of epigenome, for example, is usually studied by looking at the substrates of epigenetic enzymes, but such targeted approaches leave out metabolites that could have remote regulating effects.

Experimental approach: In this work, we propose a new way to systematically identify metabolic pools that regulate stem cell fate, by finding those whose intracellular concentrations are more tightly controlled. We applied principles of process dynamics and control to the intracellular metabolite pools of human pluripotent stem cell lines (hiPSC) and human neural stem cell lines (NSC) challenged with a step increase in extracellular glutamine.

Results and discussion: Over 200 metabolites were identified and quantified in independent experiments with 2 hiPSC and 2 NSC cell lines. Dynamic metabolic responses to the glutamine step were found to be reproducible and cell-specific by comparing steady-states before and after the challenge as well as dynamic response profiles. Importantly, many amino acids had conserved dynamics and readjusted their steady state concentration as a response to the increased glutamine influx. On the other hand, some metabolites present clearly distinct dynamic responses between hiPSC and NSC. The characterization of these differences allows for the identification of key metabolites in each cell type which could be critical for the design of targeted approaches for stem cell processing.

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AUTOMATED MINI-POOL APPROACH FOR HIGH PRODUCING CELL LINE DEVELOPMENT

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Background and novelty: Chinese hamster ovary (CHO) cells are widely used as host cell line for the production of therapeutic recombinant proteins. However, Cell Line Development (CLD) process still requires improvement to find an efficient method for the selection of high-yield producers. In this context, the mini-pool (MP) approach consists in dividing a transfected cells population into several tiny populations. The detection of high producing cells is then facilitated thanks to the reduced competition occurring within these MP.

Experimental approach: The MP approach was implemented by plating the cells in 96-well plates after transfection. In order to efficiently screen and expand the mini-pools in static cultures and reduce the amount of work, a technological platform was dedicated to MP's growth and productivity monitoring. The system is composed of a cell imager [Cell Metric] associated to a robot [Orbitor] and an automated incubator [Cytomat]. The imager software which records the cell confluence of each well makes the documentation and growth monitoring easier. After recovery, all mini-pools are directly screened in 96-well plates to measure their productivity performances using the [Octet], a high-throughput analyzer.

Results and discussion: This semi-automated platform allows the simultaneous screening of 3150 MP. The highest the number of mini-pools screened is, the highest the probability to isolate a high producer is. Beyond the technical capabilities of the platform, statistics were combined to CLD to reduce the number of MP to screen to ensure the same performance results.

This platform demonstrated the importance of automated systems to achieve high-yield CLD thanks to the significant increase of data generated and analyzed. These automated methods combine to statistics are today necessary to efficiently create, screen and isolate high producing clones with reduced cost.

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STABILIZING EXPRESSION BY (EPI)GENETIC EDITING IN CHROMOSOMAL SITES

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Background and novelty: High and stable transgene expression is often compromised by epigenetic silencing while the underlying mechanisms usually remain elusive. Both the chromosomal environment and the sequence of the incoming cassette contribute to the degree of epigenetic repression of a promoter. Thus, the optimization of constructs to the site of integration and to the cell line is thought to provide a robust production of the transgene. We developed a strategy to optimize transgene cassettes for defined integration sites to obtain stable and high transgene expression

Experimental approach: We investigated epigenetic restrictions of different chromosomal sites upon transgene integration. We focused on chromosomal sites both in CHO K1 production cells as well as in embryonic stem (ES) cells that are affected by epigenetic repression. Employing Flp recombinase mediated cassette exchange (RMCE) we targeted various expression cassettes for locus specific optimization and identified the mechanisms of epigenetic restriction.

Results and discussion: Utilizing Flp mediated cassette exchange and treatment with specific modifiers w**e** identified different epigenetic mechanism that limit or even silence transgene expression in the various cell lines. Several genetic elements were investigated upon targeting into the particular sites. The integration of an UCOE 5' to the transgene promoter supported higher transgene expression levels while its impact was depending on the nature of the integration site. By specific targeting of epigenetic modifiers (e.g. the catalytic domain of Tet1c) to the transgene promoter (epigenetic editing) we could overcome the repression. Together, this strategy provides the option to design site and construct specific optimized expression systems.

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CHARACTERISTICS OF NOVEL HOST CELL LINES FOR INTENSIFIED PROCESSES

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Background and novelty: With the increasing demand for efficacious, safe, and affordable biologicals, process intensification and advanced process strategies are increasingly employed in biomanufacturing. Both high cell density fed-batch and perfusion cultures can be implemented to achieve cell concentrations of greater than 50x106 cells/ml. Routine implementation however, is challenged by a limited understanding of cellular behavior, metabolism and environmental stresses placed these culture processes. Here we discuss the selection and engineering of host cell lines with desired characteristics for an intensified culture process.

Experimental approach: The cellular state and metabolic behavior of recombinant protein producing CHO cell lines and factors affecting specific growth, productivity, protein quality profiles were evaluated in high-density fed-batch and perfusion processes. Cellular state and metabolic behavior is greatly affected by shear stress. Molecular mechanisms involved in the response of CHO cells to mechanical forces and effects on the cell cycle are discussed. Additionally, lipid composition and membrane structure and strategies to favorably manipulate them are discussed. Other cell line characteristics such as oxygen consumption rate and response to oxidative stress are currently areas of focus.

Results and discussion: As efforts to create higher performing CHO host cell lines in intensified processes continues, a review of the environmental stresses and demands on producing cell lines indicates a need for developing novel cell lines with modulated growth, metabolism and productivity characteristics. Advances in cell line gene editing technologies allow for engineering in various desired phenotypes and traits into manufacturing cell lines. This review focuses our attention on aspects of host cell line engineering for the development of robust and stable cell lines for superior performance in intensified processes.

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ACCELERATE CLINICAL ENTRY BY AUTOMATING CELL LINE DEVELOPMENT

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Background and novelty: Despite the race to bring new biotherapeutics into clinical trials, the cell line development process requires much manual work and is often associated with long development timelines and high costs. There is a great need for a workflow-based data integration solution to increase experimental throughput and to support complex, multi-stage cell line development for any type of novel biotherapeutic modalities in order to more efficiently address unmet clinical needs.

Experimental approach: We have developed a new end-to-end platform (Genedata Bioprocess®) for bioprocess development of all types of novel biotherapeutics: IgGs, novel bi- and multi-specific formats, fusion proteins, new scaffolds and cell therapeutics such as CAR-T cells. The system supports all steps in the cell line development workflow including seeding, selection, passaging, analyzing, cryo-conservation and processing in micro-bioreactors, such as ambr®. It tracks manual or fully-automated cell pool and clone selection as well as cell maintenance workflows in plates and shake flasks. 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 (liquid handling systems, measurement devices, and bioreactors).

Results and discussion: Here, we present a full cell line development campaign data set compiled using this platform and highlight the clone selection decisions and clone history reporting capabilities. We will discuss how the platform streamlines generation and assessment of mammalian production cell lines.

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HIGH-EXPRESSING CHO CELLS VIA EXPRESSION CASSETTE DESIGN

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Background and novelty: The search for the best-producing clone is often compared to seeking a needle in a haystack. While much effort is made on clonal selection, typically, expression cassette optimization, including promoter and enhancer selection, are often overlooked; a generic CMV promoter is used instead. Also, often overlooked is a statistical approach on number of clones to screen to get a good producer. The number of cell lines derived from Chinese Hamster Ovary (CHO) cells containing an expression vector such as CHOK1, CHOS, CHOM... illustrate the diversity of CHO-based systems; however, there is little diversity in the expression cassettes. Mammalian cell-based expression systems can use many combinations of promoters/enhancers from diverse origins including endogenous and viral sequences driving constant or inducible expression. These regulatory elements can be used in a single expression cassette or two based on the need, for example, to express 2 antibody chains.

Experimental approach: We tested 61 combinations including promoters/enhancers from various origins, endogenous and viral, signal peptides, polyAs, introns, to come up with 15 best combinations. These combinations were tested within a CHO-K1 system, then tested with different model antibodies to confirm the high expression level observed was constant regardless of molecule. We extensively assessed our screening scale-down model through a statistical approach ensuring that enough pools, mini-pools, clones were selected at each step to get to the optimal expression levels. This statistical approach also helped in reducing the overall timeline of cell line generation.

Results and discussion: The results generated by the molecular approach, with a better selection of promoters/enhancers and other genetic elements combined to a statistical approach, new protocols, mini-pool approach and optimized flow demonstrate that some significant productivity improvements can be achieved using a CHO-K1 expression system.

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ENHANCED CHO CLONE SCREENING USING NGS + TARGETED LOCUS AMPLIFICATION

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Background and novelty: Early analytical clone screening is important during CHO cell line development of biotherapeutic antibodies to select clones with most favorable stability and product quality. While sensitive sequence confirmation methods using mass spectrometry have limitations in throughput and turnaround time, next-generation sequencing (NGS) technologies have emerged as alternatives for clone analytics. Here, we report an efficient NGS workflow applying the Targeted Locus Amplification (TLA) strategy for genomic screening of antibody expressing CHO clones.

Experimental approach: In contrast to previously reported RNA sequencing approaches, TLA allows for targeted sequencing of genomic integrated transgenic DNA without prior locus information with only a few primer pairs, thus enabling robust detection of single nucleotide variants or genomic rearrangements.

Results and discussion: During selection of clones for a monoclonal antibody project, TLA/NGS revealed a high-level missense point mutation within the antibody heavy chain gene in several CHO clones, which could be confirmed at protein level by LC-MS. We determined detection limits and quantification thresholds of the TLA method for single nucleotide variants and for potential contamination of clones with different transgene integration sites. We further demonstrate the capability of the method to detect genetically identical "sibling clones". In another case, TLA/NGS enabled the identification of a rearrangement on plasmid level resulting in a truncated version of the light chain in some of the plasmid copies. In summary, we show that TLA is a robust screening method useful for routine clone analytics during cell line development with the potential to process up to 24 CHO clones in less than seven workdays including data analysis.

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ENGINEERED TRANSPOSASE DOUBLES TITER OF CHO PRODUCER CELL LINES

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Background and novelty: Most systems for generation of stable CHO producers still rely on random integration of transfected plasmid vectors. Yet, as active chromosomal loci are rare thousands of clones have to be screened to obtain high producers. The alternative targeting of pre-selected genomic sites is intriguing because it offers a largely reduced screening. However, despite extended effort to identify such sites very few systems approach productivities of clones obtained by random integration. In this context transposase-mediated gene integration provides a new option. Multiple integrations of single cassettes occur simultaneously yet integration sites are considered preselected for expression by the respective transposase. Here, we not only compare our CHO.RiGHT platform based on random integration with a piggyBac transposase approach but have further engineered the transposases for higher performance.

Experimental approach: Engineered and conventional piggyBac transposase genes were synthesised and introduced into vectors for transient expression. CHO.RiGHT platform vectors containing heavy and light chain genes of an antibody were equipped with transposase ITRs and transfected with or without the respective transposase vectors into DG44 cells. After puromycin/dhfr selection resulting clone pools were compared in shaken fed batch processes.

Results and discussion: To our surprise, clone pools derived from the CHO.RiGHT platform approach significantly outperformed all pools generated with the unmodified piggyBac transposases. However, for one of the engineered transposases a 10-fold higher titer than for the respective unmodified version was obtained. The best engineered transposase reproducibly provided clone pools that exceeded productivity of those generated using the CHO.RiGHT platform by a factor of two. The novel design may take cell line engineering by transposons to a new level.

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THE CELLULAR IMPACT OF GLYCOENGINEERING

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Background and novelty: Protein glycosylation is fundamentally important to most biological processes and it is often important to regulate it in biopharmaceutical development. Thus, substantial efforts have been taken to engineer glycosylation of a variety of biologics. However, the diversity and complexity of glycosylation have made it difficult to control glycosylation and unravel how engineering efforts impact the host cells.

Experimental approach: To enable rational glycoengineering and elucidate how such strategies impact the host cell, we have comprehensively studied the impact of glycoengineering on more than 180 CHO cell clones, wherein each has single or multiple glycosyltransferase genes knocked out. First, the clones were all glycoprofiled, and we developed a novel computational platform to rapidly study the changes in glycosylation across all mutants. Second, we quantified the impact of different glycosyltransferase knockouts on the bioprocessing phenotypes of the CHO cells (e.g., cell size, growth, viability, and metabolism). Finally, we conducted a large-scale RNA-Seq study of the clones to study the molecular basis of the phenotypic changes.

Results and discussion: Here we identified dominant glycosyltransferses in CHO cells, and studied instances wherein the cells differentially expressed isozymes in response to a knockout. Furthermore, we identified groups of glycosyltransferases whose deletion had a more severe impact on cell glycoprofiles and phenotype. We also found specific molecular pathways were perturbed when different glycosyltransferase families were perturbed. Through this effort we are gaining a more comprehensive view of the impact of glycoengineering on biopharmaceuticals and the host cells producing the recombinant protein drugs.

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OPTIMIZATION OF 2G UNIC TECHNOLOGY FOR DTE PROTEINS IN CHO GS CELLS

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Background and novelty: 2G UNic™ technology consists of a combination of optimized genetic elements which synergistically improve transcription and translation of recombinant proteins. The elements are inserted into existing (platform) vectors or provided as complete ready-to-use vectors to boost protein production in stably transfected CHO cell lines. With antibiotic or DHFR selection the technology routinely results in 2-3 fold increased expression in pools and clonal cells.

We successfully combined 2G UNic™ technology with glutamine synthetase (GS) selection for high titer (>5 g/L) mAb expression in different CHO GS-/- cells. Here we present further development of vectors for increased expression of difficult-to-express (DTE) proteins. We also show that multigene expression vectors can be generated by single step plasmid cloning.

Experimental approach: Expression vectors with 2G UNic™ genetic elements were modified by introducing Type IIS restriction sites to enable single step insertion of heavy and light chain genes.

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: We present case studies of the application of 2G UNic™ technology in expressing DTE pharmaceutical proteins in the CHO GS platform by adding the elements to an existing client vector as well as by using the convenient single step cloning ProteoNic vectors. The data show that the 2G UNic™ genetic elements can be successfully used to obtain significantly increased titers of DTE proteins, including fc-fusion proteins, bi-specific Abs and large multi-subunit complex biotherapeutics.

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COMPARISON OF CHINESE HAMSTER MULTI-TISSUE AND OVARY CELL PROTEOMES

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Background and novelty: Chinese hamster ovary (CHO) cells are the predominant production vehicle in the biotechnology industry. Quantitative proteomics data were obtained from CHO cell lines (CHO-S and CHO DG44) and from multiple Chinese hamster tissues including liver and ovary. This multi-tissue and multi-cell line analysis aims to improve our understanding of both hamster as the original tissue source for CHO cell lines as well as CHO cells through a comparison elucidating similarities and differences across cells and tissues.

Experimental approach: In this study, comparative proteomics was employed to compare two representative CHO cell lines and different tissues from Chinese hamster. Briefly, proteome samples were sonicated and subjected to reduction, alkylation, digestion, fractionation, and MS identification. CHO-S and CHO DG44 cell lines were used as model cell lines for comparison to hamster tissue expression patterns. In addition, different organs including liver and ovary were used to generate the tissue proteome.

Results and discussion: 11,801 unique proteins were generated including 9359 proteins from cell lines and 6663 protein from liver and ovary tissues. Proteins upregulated in cells compared to tissues using gene ontology and KEGG pathway analysis indicated increased cell cycle activities in cells while tissue functions increased included glycosylation and lipid transporter. Cellular components upregulated in tissues included the Golgi apparatus. In conclusion, this experiment expands on CHO knowledge by establishing a catalogue to differentiate between cells and tissues, thus creating the most extensive Chinese hamster proteome to-date. In addition, this resource provides insights into potential targets of intervention for improving the application of CHO cells for production of biopharmaceuticals.

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A COMPARISON OF CHINESE HAMSTER MULTI-TISSUE AND OVARY CELL PROTEOMES

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Background and novelty: Chinese hamster ovary (CHO) cells are the predominant production vehicle in the biotechnology industry. In this manuscript, quantitative proteomics data were obtained from CHO cell lines (CHO-S and CHO DG44) and from seven Chinese hamster tissues (brain, heart, kidney, liver, lung, ovary and spleen) by tandem mass tag (TMT) labeling followed by mass spectrometry (MS). This multi-tissue and multi-cell line analysis aims to improve our understanding of both hamster as the original tissue source for CHO cell lines as well as CHO cells through a comparison elucidating similarities and differences across cells and tissues.

Experimental approach: In this study, we employed comparative proteomics to compare two representative CHO cell lines and different tissues from Chinese hamster. Briefly, proteome samples were sonicated and subjected to reduction, alkylation, digestion, labeling, fractionation, and MS identification. CHO-S and CHO DG44 cell lines were used as model cell lines for comparison to hamster tissue expression patterns. In addition, multiple different organs (brain, heart, kidney, liver, lung, ovary and spleen) were used to generate the tissue proteome and identify characteristics of each that are highly abundant.

Results and discussion: Our experiment has generated an expansive tissue map of the Chinese hamster proteome. In conclusion, this experiment expands on CHO knowledge by establishing a catalogue to differentiate between cells and tissues, thus creating the most extensive Chinese hamster proteome to-date. In addition, this resource provides a holistic view on how expression patterns vary according to critical functionalities in different tissues and cells and provides insights into targets of intervention for improving the application of CHO cells for enhanced production of biopharmaceuticals.

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DOES CHO GENE EXPRESSION CHANGE IN RESPONSE TO EXPRESSED MAB VARIANT?

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Background and novelty: Despite recombinant Chinese hamster ovary (CHO) cell lines are developed and cultivated under comparable conditions, the antibody expression potential of individual monoclonal antibodies (mAbs) can differ significantly. As IgGs are secretory proteins, we considered that insufficient secretion might result from the intrinsic antibody structure and its interaction with cellular compartments of the folding and secretion machinery [1,2]. This could lead to an accumulation of antibody fragments in the ER lumen, which might induce ER stress and hence activate the unfolded protein response. Therefore, we analyzed antibody secretion levels as well as changes in gene expression in response to the expressed mAb variant.

Experimental approach: To explore responsible factors, the "difficult-to-express" anti-HIV1 antibody 2G12 and its germline-derived cognate mAb, 353/11, were compared. Both mAb variants were stably expressed in a defined chromosomal environment in CHO cells. These cell lines were cultivated in a semi-continuous perfusion process to supply the cells daily with nutrients and avoid accumulation of toxic by-products. In semi-continuous perfusion, differences in secretion and gene expression were evaluated in the quasi-steady state. To induce ER stress, CHO cells were treated with tunicamycin, an ER stress-inducing agent that inhibits N-glycosylation.

Results and discussion: Overall, distinct differences in mAb productivities were observed. Intracellular and secreted product was analyzed by immunoblotting to enable tracking of heavy and light chain fragments as well as accumulation of certain antibody fragments. We evaluated process-relevant markers in response to the expressed mAb variant and to imposed ER stress. These methods help us to investigate the phenomenon of differential antibody expression levels and may provide details on targets to modulate recombinant antibody production in CHO cells.

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THE INFLUENCE OF THE CELL CULTURE MEDIUM ON MEASLES VIRUS PURIFICATION

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Background and novelty: Oncolytic viruses, such as the Measles virus (MV), directly lyse cancer cells and thereby stimulate the patient's immune system. For a single therapeutic dose, 10^8 to 10^{11} infectious and pure MV particles are needed (Russell et al. 2014). Therefore, one bottleneck for a widespread cancer therapy with oncolytic MV is the manufacturing process. By using modern PAT and bioreactor technology, we are able to produce MV titers of 10^{10} $TCID_{50}$ mL^{-1} (Grein et al. 2017). At present, we are developing downstream processing (DSP) strategies to efficiently concentrate oncolytic MV and reduce the impurities (e.g. host cell proteins and DNA, non-infectious virus particles) at the same time to appropriate limits set by regulatory authorities. In this context, we investigate the influence of the feed stream composition on the DSP outcome.

Experimental approach: We produced oncolytic MV in a serum-containing and a serum-free medium. We used the MV-containing supernatants as feed streams in different DSP unit operations.

Results and discussion: The composition of the feed stream had a major impact on the DSP outcome. We determined an unwanted virus reduction of ~1 log order after clarification by depth filtration when we switched the production medium from serum-containing to serum-free. We studied the effect of several additives on the MV recovery in the clarification process, but none could reduce the MV loss. The virus-membrane adsorption was complex and not solely of electrostatic or hydrophobic nature. Similar effects were found in the purification step using chromatography- and filtration-based DSP unit operations. During filtration, it was possible to reduce unwanted adsorption by cross flow filtration. Our work impressively showed that changes in the upstream process, e.g. of the cell culture medium, was able to generate a big impact on the DSP outcome.

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CHALLENGING 2 CELL RETENTION DEVICES FOR VIRUS PRODUCTION IN PERFUSION

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Background and novelty: Perfusion systems allow vaccine and viral-based gene therapy manufacturing to shift to more flexible and intensified virus production. Currently, the preferred cell retention technology is the membrane-based ATF system, yielding high virus titers [1, 2]. However, virions generally do not efficiently pass through the membrane, leading to potential virus degradation in the bioreactor. Here, we compared virus production performance of an ATF system with an acoustic settler technology allowing continuous harvesting.

Experimental approach: Suspension avian cells (AGE1.CR.pIX) were cultivated in CD-U3 chemically defined medium in 0.6 L and 1.2 L Biostat bioreactors (Sartorius). Perfusion rates were manually adjusted to achieve a constant cell-specific feed. Hollow-fiber based perfusion was done with an ATF2 (Refine) system with a 0.2 µm PES membrane (Repligen). Perfusion cell culture with continuous harvest was operated using an acoustic settler system (SonoSep). For infection, influenza A/PR/8/34 H1N1 (RKI) or a recombinant modified vaccinia Ankara virus (MVA-CR19.GFP) (ProBioGen) was used.

Results and discussion: Successful cell culture was performed using both perfusion systems. Cell concentrations up to 50 x 10^6 cells/mL with a doubling time similar to batch cultivations was reached. When producing influenza A virus using the acoustic settler, productivity was increased by more than a factor of 2 (1.4 x 10^{12} virions/L/day). A similar productivity was observed for MVA production using both systems. Cell culture recirculation strategy, perfusion harvest rate and residence time in the acoustic settler were found to be critical parameters to achieve the desired goals. Additional process optimization and further parameter analyses including product quality and process robustness will be performed. Overall, it seems that continuous harvesting of virions using an acoustic settler is highly beneficial.

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INTEGRATED PROCESS FOR MDCK-BASED INFLUENZA VACCINE MANUFACTURING

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Background and novelty: Human influenza virus infections are not only a threat for the health of millions of people, but also have a significant impact on local and global economies. Cell culture-based viral vaccines could reduce manufacturing time to improve pandemic preparedness. In this work, we analyze cell growth, cell metabolism, virus infection, and virus purification to optimize a MDCK cell-based process for influenza virus production. We demonstrate a productive and scalable integrated platform for next-generation influenza vaccine manufacturing.

Experimental approach: MDCK suspension cells were cultivated in three parallel DASGIP bioreactors (0.3-0.6 L wv) in batch mode. For virus propagation, MDCK cells were diluted with medium, supplemented with trypsin, and influenza virus (A/PR/8/34 H1N1) was added at a MOI of 10^{-3}. Cell, metabolite, influenza virus, and host cell contaminant levels were measured to identify the optimal time for harvest. Clarified virus broth was purified using single-use steric exclusion chromatography (SXC) and membrane-based pseudo affinity chromatography (SCMA) to analyze final product quality and purity.

Results and discussion: MDCK cells cultivated in chemically defined medium showed excellent growth performance in bioreactor replicates (t_D <21 h) reaching concentrations of almost 10^7 cells/mL within 72 h. Very high total (3.6 lg(HAU/100 µL)) and infectious (>10^9 virions/mL) virus titers were reached for the optimal harvest point at 21-24 hours post infection. With only one DNA digestion, one capture (SXC), and one polishing (SCMA) step, we achieved not only a high recovery (>85 %), but also a high product purity, complying with regulatory demands. Based on the final HA-antigen content about 300 vaccine doses per liter of cultivation volume were produced. Advances in cell growth, cell-specific virus yield (12,000 virions/cell), and overall productivity make this process unique for cell culture-based influenza vaccine manufacturing.

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EXPRESSION OF HEPATITIS B VIRUS SURFACE ANTIGEN IN MAMMALIAN CELLS

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Background and novelty: Up to 6% of population is chronically infected with Hepatitis B virus (HBV). New cases can be prevented by vaccination. The main antigen of HBV is the surface antigen (HBsAg) that consists of three co-carboxyterminal surface glycoproteins, Large (L), Middle (M), and Small (S) that share one open reading frame with three translation initiation codons. Currently available vaccines in Latin America for Hepatitis B are produced in yeast, including only a non-glycosilated form of S. Although highly efficacious, about 10% of population are non-responders to this vaccine. One approach to overcome this, is the development of new generation vaccines where L, M and S glycoproteins are included as Virus-Like Particles (VLPs). Thus, we generated stable CHO-K1 and HEK293 mammalian cells lines expressing the three HBV surface glycoproteins, as potentials substrates for a production system of a new generation vaccine for Hepatitis B.

Experimental approach: The coding sequence of S glycoprotein was cloned into third generation lentiviral vectors and used to transduce CHO-K1 and HEK293 cells. Later, the cells were transfected with a plasmidic expression vector carrying the coding sequences of M and L glycoproteins. Expression levels and localization of glycoproteins were analysed by flow cytometry and fluorescence microscopy. VLPs budding to supernatant from producing cells were quantified by ELISA and their antigenicity was analysed by Western blot.

Results and discussion: The expression levels and the subcellular localization of L, M and S glycoproteins were similar in CHO-K1 and HEK293 cell lines. VLPs composed of the three glycoproteins were detected at supernatants of both cell lines. Since the selection of a cell substrate is a key step in the development of a vaccine candidate, these results encourage further studies to evaluate biochemical and biophysical characteristics of these recombinant VLPs, as well as the analysis of their immunogenic properties.

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EXPLORING GENE TRAIT OF SUPERIOR INFLUENZA VIRUS PRODUCING MDCK CELLS

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Background and novelty: Madin-Darby canine kidney (MDCK) cell-based influenza vaccine production is substituting the traditional egg-based processes owing to many remarkable superiorities. Though process strategies have been established to achieve some improvements in virus titer, cell engineering is a prospective field to be explored in pursuing hyper-productive cell lines. However, the markers of superior virus producing cells and the mechanisms of interactive regulations remain to be elucidated. Thus, we aim to reveal the extraordinary talent of high producer on viral propagation for the first time, and lay the foundations for robust cell line engineering and rational process optimization.

Experimental approach: 130 MDCK clones were obtained and examined for influenza viral productivity. A high-producer was expanded to investigate the viral propagating process, and the cellular and viral gene expressions were quantified by transcriptome analyses using RNA-seq. Functional analysis of deferentially expressed genes and Gene Set Enrichment Analysis (GSEA) were applied to examine the vital Gene Ontology (GO) terms and metabolic pathways.

Results and discussion: Viral propagation and cellular responses were evaluated during a synchronized single-cycle infection. Higher viral titer and better cell maintenance were observed in the high-producer. Further exploration in the global transcriptome showed that the high-producer had more expression in ribosomal, mitochondrial and redox processes, moderate antiviral responses and endoplasmic reticulum stress to virus, and comparable viral mRNA content. Besides, the transcriptome data showed a greater divergence in time-serial than cell strains. These results paved the way for a better understanding on the cellular mechanism of higher viral productivity, and reveal latent gene markers, which provide potential targets for cell engineering and the selection of superior cell lines for influenza vaccine manufacturing.

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PRODUCING A NEW CLASS OF DEFECTIVE INTERFERING PARTICLES AS ANTIVIRAL

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Background and novelty: Influenza A virus is a major human pathogen that causes annual epidemics. Recently, defective interfering particles (DIPs) were proposed for use in antiviral therapy. Influenza DIPs typically contain a large internal deletion in one of the eight genomic viral RNA (vRNA) segments. Thus, they can only replicate in a co-infection with fully infectious standard virus (STV), which complements the "defective" gene segment. Concurrently, however, the defective interfering genome interferes with and suppresses STV replication. Recently, we discovered a novel type of influenza DIP, called OP7, which has nucleotide substitutions in segment 7 vRNA instead of large internal deletions [1].

Experimental approach: As OP7 replication relies on a co-infection, the produced material will contain STVs. For the establishment of a process with a high ratio of OP7 to STV, different multiplicities of infection (MOI) from 1E-1 to 1E-4 were screened in shake flasks. As an option for DIP manufacturing, suspension Madin Darby canine kidney (MDCK) cells in two chemically defined media (Smif8- and Xeno-CDM) were compared regarding the production of material with a high interfering potency. The potency was evaluated in an interfering assay, which quantifies the OP7-induced virus titre reduction.

Results and discussion: The interfering potency is strongly dependent on the MOI applied for DIP propagation. Higher MOIs increase the proportion of formed OP7, but also lead to overall lower virus titres and vice versa. The most potent material was produced with a MOI of 1E-2 in Xeno-CDM. In the interfering assay, this material reduced the infectious virus titre by a factor of almost 25,000 from 2.16E9 viruses/mL to 8.8E4 viruses/mL. This evaluation does not include the potential further reduction in titre caused by an enhanced innate host immune response. To inactivate the remaining STV, the material was further treated with UV-light, and will now be tested in animal trials.

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UNDERSTANDING VIRUS-LIKE PARTICLE (VLP) GENERATION IN HEK 293 CULTURES

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Background and novelty: Production of virus-like particles (VLPs) is of high relevance in the development of new recombinant vaccines. Like native viruses, VLP generation arises from the ability of structural viral proteins to self-assemble. In the case of HIV-1 Gag VLPs, this process occurs underneath the cell membrane, where the VLP finally buds to the extracellular space. The study of these dynamic processes by confocal microscopy has been traditionally limited by the Abbe diffraction limit. In this work, super-resolution has been applied to gain insight into VLP formation in HEK 293 cultures.

Experimental approach: We have developed a novel quantification method based on super-resolution confocal laser scanning microscopy (CLSM) and 3D-image analysis. The use of fluorescent GFP in frame with the Gag polyprotein enabled the evaluation of VLP production. Indeed, orthogonal characterization studies on harvested VLPs were also performed to assess product quality.

Results and discussion: Budding of individual VLPs and their attachment to cell filopodia were observed in HEK 293 cells. Early and late assembly sites were detected within the lipid membrane. Around 5E+11 assembly sites per mL of cell membrane were quantified at mid-production phase. This value represented less than 25% of the membrane volume, showing a window of improvement in terms of particle production in HEK 293 cells. Harvested VLPs were also directly observed and quantified by CLSM, obtaining concentrations in the same range as the reference techniques. Interestingly, nucleic acids within the nanoparticles, as well as the lipid envelope, could be detected by the CLSM through common staining methodologies. These results open the window towards the development of novel detection, diagnosis and quantification methods in the nanoscale range based on super-resolution image analysis.

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INCLINED SETTLER FOR INFLUENZA A VIRUS PRODUCTION IN PERFUSION MODE

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Background and novelty: Bioreactor operation in perfusion mode allows for high cell density processes with high yields in virus vaccine production. Previous process developments using ATF systems led to increased productivities compared to batch mode but direct virus harvesting through hollow-fiber membranes was not possible [1]. Given that a continuous harvest is desirable to reduce residence time and degradation of viruses in the bioreactor, we evaluated an inclined gravitational setter for influenza A virus production.

Experimental approach: AGE1.CR.pIX cells were cultivated in CDM in a 650 mL STR using an inclined settler for perfusion with peristaltic pump recirculation. The heat exchanger was cooled with water recirculation either using a thermostatic bath or from a container kept at room temperature (RT). Following the growth phase, cells were infected with influenza A/PR/8/34 H1N1 virus.

Results and discussion: Initial cultivations showed reduced cell growth in perfusion mode, which was probably due to cooling of the settler using the water bath. Doubling times (t_D) up to 68 h were obtained (reference using ATF [1]: 27-41 h). Growth was improved by operation of the settler at RT and by starting the recirculation shortly after inoculation (t_D = 27 h). High cell concentrations ($3x10^7$ cell/mL) and viabilities (91-96%) were achieved. After infection, viruses were continuously harvested at high titers enabling a significant increase in cell-specific virus yield (2663 virions/cell) and volumetric productivity ($1x10^{12}$ virions/d/L), compared to previous experiments performed with ATF [1]. Despite the initial challenges associated with recirculation of cells, we could demonstrate successful virus production using inclined settlers. As a simple and robust technology, which is used for recombinant protein production from lab to industrial scale, inclined settlers should also be considered for viral vaccine production.

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BACULOVIRUS-FREE RECOMBINANT PROTEIN PRODUCTION IN INSECT CELL LINES

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Background and novelty: The baculovirus/insect cell system has become a standard system for r-protein production. Although a powerful system, several limitations are associated to the presence of contaminating BVs and the viral infection itself. Hence, exploiting the potency of these hosts devoid of BV is of great interest. Here, a baculovirus-free, plasmid-based methodology with the cheap transfection carrier polyethylenimine has been developed for suspension-adapted Hi5 and Sf9 cells.

Experimental approach: In the present study, both insect cell lines were compared for the production of three different recombinant proteins: eGFP (intracellular), hSEAP (extracellular) and HIV-1 Gag-eGFP Virus-Like Particle (extracellular protein complex). To do this, a combination of statistical design (DoE) and nanoscale characterization techniques (Cryo-EM and DLS) were used. In the case of VLPs, several approaches based on live tracking (NTA and flow virometry) were employed to quantify and characterize the nanoparticles obtained in each system.

Results and discussion: The optimization of transfection conditions revealed that low cell densities were optimal for Hi5 cells (~60% transfection), whereas high cell concentrations were required for efficient Sf9 cell transfection (~50%). In addition, minimal DNA:PEI complexing time (< 1min) showed the best transfection yield in both cases. An increase in specific productivity was obtained in Hi5 over Sf9 cells regarding eGFP (0.8 vs 0.6) and hSEAP (1.9 vs 1.1 mg/10^6 transf. cell · day). However, higher productions of 11.9 and 23.6 mg/L were respectively achieved for both proteins in the Sf9 cell line. Interestingly, though VLPs were correctly self-assembled in both systems, Sf9 cells proved to be more efficient at assembling and producing these nanoparticles (1.9 vs 0.4·10^9 FP/mL) and also in terms of specific productivity (1.5 vs 0.5·10^8 FP/10^6 transf. cell ·day).

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TRANSIENT YELLOW FEVER VLP PRODUCTION USING HEK 293T IN A BIOREACTOR

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Background and novelty: Yellow fever (YF), an acute hemorrhagic disease in the tropics and subtropics, causes mortality rates between 20 % and 50 % and, thus, up to 60,000 deaths p. a.. At present, there is no treatment available for already infected people at all. In order to respond to the global issue of YF outbreaks, viruslike particles (VLPs) have proved to be a promising possibility to produce YF-antibodies for a passive immunization of already infected people.

We developed a production platform for the production of YF-VLPs, using HEK 293T cells on a bioreactor scale.

Experimental approach: Bearing in mind criteria such as optimal growth, a high transfection rate and the waiver to exchange the medium before/after the transient transfection, we established a YF-VLP production process, and optimized the transfection with the reagent PEI (polyethylenimine) in a bioreactor.

The production kinetics of the YF-VLPs was analyzed, using the fluorescent active eGFP, whereas a specific detection of the YF-VLP protein E was carried out using Western Blot analysis.

Results and discussion: Fed-batch and perfusion were applied to optimize the HEK 293T cell growth and to increase the cell number at transfection. Transfection studies were done with suspension cells and adherent growing cells on microcarriers. We investigated different media and transfection strategies on a static and a dynamic scale.

In order to guarantee a consistently high YF-VLP stability, quality and quantity, further studies are required. Parameters such as the T, pH or the salt concentration, e. g. during the transient transfection in the bioreactor, may have a significant influence on the product. Additionally, the impact of hydrodynamics in the stirred bioreactor on the YF-VLP quality and quantity will be verified.

We presume that the application of the appropriate medium, the bioprocess parameters and the running mode will further enhance the yield of YF-VLPs for a passive immunization against YF.

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VIRUS HARVESTING IN PERFUSION CULTURE: CHOOSING THE RIGHT MEMBRANE

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Background and novelty: Virus particle retention in membrane-based high cell density perfusion systems is poorly understood. Typically, the permeate flow goes into the waste while fragile products accumulate in the bioreactor. Most perfusion processes established for virus production use only one type of membrane, i.e. PES with 0.2 µm pore size. However, to harvest virions continuously through a membrane, different materials should be tested and properties such as pore sizes, surface charge and roughness must be taken into account for membrane fouling and virus retention.

Experimental approach: In this study we tested various membrane materials (ME, PES, mPES, PS, PE with pore sizes from ~0.05 to 10 µm) for harvesting yellow fever (YF) virus (~50 nm) propagated in BHK-21 cells (~14 µm) in cross-flow filtration mode. Membranes were operated at constant permeate fluxes and permeate-side pressure increases were recorded. In addition, virus, DNA and protein concentrations were measured in the permeate flow. New and blocked membranes were subjected to SEM to visualize surface structures.

Results and discussion: Despite identical surface-specific fluxes, fouling dynamics differed strongly in dependence on the roughness but little on the pore size. Initial virus retention was observed especially for membranes with small pore sizes (~0.1 µm), and increased with progressing filter fouling. Material-related DNA or protein rejections played a subordinate role. SEM analysis showing a smooth surface structure complemented observations regarding the best performing membrane (PS, 0.4 µm pore size) with a homogeneous, large-pores surface and very low virus retention. In consequence, results indicated that by correct choice of the membrane, virus particle harvesting over the complete production phase of perfusion cultures might be feasible.

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CELL-BASED HCV VACCINE ELICITS NEUTRALIZING ANTIBODIES IN MICE

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Background and novelty: Each year ≈2 million new infections with the hepatitis C virus (HCV) occur. Acute infection results in chronic infection in ≈80% of the cases, both causing unspecific minor or no symptoms. However, chronic infection can result in liver cirrhosis and cancer. There is no vaccine against HCV, considered to be required to control HCV globally. Infectious human hepatoma cell line (Huh7.5) culture systems might facilitate the development of an inactivated whole particle HCV vaccine.

Experimental approach: A highly cell culture adapted HCV recombinant with genotype 5a specific core-NS2 [1] was used for virus production in Huh7.5 cell culture in 10-layer cell factories without serum [2]. HCV from 8-80L supernatant was purified/concentrated by cross flow filtration, iodixanol ultracentrifugation and sephadex chromatography. UV-inactivated HCV equivalent to 7.0-8.1 log10 focus forming units (FFU) was formulated with Alum+monophosphoryl lipid A or Addavax and used for 4 immunizations of BALB/c mice. Purified mouse serum IgG was used in neutralization assays in vitro [3].

Results and discussion: Each cell factory yielded 5 harvests of 800mL supernatant with infectivity titers of ≈6.0 log10 FFU/mL. Up to 25% of input HCV was recovered and 2000-fold concentrated in the purification process. Serum IgG showed up to 90% or 80% neutralization of the genotype 5a virus with or without hypervariable region 1 (HVR1) [4], respectively, and up to 60% neutralization of a genotype 1a virus with HVR1. In conclusion, we show immunogenicity of cell-based inactivated whole HCV particles. To increase vaccine antigen amounts and to work towards a human vaccine, establishment of GMP-compatible and scalable high cell-density Huh7.5 cultures [5] and scalable downstream processes will be a future focus area.

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BIOPROCESS STRATEGIES TO ENHANCE VLP PRODUCTION IN STABLE INSECT CELLS

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Background and novelty: Stable expression of virus-like particles (VLPs) in insect cells has been increasingly explored to circumvent the bottlenecks of the baculovirus expression vector system (BEVS), but protein productivities achieved up to today are still low.

In this study, aiming at increasing productivities, two bioprocess engineering schemes were designed and evaluated: (i) adaptive laboratory evolution (ALE) of insect cell to hypothermic culture conditions and (ii) high-cell-density perfusion process.

Experimental approach: Sf-9 and High Five cells stably producing Gag-VLPs were adapted to hypothermic culture conditions by sub-culturing over 3 months. The generated adapted cell lines were pseudotyped with Influenza HA protein and their performance benchmarked against IC-BEVS for production of Gag-HA VLPs. The scalability of the process was assessed in stirred-tank bioreactor. A high-cell-density perfusion process was implemented using XCell™ATF system for the production of Gag-HA VLPs in Sf-9 cells.

Results and discussion: Adapted cells expressed up to 26- and 2-fold more Gag-VLPs and Gag-HA VLPs, respectively than non-adapted cells. VLPs production in adapted cells was successfully demonstrated in stirred-tank bioreactors. Transmission electron microscopy images confirmed the presence of particles resembling Gag-VLPs and Gag-HA VLPs, both in size and morphology, thus confirming the null impact of the adaptation process on particles formation.

In perfusion, we were able to maintain the specific productivity for more than 20 days, reaching a maximum cell concentration of $100x10^6$cells/mL which represents a 10-fold increase comparing to stirred-tank bioreactor operated in batch mode.

Overall, the stable insect cell platform herein proposed can be applied for the production of other VLP-based products.

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ADAPTIVE EVOLUTION OF INSECT CELLS FOR IMPROVED PRODUCTION OF HA VLPS

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Background and novelty: Adaptive laboratory evolution (ALE) has been widely used for the selection of cell populations with improved phenotype, this being increased tolerance to a certain selective pressure, optimization of substrate utilization or higher recombinant protein titers. In this study, we used ALE to select a high-performance insect cell population adapted to grow at neutral pH, capable of over 2-fold higher specific productivity of Influenza hemagglutinin-displaying virus-like particles (HA-VLPs), using the insect cells-baculovirus expression vector system (IC-BEVS).

Experimental approach: An insect cell line adapted to grow at neutral pH was established by sub-culture of High Five cells in increasingly higher culture pH, in a step-wise manner, for over 2 months. HA-VLPs were produced by infecting cells with an M1/HA-encoding recombinant baculovirus, at different cell concentrations at infection (CCI) and multiplicities of infection (MOI). HA titer was assessed by hemagglutination assay, HA and M1 were identified by western blot and VLPs morphology evaluated by transmission electron microscopy. Scale-up from shake-flask to bioreactor was performed.

Results and discussion: Adapted cells yielded higher HA titers than non-adapted cells in CCI/MOI tested, reaching over 2-fold increase in specific HA productivity. The adaptation process, rather than the neutral pH adaptation medium, was found to be the crucial factor contributing to maximize HA titers. VLPs produced by adapted cells were morphologically concordant with those from common industrial cell lines. Specific HA productivity of adapted cells was maintained for several generations and, importantly, when scalep-up to bioreactor scale, showing the robustness and applicability in a controlled and readily scalable system. These results demonstrate the potential of ALE to select insect cell populations with improved fitness for the production of VLP-based vaccines using IC-BEVS.

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PHYSIOLOGICAL CHARACTERIZATION OF HEK293 USING A PROTEOMIC APPROACH

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Background and novelty: The use of mammalian cells such as HEK293 along with transient gene expression (TGE) is becoming one of the platforms of interest to produce safer candidates for new and existing vaccines[Office1] . Today, the advances in techniques like quantitative proteomics allow to develop a detailed metabolic and physiological profile of cell cultures in different conditions This work is focused on HEK293 cultures producing HIV-Gag polyprotein virus-like particles (VLPs), which represent a potential candidate for a future HIV therapy. Different conditions have been tested: growing cells without transfection, to define the physiology of the cell density effect, cells transfected with an empty plasmid to characterize the effects of transfection and cells transfected with a plasmid codifying for Gag polyprotein to determine the VLP production effect on cell physiology.

Experimental approach: A quantitative proteomic approach using TMT-10plex and further statistical data analysis and bioinformatic tools were applied to obtain all proteins and functional categories up and down-regulated in each condition. In addition, VLPs and exosomes produced by the cell were purified by ultracentrifugation and also analyzed.

Results and discussion: A total of 7280 proteins and 265 biological processes were identified. An increase in mitochondrial activity as well as a decrease in lipid biosynthesis and nuclear intracellular protein transport are assigned to the cell density effect. An overall disruption of cellular homeostasis and cell viability was found after transfection and an increase in exosome production correlated with VLP production. Metabolic engineered solutions were assessed, such as the overexpression of proteins from the pathway targeting Gag to the cell membrane or the addition of lipids to the culture so as to improve VLP production towards a more sustainable, intensified and efficient bioprocess.

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EXTRACELLULAR VESICLE CHARACTERIZATION DURING LENTIVIRAL PRODUCTION

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Background and novelty: Lentiviral vectors (LVs) are a powerful tool for gene therapy. Human embryonic kidney cells (HEK293) have been used extensively as a platform for viral vaccine and viral vector production. Similar to most cells and body fluids, HEK293 cells release extracellular vesicles (EVs). EVs released by cells share similar size, biophysical characteristics and even biogenesis pathway with cell-produced enveloped viruses, making it a challenge to efficiently separate EVs from LVs. Thus, EVs become "impurities" in the context of gene therapy viral vectors, as they co-purify with LVs during downstream processing.

The aim of this study is to characterize EVs during LV production and to identify their changes upon lentiviral production from a proteomic, lipidomic and transcriptomic perspective.

Experimental approach: The first step is to optimize the EV isolation process and to separate EVs from LVs as the proteomic and RNA profiles are clearly affected by the isolation method. After isolation, EVs are characterized by transmission electron microscopy (TEM) and western blot (WB). Additionally, nucleic acid content is assessed as it is critical to quantify the amount of residual host cell DNA in biologics destined to the clinic. Mass spectrometry (MS) is used to determine the proteomic content of EVs.

Results and discussion: Preliminary data showed size exclusion chromatography (SEC) as an improved isolation method when compared to the gold standard ultracentrifugation. SEC yielded more intact and purer EVs as shown by TEM. WB and MS analysis confirmed the presence of EV enriched proteins (e.g. Alix, TSG101, CD9, CD63 and CD81). MS results showed that the presence of some proteins was medium dependent. However, it was observed that protein detection by MS was affected by sample preparation (e.g. addition of detergent). Results also showed that GFP was detected in EVs since the cell line used in this study expresses GFP constitutively.

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GENERATION OF A HEK293 PLATFORM TO PRODUCE ANTIGEN-PRESENTING GAG VLPS

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Background and novelty: Virus-like Particles (VLPs) are molecular structures that mimic the native conformation of viruses without carrying genetic information. Display of foreign antigens on Gag-based VLPs is an attractive approach for the development of recombinant vaccines, since the dense protein arrangements significantly enhance the response to otherwise low immunogenic epitopes. Therefore, having a HEK293 cell line that stably produce Gag-VLPs offers a great potential since 293 cells generate human post-translational modifications and can be easily transfected to test the immunogenicity of different epitopes.

Experimental approach: A HEK293 cell line was generated by transfection of a GagGFP expression plasmid, selected by gentamicin (G418) resistance and isolated clonally by FACS cell sorting. The clones showing the highest GagGFP production were adapted into suspension and serum-free conditions for a deeper characterization. Growth kinetics of each clone was studied in batch culture and Gag-VLP production was assessed by ELISA analysis of the supernatants. Finally, to assess its potential to co-express viral epitopes, the 10H9 clone was transfected with HIV-1 Envelope (Env) protein.

Results and discussion: The selected clone, 10H9, showed the highest specific productivity and reached a maximum cell density of $5{\cdot}10^6$ viable cells/mL with a duplication time of 26.7 hours. Stability analysis during 36 passages showed that 10H9 cultured without antibiotic selection reduced its fluorescence while 10H9 under G418 maintained almost unaltered its expression. When the 10H9 clone was transiently transfected with the Env protein, FACS analysis at 72hpt showed a 92% of double positive population for GagGFP and Env. Microscopy analysis confirmed its membrane co-localization, suggesting that 10H9 is a suitable platform for the expression of viral epitopes to generate functional GagGFP-based VLPs.

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IMPROVING GENE THERAPY VECTOR BIOPROCESS THROUGH METABOLIC ENGINEERING

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Background and novelty: Retroviruses (RV) and lentiviruses (LV) are among the most used vectors in gene therapy due to their efficient cell transduction and long-term gene expression. Recently, RV and LV based gene therapy products reached the market. Yet, their bioprocess is challenging and provides low yields of infectious virus.

Manufacture improvements can be accomplished using metabolic gene engineering to enhance cell performance and product quality. Previously, transcriptomic studies identified the metabolic pathways influencing vector productivity[1]. This work aimed to study the effect of those pathways – glutathione, endoplasmic reticulum protein processing and apoptosis – on RV and LV yields by overexpressing key genes in 293 derived cell lines.

Experimental approach: Candidate genes[1] targeting the metabolic pathways above mentioned, were overexpressed at incremental levels. Two human cell lines used for transient LV and stable RV production, 293T and 293 FLEX, respectively, were used. The delivered metabolic gene copies were controlled by transduction. Populations with different gene expression levels were characterized for cell growth, vector productivity, metabolic and viral gene expression.

Results and discussion: Depending on the vector, the metabolic gene and its overexpression level, different productivity outcomes were observed. Stable RV production yield changed from 0 – 10 fold. The best improvements were obtained for the glutathione engineering strategy, possibly by relieving cell redox burden. LV transient production was only increased when overexpressing an anti-apoptotic gene improving cell viability.

This work elucidates the influence of metabolic pathway functions on viral vector producer cells performance, vector yield and quality. Enabling to engineer robust and enhanced producer cells, contributing for improved manufacture of gene therapy products.

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CELL CULTURE SCALE-UP IN BIOBLU SINGLE-USE VESSELS

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Background and novelty: Bioprocess development is usually carried out at small working volumes. Subsequently, the bioprocess is scaled-up to larger volumes to produce more material for characterization, trial runs, and finally for commercialization. To maintain product yield and product quality while scaling up, bioprocess engineers usually aim to keep one or more process engineering parameters constant across scales. Keeping the power consumption per liquid volume (P/V) constant is a widely used strategy for scale-up. To calculate P/V, one needs to know the impeller power number (Np), a constant associated with different types of impellers. To support constant P/V-based bioprocess scale up, we determined the impeller power numbers of differently sized BioBLU c single-use bioreactors.

Experimental approach: We have experimentally measured the impeller torque of differently sized BioBLU c Single-Use Vessels by using a rotational torque sensor and calculated the vessels' impeller power numbers. For all BioBLU vessels tested, the impeller power numbers measured at different tip speeds were comparable. Therefore, a mean power number could be used to calculate P/V. Taking the tip speed range of each bioreactor into account, we identified a P/V zone within which constant P/V values can be maintained among different bioreactors in a certain range.

Results and discussion: Our results can help bioprocess engineers in setting up a P/V based scale-up strategy within the BioBLU c Single-Use Vessel family and beyond.

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DEVELOPMENT OF SUSPENSION ADAPTED VERO CELL CULTURE PROCESS TECHNOLOGY

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Background and novelty: Vero cells are considered as the most widely accepted continuous cell line for the manufacture of viral vaccines. The growth of Vero cells is anchorage-dependent. Scale-up and manufacturing in adherent cultures are labor intensive and complicated. Adaptation of Vero cells to grow in suspension will simplify subcultivation, process scale-up and manufacturing significantly, and therefore reduce the production cost. Here we report on a successful adaptation of adherent Vero cells to grow in suspension in serum-free media (IHM03) developed in-house and high yield productions of viruses at high cell density.

Experimental approach: An ATCC originated Vero cell line was adapted to grow in suspension in IHM03 using a process developed in-house. Suspension adapted Vero cells were then tested for authenticity and tumorigenicity. Virus productivity of the suspension cells was evaluated under various culture conditions using vesicular stomatitis virus (VSV) and influenza virus as models.

Results and discussion: The suspension adapted Vero cell culture in IHM03 grew to similar or better maximum cell density as what observed for the adherent Vero cells grown in commercial serum-free media. Much higher cell density ($8x10^6$ cells/mL) was achieved in a batch culture when three volumes of the culture medium were replaced. DNA samples of suspension Vero cells from various stages had 100% concordance with the Vero DNA control sample, indicating the suspension cells maintained their genetic stability. Furthermore, suspension Vero cells at a passage number of 163 were not found to be tumorigenic.

The suspension cell culture showed a better VSV productivity than the adherent Vero culture. In addition, the suspension culture could be infected at higher cell densities, thus improving volumetric virus productivity. More than one log of increase in the VSV productivity was achieved in a 3L bioreactor perfusion culture infected at a cell density of $6.8x10^6$ cells/mL.

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NON-VIRAL TRANSFECTION OF HUMAN T LYMPHOCYTES

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Background and novelty: Genetically engineered primary human T cells are relevant in basic research and in medical therapy. Their efficient transfection relays on viral or physical methods, both associated to some drawbacks; chemical methods are so far fairly inefficient. The results presented here show that it is possible to chemically transfect T cells with both transfection efficiency (TE) and viability above 80%.

Experimental approach: T cells were transfected with poly(2-dimethylamino) ethyl methacrylate (PDMAEMA) nanostars, while varying the amount of polymer per cell, the transfection volume and vessel, and the incubation time. Polyplexes-based procedures and direct addition of the transfection agent to the cell/pDNA mixture (high cell density transfection, HCD) were used for transfection.

Results and discussion: This study summarizes a systematic optimization of transfection strategies for genetic engineering of human T cells using Jurkat cells. In the polyplexes-based standard transfection protocol, TE >70% could only be achieved at severely reduced viabilities. Reducing polymer density and incubation time, in a tube-based format, improved TE and viabilities (both > 80%, shift towards middle and high producers). The HCD transfection protocol led to TE and viabilities >70%. In primary T cells, TE were about 4-fold lower than for Jurkat cells, but significantly better than with l-PEI. We argue that the polyplexes-based tube protocol could in future be used to effectively transfect T cells at small scale for research purposes. The HCD protocol has excellent scale up potential and could become the basis for medical applications of T cell therapies.

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SCALABLE SINGLE-USE TECHNOLOGY TO MEET GENE THERAPY PRODUCTION DEMANDS

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Background and novelty: The demand for viral vaccines and viral vectors for gene therapy is growing exponentially pushing for new production capacities. However, producing these therapies using traditional technology face a number of limitations including scalability, the need to be flexible enough to accommodate a range of therapies and vectors with ease and production at reasonable costs.

Experimental approach: To address these challenges, we have developed a portfolio of single-use, fixed-bed bioreactors – the scale-X™ systems – accommodating viral production from process development, pilot scale, to medium and large scale industrial production. The scale-X bioreactor systems are operated in fed-batch or perfusion modes, with in-line clarification and capture processes operated in simulated continuous & automated mode reducing therefore the number and complexity of operations compared with traditional equipment. Direct linear scalability is ensured by applying concepts similar to those found in the scale-up of chromatography columns, whereby the height of the reactor is kept the same with its diameter increased as a function of the scale. In addition, the physical and chemical conditions are kept the same across scales, ensuring a smooth and risk-free scale-up.

Results and discussion: This innovative solution will allow to reduce significantly the cost of manufacturing of viral gene therapy and cell-based vaccines and offers an excellent opportunity for emerging countries & producers to enable local manufacturing of affordable costs. Based on case studies using VERO and HEK293 cells for sIPV and adenovirus production, this presentation will demonstrate how scale-X system can improve reproducibility, reduce both capital and operating costs and remove the scalability bottleneck between clinical trials and full commercial production. Results such as cell culture dynamics, productivity, and key process quality indicators will be demonstrated as well.

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A LOW-FOOTPRINT, INTEGRATED & AUTOMATED PLATFORM FOR VIRAL PRODUCTION

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Background and novelty: The world is facing an under-supply of some key vaccines due to poor synergies between growing market demands and aging production models.

Experimental approach: In this light, we have developed a proof of concept of a vaccine manufacturing platform aiming at increasing availability and affordability of vaccines – the NevoLine™ system.

This simulated continuous and automated platform integrates both USP[1] and DSP[2] processes and is encapsulated into an isolator, making it a self-contained production unit (6m²).

The technology relies on a single-use, high-density fixed-bed bioreactor operated in perfusion chained with downstream filtration, clarification and polishing steps to (a) decrease batch time, (b) reduce equipment utilization, (c) optimize utilities consumption and (d) intensify operations. By optimizing single-use technologies we are able to drastically reduce CAPEX[3], CoGs[4] and footprint and increase production capacity. Such manufacturing platform can easily be implemented into flexible facilities with simplified infrastructure, increasing adaptability in production and capacity for record time-to-market.

[1] Upstream
[2] Downstream
[3] Capital expenditure
[4] Cost of goods

Results and discussion: This study will present the platform proof of concept on Vero line and trivalent inactivated polio vaccine (sIPV) production, achieving low CoGs (0,28$/dose for a trivalent sIPV) and large capacity. The presentation will feature the description of engineering development, but also results of cell growth, infections and product quality, as well as a description of the CAPEX, CoGS and capacity calculations. This manufacturing platform is undergoing sIPV process scale-up and pre-clinical bulk production.

The NevoLine system is expected to produce any type of viral vaccine at a very low cost and large capacities to face global health challenges.

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A NOVEL TYPE OF DEFECTIVE INTERFERING PARTICLE FOR ANTIVIRAL THERAPY

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Background and novelty: Defective interfering particles (DIPs) usually harbor a heavily deleted form of the viral genome, which makes them defective in virus replication. The co-infection with standard virus (STV) can complement this defect. Yet then, interference with the normal viral life cycle takes place, which leads to a suppressed STV replication and the predominant production of non-infectious DIPs. Previous research suggests that DIPs may be utilized as an antiviral agent. We report the discovery of a yet unrecognized form of influenza A virus (IAV)-derived DIP (termed "OP7" virus) that contains various point mutations instead of large deletions in the genome [1].

Experimental approach: OP7 virus was discovered by single-cell analysis [2]. Briefly, IAV-infected Madin-Darby canine kidney cells were isolated in 384-well plates, whereby we identified wells containing single cells by microscopy. After incubation, produced virions were quantified by plaque assay and intracellular genomic viral RNAs (vRNAs) by real-time RT-qPCR. OP7 virus was then enriched, and further characterized by genome sequencing and STV co-infection experiments.

Results and discussion: The genomic vRNA of segment 7 of OP7 virus (S7-OP7) contained 37 point mutations, affecting several functional regions. Upon STV co-infection, an over-proportional level of S7-OP7 compared to other vRNAs was observed, both intracellularly and in the produced virions. Further, OP7 virions lacked a large fraction of other vRNAs, which appears to explain its defect in virus replication. Finally, it showed strong interference with replication of various IAV strains in different cell lines. Thus, we believe that it might be a promising antiviral agent. Future research efforts comprise animal trials and R&D of cell culture-based manufacturing of OP7 virus.

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ACCELERATING LENTIVIRUS MANUFACTURING TO GMP COMPATIBLE BIOPROCESSES

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Background and novelty: Lentiviral vectors (LVVs) have been used increasingly as gene and cell therapy vectors for the treatment of acquired and inherited diseases. LVVs face the downstream challenges common to retroviridae family of vectors namely short half-lives at room temperature, sensitivity to pH variations and salt concentrations, and shear stress that compromises the translation to the clinic. Despite all the advances, there is the need to improve bioprocesses before lentivirus can be routinely used in preclinical and clinical research.

Experimental approach: To address the downstream bottlenecks we report an improved lentivirus purification process for phase I and II clinical trials and present a case on the use of new materials as a replacement for traditional centrifugation. 3D printing to produce customized matrices will contribute to the available toolbox of chromatography-based downstream processing. Furthermore, alternatives to traditional chromatographic methods and predictive modeling based on deterministic knowledge improve the downstream unit operations and build a continuous purification process. Product recovery, potency, purity and the effect of additive materials will be discussed.

Results and discussion: We implemented a scalable protocol for LVVs that is easy to transfer to GMP environment, combining microfiltration, anion-exchange, and ultrafiltration membrane technologies towards maximization of infectious virus recovery without the need for cleaning validation in a cost-effective manner. The process developed allowed a volume reduction of up to 100 and a maximum concentration of infectious particles up to $2x10^8$ IP/ml. Assessment of LVVs quality was carried out and infectivities from 8 $x10^4$ and 5 $x10^4$ IP/ng p24 indicate that purified clinical vectors show high infectivity. The work under development can be further extended in the future to redesign biomanufacturing strategies for other viral particles or other fragile macromolecules.

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PRODUCTION OF INFLUENZA VIRUS-LIKE PARTICLES IN INSECT CELLS

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Background and novelty: Virus-like particles (VLPs) consist of one or several recombinant viral surface proteins that spontaneously assemble into particulate structures similar to authentic virus particles [1]. VLPs can be used as a highly effective vaccine that elicits strong immune responses. The baculovirus–insect cell system has been widely used for the VLP production, but it has several inherent limitations including contamination with progeny baculoviruses. Recombinant insect cells can be employed as an attractive alternative to the baculovirus–insect cell system. In the present study, we investigated the production of influenza VLPs in recombinant insect cells.

Experimental approach: The DNA fragments encoding hemagglutinin (HA) and matrix protein 1 (M1) of an influenza virus A (H1N1) were individually cloned into the plasmid vectors pIHAbla and pIHAneo. The pIHAbla and pIHAneo 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, together with either a blasticidin or a neomycin resistance gene for use as a selectable marker, respectively [2]. After cotransfection with the resulting plasmids, *Trichoplusia ni* BTI-TN-5B1-4 (High Five) cells were incubated with blasticidin and G418, and cells resistant to the antibiotics were obtained. Recombinant High Five cells were incubated in static or shake-flask cultures.

Results and discussion: Western blot analysis of a culture supernatant revealed that transfected High Five cells secreted HA and M1 molecules. Sucrose density-gradient sedimentation analysis and dynamic light scattering of the culture supernatant suggested that secreted HA and M1 were in a particulate form. Hemagglutination assay using chicken erythrocytes showed hemagglutination activity in the culture supernatant. Recombinant insect cells may offer a promising approach for efficient production of influenza VLPs.

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P-226

ANIMAL COMPONENT FREE ROTAVIRUS PRODUCTION USING MICROCARRIERS

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Background and novelty: Worldwide, Rotavirus infection is a major cause of acute gastroenteritis with dehydrating diarrhea in infants and young children throughout the world. A number of live-attenuated, oral rotavirus vaccines are prequalified and available globally. Improved production technologies are needed to reduce costs and increase quality.
Rotavirus is mainly produced in T-flasks, cell factories, or roller bottles using adherent Vero cells as cell substrate and porcine trypsin for rotavirus activation.
As regulatory requirements become stricter, live vaccine production is moving away from animal-derived materials. Furthermore, closed bioreactor systems will help mitigate cross-contamination risks associated with open handling in current methods. Improved methods and solutions arising from academic and industrial research will result in updated production processes. These will benefit rotavirus production in the future.

Experimental approach: In this work, we developed methods for rotavirus production in bioreactors using microcarriers and animal component-free materials. We describe different approaches for serum-free Vero cell cultivation using pre-sterilized microcarriers. Vero cells were grown in spinner flasks and a single-use WAVE bioreactor™ system, then infected with rotavirus during exponential growth. Infectious virus concentration was determined by fluorescence focus assay.

Results and discussion: The results show significant differences between tested cell culture media, as well as suitability of recombinant trypsin for rotavirus activation. Pre-sterilized microcarrier cultures give similar cell-specific productivity compared with cells grown in T-flasks. Finally, the described culture conditions successfully propagate rotavirus in a WAVE bioreactor system.

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POLYMER VECTORS FOR GENE DELIVERY: INFLUENCE OF AMINE MOIETIES

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Background and novelty: Gene delivery has become a powerful tool for manipulating cells. Promising approaches as CAR-T therapy or the tuning of suspension-adapted cell lines emphasize the need of efficient gene vectors. Cationic polymers are a promising class of non-viral vectors, since they can effectively form complexes with genetic material and promote the interaction with cell surfaces. Due to the high cytotoxicity of poly(ethylene imine), the "gold standard" among cationic polymers for gene delivery, alternatives need to be developed. Here, a novel library of polyacrylamide-based polymers with different amine substitution patterns was investigated.

Experimental approach: A range of tailor-made polymers was synthesized bearing primary amine, tertiary amine, or guanidinium functionalities, known from arginine in cell penetrating peptides. The polymer library and their complexes with plasmid DNA were investigated in HEK cells for toxicity, uptake properties and protein expression. Additionally, interaction with membranes as well as specific lipids were studied. The bottlenecks of transfection, cellular uptake and endosomal release were studied in more detail using calcein release studies, live cell confocal laser scanning microscopy and flow cytometry.

Results and discussion: All obtained polyacrylamides exhibited similar pDNA binding properties and are taken up into cells *via* energy dependent pathways. Among the different amino substitution patterns, the guanidinium-based polymers exhibit superior transfection efficiency. Because the guanidinium moiety is not pH-dependent, the classical mechanism of endosomal release needs a re-think. Specific interaction with endosomal elements will be discussed, identifying guanidinium-based polymers as a promising gene delivery tool.

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DEVELOPMENT OF A GMP ONCOLYTIC VIRUS MANUFACTURING PLATFORM PROCESS

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Background and novelty: The technology to tailor-design oncolytic viruses as well as the understanding of the interactions of viral replication in the tumor cell, virus-induced tumor lysis and sustained immune activation has seen a tremendous progress in recent years. Accordingly oncolytic viruses receive rising interest as potent and economically attractive pharmaceuticals. To assist in their clinical development, Fraunhofer ITEM aimes to establish a cell culture based platform process for GMP compliant manufacturing of oncolytic viruses.

Experimental approach: Using a recombinant herpes simplex 1 (HSV-1) oncolytic virus as a first model, a completely serum free, scalable bioreactor cultivation process was set up using the adherent Vero-B4 cell line. To optimize virus yields, various process parameters, e.g. pH, temperature, multiplicity of infection, time of infection, time of harvest were systematically assessed in parallel shaking flask and bioreactor cultivations. In order to prevent the accumulation of toxic metabolites as well as the deprivation of glucose, various medium exchange strategies were considered.

Results and discussion: Results demonstrated that virus propagation can be facilitated up to 25-fold applying one single medium exchange step at time of infection. So far, virus yields in the range of 5 x 10^7 (Plaque Forming Unit) PFU/mL can be obtained consistently in batch cultivations. These yields are well in line with recent literature data for similar processes. Using depth filtration, a scalable primary harvest strategy was established and stability of the bulk harvest was evaluated.

As the present data already indicates promising potential for higher virus yields, a perfusion strategy will be established in the next step. Yet even at the current development state, process yields are already high enough to meet the material requirements of a typical phase I/II study from one typical pilot scale fermentation run.

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HEK293 CELL CULTURE AND ADENOVIRUS VECTOR PRODUCTION PLATFORM

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Background and novelty: Current viral vector manufacturing processes face many significant challenges. Typical production schemes involve propagating seed trains and viral amplification using serum containing media. Although these systems deliver robust vector yields, the serum component has several disadvantages such as performance variability, high costs, and risk of adventitious agents. Utilizing serum-free medium for growth and amplification answers these concerns and can lead to significant process improvements.

Experimental approach: We evaluated multiple chemically defined, serum-free media formulations for adenovirus vector production in the iCELLis® bioreactor. These media were evaluated for ease of adaptation and growth characteristics with suspension HEK293 cells. Process parameters such as multiplicity of infection (MOI) and duration of infection (DOI) were optimized in shake flask. The process was then transferred into the iCELLis® Nano bioreactor. The HEK293 cells were expanded to a density of around 200,000 cells/cm^2. Complete medium exchange was performed in the bioreactor without cell removal, followed by infection at MOI 50 and harvested after 72 hours.

Results and discussion: Viral vector productivity was generally consistent between shake flask and the iCELLis® Nano bioreactor system. We identified one chemically defined media formulation which resulted in viral productivity similar to serum containing media. This data demonstrated that high cell densities can be achieved and maintained under adherent conditions in serum-free media in the iCELLis® Nano bioreactor system. This data also demonstrated viral productivities seen with serum containing media can be achieved utilizing chemically defined media.

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DEVELOPMENT OF AN OPTIMIZED ACF PRODUCTION PROCESS FOR SABIN IPV

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Background and novelty: A production process for Inactivated Polio Vaccines based on Sabin strains (sIPV) was previously developed [1]. Clinical trials were successful regarding safety and immunogenicity [2, 3]. However, with DSP yields <15% for Sabin poliovirus (PV) type 2 [4], the process was not economically feasible.

The approach aimed at optimizing and modernizing the process as a whole. A design of experiment (DoE) approach was used to assess the effects of changes in upstream unit operations (UO) to those downstream. The newly developed process is completely animal component free (ACF) and overall production yields showed a major improvement.

Experimental approach: Process optimization was done using the scale-down model for IPV [4]. The process consists of 7 UO [1]. Optimizations per UO were evaluated using DoE. In the final DoE, the entire process was run 10 times to assess impact of minor variations.

Results and discussion: Setting up a ACF process required major changes in the USP to replace the serum containing (SC) media. Also, a different filter and cassette were selected for the concentration unit to allow cleaning with NaOH instead of trypsin.

Main yield improvements were achieved in chromatography, for which previously major losses were reported (yields <20% for PV type 2) [1]. These losses could be attributed to product self-aggregation as the running buffer's pH was close to the product isoelectric point. To overcome this, elution buffers containing L-Arginine were used [5]. Also, a different resin for ion exchange was introduced to enable scale-up. Resulting recoveries of the new chromatography units are >80% for PV type 2.

The sIPV product from the ACF process has a comparable quality (rat potency; D-antigen, HCP & HC-DNA concentrations) to the sIPV produced in the SC process, and to the conventional Salk IPV product. Moreover, the developed process is robust, low-risk and efficient with overall yield of approx. 45% for Sabin PV type 2.

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PRODUCTION OF SEASONAL INFLUENZA VACCINES USING SUSPENSION MDCK CELLS

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Background and novelty: Influenza-related illnesses have caused an estimated over million cases of severe illness, and it has about hundred thousands of deaths worldwide annually. In response to rapid antigenic drift in influenza viruses, vaccination is considered the most effective intervention. Traditionally, seasonal influenza vaccines are produced in embryonated chicken eggs. However, egg-based production system requires long logistic planning and labor-intensive. These limitations resulted in the spurred exploration of alternatives. MDCK cells are widely considered as an alternative host to embryonated eggs for influenza virus propagation. Although MDCK cells were considered to be a suitable host for the virus production, their ability to grow in suspension MDCK cells still unclear.

Experimental approach: In this study, suspension MDCK (sMDCK) cell line was adapted in adapted in a chemically-defined (CD) media prototype. Experiments were conducted to evaluate the possibility of 2017/2018 several seasonal egg_ and cell_ derived influenza A/B strains grown in the adapted sMDCK cells. The viral productivity was measured by Hemagglutinin (HA) titer and Tissue Culture Infectious Dose ($TCID_{50}$/ml) in 125ml spinner flasks compared to its adherent MDCK cells in microcarrier-based cultures.

Results and discussion: The sMDCK cell concentration reached to 2×10^6cells/ml after 96 hrs, and the doubling time was found very similar to the aMDCK cells cultivated on microcarriers (5g/L). No fresh medium replacement was necessary in the sMDCK cell culture during the cell growth stage. Two cell_derived influenza B strains from VIDRL grew well in the sMDCK cells, but H3N2 and H1N1 strains from egg_ or cell_derived did not grow well. Our data showed that egg_derived candidate vaccine virus (CVV) strains did not grow well directly in the sMDCK cells. This study indicated that WHO ERL labs should provide cell_ derived CVVs for manufacturers for the cell_based vaccine.

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HIGH QUALITY TRANSFECTION REAGENTS FOR THERAPEUTIC VIRUS PRODUCTION

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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®, the unique PEI-based transfection reagents available in different quality grades, allowing a seamless transition from process development with PEIpro®-HQ to cGMP biomanufacturing with PEIpro®-GMP.

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

Results and discussion: PEIpro® and its high-quality counterparts PEIpro®-HQ and PEIpro®-GMP are particularly well suited for therapeutic virus production. Both reagents are PEI (Polyethylenime) based and are free of components of animal-origin. They have been selected for their high transfection efficiency using low DNA amount. PEIpro®-HQ 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. Finally, PEIpro®-GMP is manufactured according to validated manufacturing process with GMP guidelines to ensure tracability from starting material to the final product, making PEIpro®-GMP perfectly suitable for use as a qualified raw material for the production of clinical batches of viruses in GMP processes.

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SUSPENSION CAP-GT CELLS AS PLATFORM FOR AAV PRODUCTION

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Background and novelty: Recombinant adeno-associated virus (rAAV) has shown great promise as a gene therapy vector in multiple aspects of preclinical, clinical and commercial applications. However, a major remaining challenge is establishing large-scale manufacturing technologies in accordance with current good manufacturing practices (cGMP) to yield the purified vector quantities and quality needed for the expanding clinical and further commercial needs.

Experimental approach: To address this challenge CEVEC pursued two approaches. i) For rapid production of rAAV gene therapy vectors the proprietary suspension CAP-GT cell line was optimized for the PEI-based transient transfection production of rAAV. ii) To simplify large-scale manufacturing, CEVEC generated a helper virus-free packaging cell line that can easily be turned into a producer cell line by only one additional step of cell line development.

Results and discussion: Process development for the transient transfection process was performed using the ambr system as scale-down model to accelerate the development and optimization of a robust and scalable process for rAAV production. Subsequently, this optimized process was successfully scaled-up.

For the generation of stable producer cell lines, suspension CAP-GT cells were genetically modified to stably express Rep proteins, as well as the adenoviral helper functions E2A, E4orf6 and VA RNA. Resultant SCCs were further genetically modified by integration of AAV capsid and GFP as transgene flanked by the ITRs resulting in a proof of principle producer cell line. In ongoing experiments, the previous obtained process parameters from our transient transfection process are now transferred to the stable system and further optimized.

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THE IMPACT OF CELL DENSITY EFFECTS ON FOOT-AND-MOUTH DISEASE VIRUS.

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Background and novelty: Foot-and-mouth disease is a highly contagious and economically devastating animal disease with endemic occurrence in many parts of the world. Vaccination is the method of choice to limit viral spread and eradicate the disease, but the short shelf life of vaccines and the need for frequent re-vaccination currently make vaccination programs cost-intensive. Animal-component-free (ACF) or chemically defined media (CDM) at high cell densities are a promising approach for the production of inexpensive high-quality vaccines, but the occurrence of "cell density effects" has been reported for various virus-cell production systems. For FMDV, however, the use of CDM or ACF media for vaccine production has not been studied and no information about cell density effects is available. This work describes the propagation of FMDV in ACF media and in prototypes of CDM with different cell densities in spin tubes and a stirred-tank bioreactor.

Experimental approach: Cells were cultured in Cellvento™ BHK200 (ACF) or in BHK300B/G (CDM). Spinner tubes were inoculated with FMDV at increasing cell densities and either 100% media exchange or addition of 30% fresh media was performed prior infection. Cells in Mobius® 3L bioreactors were infected at 3×10^6 cells/mL with 30% fresh media prior infection. Virus was harvested 20 hpi and the viral yield was quantified by endpoint titration.

Results and discussion: Increasing cell densities reduce the viral titer and increase yield variability in all media except BHK300G. This effect can be mitigated by performing a 100% media exchange before infection or when using the controlled environment of a bioreactor. Further studies are necessary to investigate the reasons for the diminished viral growth more deeply.

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SYNERGISTIC EFFECT OF GLYCO-ENGINEERING AND VLP-DISPLAY OF VACCINE AG

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Background and novelty: The goal of glyco-engineering cell lines is often to remove unwanted glycan structures or to produce recombinant protein with a more human-like glycan pattern. The novelty of our work lies in a fundamentally opposite approach – namely to glyco-engineer recombinant proteins to become more foreign and more immunogenic to the human immune system. Under the hypothesis that foreign glycans will result in more potent vaccines, we used the placental malaria vaccine antigen, VAR2CSA, as model protein and targeted it to various mannose recognizing receptors/lectins by altering the glycan pattern. Additionally, we coupled this glyco-engineered antigen to VLPs, to further enhance the immune response.

Experimental approach: *Drosophila* S2 insect cells were genetically engineered to produce high-mannose (HM) glycans by deleting the Golgi α-*Mannosidase Ia (ΔMan-Ia),* which is responsible for trimming down the Man_9 structure to Man_5. We expressed VAR2CSA in S2 cells and in the S2-Δ*Man-Ia* cell line and coupled the antigens covalently to VLPs using SpyTag/SpyCatcher. Vaccines were formulated w/wo VLPs and w/wo glyco-engineered VAR2CSA and tested in mice.

Results and discussion: The glycan analysis confirmed that S2 produced VAR2CSA predominantly carried fucosylated Man_3, while S2-Δ*Man-Ia* produced VAR2CSA consisted of 76% HM glycan structures. The immunological effect of HM glycans and presence of VLPs was evaluated in mice, where blood samples were analyzed for placental malaria specific antibodies. The total level of VAR2CSA specific IgGs did not vary between the Man_3 and HM groups. However, the functionality of antibodies varied significantly between the groups and showed a clear picture, with significantly increased functional parasite binding blocking for VAR2CSA carrying HM glycans, which was further improved when bound to VLPs.

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IMMUNOGENICITY OF A VECTORED VACCINE AGAINST NEWCASTLE DISEASE IN MICE

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Background and novelty: Poultry are a vital village livestock asset in Sub-Saharan Africa (SSA). Newcastle disease is considered one of the most important disease effecting poultry. ND vaccines currently available, are produced in specific pathogen free (SPF) chicken embryonated eggs. These production methods are not space efficient compared to well the established mammalian cell culture technologies. Considering these limitations, a newer ND vaccine is needed. The overall aim of this work is to develop a novel ND vaccine using a non-replicative human adenovirus as a vector, which contains and expresses protective antigens, typically the HN and F glycoproteins of NDV.

Experimental approach: Recombinant non replicative adenovirus vectors expressing the Newcastle disease F and HN antigens were constructed. Bicistronic adenovirus 5 vector expressing HN and F genes under the control of CMV promoter was constructed, in addition to monocistronic adenovirus vectors harboring HN or F genes. rAd vectors were produced in HEK293 cells in shake flasks.

To select the most efficient and immunogenic rAd5-ND antigen expression vector, 3 groups of mice received via the subcutaneous route 10^7 PFU of each recombinant virus, the animals were injected two doses at two weeks interval. Two control groups were also also used; one group of mice received Phosphate Buffer Saline (PBS), the second control group received rAd expressing GFP. Humoral and cellular responses will be investigated; antibodies against HN and F antigens will be determined by ELISA and hemagglutinin inhibition test.

Results and discussion: Sera were collected at different time points; before the immunisation of the different groups, and also at day 15 and day 28 post injection. Further samples will be obtained at days 45 and 90. Analysis of humoral response and cellular response by ELISpot will be performed.

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A NOVEL VETERINARY RABIES VACCINE PRODUCED IN THE AVIAN CELL LINE

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Background and novelty: Rabies is a viral zoonosis caused by negative-stranded RNA viruses of the *Lyssavirus* genus. The aim is to develop a high-yield process to produce a low-cost veterinary rabies vaccine. In the current work, we studied rabies virus production in AGE1.CRpIX cell line in a 7L bioreactor. Different culture modes were investigated (batch, fed-batch and perfusion) to maximize the productivity of the process. The potency of the experimental vaccine was also estimated in mice according to the NIH test.

Experimental approach: AGE1.CRpIX cells Cultures were performed in a 7-L bioreactor in CD-U5 Medium. Cultures were infected in exponential phase with PV-BHK-21 rabies virus strain. The cultures were performed at 34°C, with pH regulation at 7.4

Results and discussion: AGE1.CRpIX cells were grown in CD-U5 enriched with 2 mM glutamine and 10 ng/ml of Long-R3 IG, in batch culture. The highest cell density reached was around $1x10^7$ cells/ml. At day 3, cells were infected with PV/BHK-21 virus strain at an MOI of 0.001, in the same CD-U5 medium without performing a medium exchange. The highest virus titer was obtained at day 3 post infection, and was equal $3x10^8$ FFU/ml. Viral titer of the harvest collected at the end of the culture, corresponding to 6 days post infection, was around $2x0^7$ FFU/ml. The harvest was clarified, then inactivated by BPL (beta-propiolactone). The antigenic activity of the experimental vaccine was assessed in mice according to the NIH test. The potency was equal to 3.3 IU/ml; this means that 1 ml of the clarified harvest results in 3 doses of vaccine, considering that one dose of vaccine should have a potency of 1IU, as required by WHO. To further improve the process yield, we are currently investigating the use of fed-batch and perfusion culture modes.

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IMPROVING VACCINE PRODUCTION WITH A SERUM-FREE MEDIUM FOR FIBROBLASTS

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Background and novelty: Chicken embryo fibroblasts and diploid cells are used for vaccine production, including varicella zoster (VZV), MMR, yellow fever, polio, rotavirus, rabies, and dengue virus. Vaccine manufacturers generally culture these cells in classical medium with 10% bovine serum and desire to move to serum-free formulations due to the potential regulatory and supply chain risks associated with serum.

Experimental approach: Using metabolite analysis and a design of experiment (DOE) rationale, we developed the first serum-free medium (SFM) for growth of MRC-5 and other fibroblast cells that can support direct recovery from thaw and adaptation-free expansion, while resulting in performance that is comparable to serum-containing medium. Since requirements for production of viruses are different from cell growth, we optimized the production medium separately.

Results and discussion: This animal origin-free production medium is designed to allow manufacturers to produce vaccines without concern about the bovine serum albumin limit of 50 ng/dose set by the World Health Organization. We confirmed virus production with VZV and vesicular stomatitis virus and demonstrate titers that are up to one log higher than a classical medium control. By switching to a serum-free process, vaccine manufacturers can reduce dependency on serum, production and purification costs, and increase product consistency and safety.

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IMPROVING RABIES VACCINE PURIFICATION PROCESS USING NOVEL FILTERS

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Background and novelty: Rabies is a viral zoonosis caused by negative-stranded RNA viruses of the *Lyssavirus* genus. It can affect all mammals including humans.In this work, we evaluated different grades of filters in MicroPod or Optiscale format for rabies virus clarification and defined the best filter train. By using these filters, we aim to achieve superior titers of rabies vaccine antigen compared to old processes using the classic membrane for clarification.

Experimental approach: The viral harvests were pooled and then clarified using different filters. One part of viral harvest was clarified using 8 µm cellulose filter followed by a Durapore 0.45 µm membrane. The other part was clarified using depth filters such as Millistak+ HC and other prefilters supplied by Merck. To clarify the harvest containing microcarriers, Clarisolve 60HX filter was used. The recovery of each filter was estimated based on viral titration.

Results and discussion: In the current process, rabies virus harvest clarification using 8 µm membrane followed by Durapore 0.45 µm showed a low recovery of the antigen. In order to improve the yield the Millistak+ D0HC and Millistak+ C0HC followed by one of the following filters: Milligard 1.2/0.5 µm, PolySep 1.0/0.5 µm and Durapore 0.45 µm, were assessed. The Millistak+ HC C0HC filter was the best suitable option to process rabies virus harvests. It showed the highest antigen recovery yield, equal to 60% and exhibited the best compromise in term of capacity and filtrate turbidity. At the second step of clarification polysep 1.0/0.5 demonstrated a slightly higher yield compared to the Milligard and the Durapore filters when it was used after the Millistak+ HC C0HC filter. The recovery was equal to 70%. Currently we are studying the consistency of the data generated using depth filters.

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POLYMERS FOR CELLS: SHEAR FORCE PROTECTION, NUTRIENT- & GENE DELIVERY

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Background and novelty: The interest in tailor-made polymers as delivery vector is continuously increasing, as they can possess a wide range of chemical functionality while their composition and architecture may be readily tuned. In previous studies we developed polymers based on the polymer poly(2-oxazoline), a pseudopeptide exhibiting superior shear force protection compared to poloxamers. This platform has now been further developed to deliver nutrients as well as genetic material to cells.

Experimental approach: Hardly soluble nutrients such as fatty acids (lipoic acid, linoleic acid) were covalently linked to polymers using a biodegradable ester-linker to increase their solubility and bioavailability. The basic concept to use nutrients as a prodrug approach was further investigated using different compounds (polymer backbone *vs.* polymer side chain). Cell growth, density, and viability of HEK cells were monitored and compared to conventional formulations.

For gene delivery, cationic groups such as primary amines were introduced to the poly(2-oxazoline) platform to transport and protect the genetic material. Their potential for protein expression was tuned by synthesizing block copolymers with cationic, hydrophobic and biocompatible functionalities. To understand the delivery process of the polymer micelles an in more detail, electron and confocal microscopy as well as flow cytometry were performed.

Results and discussion: The presented nutrient-polymer conjugates of lipoic acid and linoleic acid are well water-soluble, even at higher concentrations and resulted in excellent cell viability and fast growth rates of HEK cells.

Meanwhile, using block copolymers results in more successful gene expression with reduced cytotoxic effects compared to homopolymers, also enabling long-term transfections. Through the considered design and synthesis of block copolymers, multifunctional gene vectors with superior delivery potential can be designed.

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P-241

NEW VIRAL AND NON-VIRAL PLATFORMS AND PROTOCOLS FOR T-CELL ENGINEERING AND MANUFACTURING

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Background and novelty: We have developed a new suspension culture-based lentiviral production system, LV-MAX Lentiviral Production System, for the production of high titer vectors. LV-MAX provides for scalable production employs a serum-free and chemically-defined medium in which cells are cultured in suspension. The system comprises a newly developed and proprietary set of GMP reagents comprising a packaging cell line (maintained in suspension culture), a chemically-defined culture medium, a supplement, a transfection reagent and enhancers. In addition, we've developed large scale protocol for 2L, 5L, 50L production in Bioreactors In parallel, we also developed novel non viral delivery solutions using gene editing tools and electroporation platform.. For CRISPR/Cas9 gene editing in primary T cells by developing a new cas9 mutants, modified guideRNA and new electroporation buffer and large scale platform to enhance knock in efficiency and ultimately obsolete Lentiviruses as the method of choice for CART manufacturing.

Experimental approach: For the LV production system, we applied a large screening of new cells, enhancers, feeds, transfection reagents and media. DOE was applied to optimize all the critical variables to maximize the LV production in bioreactors. For the non viral approach, we optimized the electroporation conditions, the ratio between cas9 and gRNA, RNP complex to cells and homology arms to maximize knock out efficiency in primary T cells.

Results and discussion: With this new Lentiviral Production system (LV-MAX) we are able to deliver 1.5E+08 (TU/mL) of un-concentrated lentiviral vector, which is at least 10 fold higher than other published methods of lentiviral production in small (30ml) and large volumes (2L, 5L and 50L) For non viral delivery, we are able to achieve more than 90% knockout efficiency for most genes we tested, including T cell receptor (TCR). More importantly, gene knock-in efficiency can be reached to 30-50% with all-in-one electroporation. In addition, we are developing a large scale electroporation system to fulfill the needs of large scale manufacturing engineered T cell for Immunotherapy.

Acknowledgements & Funding: In this study, we will highlight the different viral and non-viral delivery approaches that we developed for the engineering of T cells in a manufacture settings.

P-300

GENERATION OF TRASTUZUMAB ANTIBODY DRUG CONJUGATES AT DIFFERENT DARS

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Background and novelty: Antibody Drug Conjugates (ADCs) represent an increasingly important tool for the treatment of cancer. Their main feature is the drug load of the molecule, characterized through the Drug Antibody Ratio (DAR). Approved ADCs tend to have a DAR close to 3-4. We have generated a HEK293 line producing Trastuzumab (Tzmb), which has been conjugated to obtain a DAR 4 heterogeneous ADC and a DAR 8 homogeneous ADC. We have also generated a HEK293 line producing a Tzmb variant, which has been conjugated to obtain a DAR 2 homogeneous ADC.

Experimental approach: Tzmb heavy (HC) and light chains sequences were cloned in the tricistronic pTRIpuro3 plasmid, which was stably transfected in HEK293 cells. For homogeneous conjugation, derivate Tzmb_cys114 (containing a substitution of Cys for Ala in the position 114 of HC) was generated by PCR mutation. Antibodies were then produced and purified with Protein A affinity chromatography. Next they were conjugated to the cytotoxic drug vcMMAE, prior reduction with TCEP. In the case of Tzmb_cys114, a reoxidation step was applied. Generated ADCs were analysed with HPLC-HIC/SEC, and their antiproliferative activity was determined through a MTS assay with breast cancer SKBR3 (HER2+) cells.

Results and discussion: Tzmb and Tzmb_cys114 were cloned and produced in HEK293 cultures, and the antibodies were purified at >99% purity. Tzmb and Tzmb_cys114 were conjugated to vcMMAE, and it was determined that reduction with TCEP is the critical step for obtaining the desired DAR. Molar proportions TCEP:Tzmb of 4 and 150 allowed to obtain DARs close to 4 (heterogeneous) and 8 (homogeneous), respectively. In the case of Tzmb_cys114, complete reduction leading to a DAR close to 2 was achieved when a molar ratio over 50 was applied. HPLC analysis revealed DARs of 1.82, 4.08, and 7.7, respectively, and *in vitro* antiproliferative activity was directly related to the DAR, with IC_{50} values of 723, 157, and 20 pM for DARs 2, 4 and 8.

P-301

COLLECTION OF STEM CELLS USING A SINGLE USE CENTRIFUGE UNIFUGE

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Background and novelty: Stem cells are aseptically concentrated and collected with the UniFuge single use centrifuge. This approach closes the process and eliminates loss and exposure from manual processes.

Experimental approach: 50 liters of Stem cells were grown in a SU bioreactor. The viability of the grown cells measured 95%. The suspension cells were grown for 4 days, doubling each day. The diameter size of the cells were 15 -20 micron. The cells reached a final cell density 4.93 million cells/ml at time of harvest. To harvest the cells, the UniFuge centrifuge parameters were feed rate (LPM) and G force. The cells were layered on a PBS buffer. The centrifuged cells were collected aseptically and automatically. After Harvest, the cells were frozen for 24 hours, thawed and then viabilities re-measured.

Results and discussion: For the Viable collection myeloid progenitor like suspension cells, the UniFuge Single use centrifuge **met all three objectives**.

1. Collect 93% of cells at 700 x G.
2. Cells maintained > 90 % viability after 24 hour freeze/ thaw.
3. No manual manipulation or hood is necessary.

P-302

STRATEGIES TO MAXIMIZE THE PRODUCTION OF CAR-T CELLS

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Background and novelty: Adoptive cellular therapy using chimeric antigen receptor (CAR)-T cells targeting CD19 has demonstrated substantial clinical efficacy in several hematological cancers. The highly demanding cell doses used in clinical trials require a scalable, efficient and GMP-compliant manufacturing process. In this work, different culture systems were evaluated in order to establish an efficient CAR-T cell expansion process

Experimental approach: T cells were isolated by magnetic selection and activated with anti-CD3/CD28 magnetic beads at a 1:1 ratio. On the next day, the cells were transduced with lentiviral vectors CAR anti-CD19 (MOI 5) and cultivated in TexMACS supplemented with 10% of AB human serum and 100 IU/ml IL-2. Afterwards, CAR-T cells were expanded in G-Rex and Xuri bioreactor (n=2, two independent donors).

Results and discussion: The presentation will highlight the differences in cell proliferation, expansion-fold, CAR expression and T-cell profile (flow cytometry) between the different systems tested. After CD19 CAR-T cell expansion, the cytolytic potential was assessed by co-culturing with B cell lines Sup-B15 and Raji ($CD19^+$) and K562 ($CD19^-$). A ratio of 10:1 effector:target cell was used and our results showed that CD19CAR-T cells produced are functionally active and highly specific, showing cytotoxic activity against $CD19^+$B cells only. Importantly, final product were negative for any microbiological contamination, including mycoplasma and endotoxin.

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P-303

ESTABLISHMENT OF 3D OVARIAN CANCER IN CO-CULTURE FOR DRUG SCREENING

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Background and novelty: Three-dimensional (3D) cell culture are now being pursued in the drug screening due to their better abillity in mimic the in vivo tumor. This work describes the establishment of spheroids using ovarian cancer cells (SKOV-3) in co-culture with mesenchymal (MCU-9) and fibroblasts (CCD27SK) as model for drug screening assay.

Experimental approach: SKOV-3:MCU-9 and SKOV-3:CCD27SK spheroids were obtained in ultra-low attachment (ULA) plates in different proportions (2:1; 3:1 and 4:1). Images were obtained daily by inverted microscope for spheroid´s diameter and roundness estimation. Resazurin was used to analyze the cell viability and the spheroid´s morphology was evaluated by SEM and confocal microscopy. The expression of genes associated with progression, hypoxia, proliferation and tumor differentiation (MMP-2 and MMP-9; HIF-1a; VEGF; SNAIL; ZEB1; Vimentin and B-catenin) was evaluated by real-time PCR. Paclitaxel drug was used to analyze the drug response.

Results and discussion: Spheroids in co-culture presented a spherical shape (0.86-0.94 roundness) and diameters around 350 µm. Cell growth was not observed in all the cell proportions evaluate, corroborating with the maintenance of spheroid diameter throughout the culture. The 3D cell architecture observed in both co-cultures and the higher presence of the microvilli demonstrated the better maintenance of *in vivo* tumor morphology, when compared with 2D culture. Gene expression analysis showed the upregulation of all the genes in 3D co-culture, when compared with 3D and 2D monoculture, demonstrating the influence of the tumor microenvironment. As expect, the 3D co-culture and mono-culture presented higher resistance to Paclitaxel drug (0.25-8µM) (90-100% viability) in relation to 2D culture (50% cell viability). These results support the hypothesis that the 3D co-culture established can better mimic the *in vivo* ovarian tumor for drug screening assays.

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P-304

AN EX VIVO TUMOUR CULTURE PLATFORM FOR FUNCTIONAL DRUG TESTING

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Background and novelty: Therapeutic drug development relies mostly on the use of 2D cancer cell line cultures and animal models. Nevertheless, these models fail in recapitulating the cellular heterogeneity and architecture of the tumour, and, consequently, tumour-associated pathways such as the oestrogen receptor (ERα) signalling in ERα+ breast cancer (BC). Organotypic cultures or tissue slices have been proposed, but they show loss of tissue architecture, along with a striking reduction both of ERα expression and cell viability after few days in culture. In order to overcome these pitfalls, we combined encapsulation of BC explants in an inert biomaterial, alginate, with dynamic culture, aiming at sustaining the tumour microenvironment and intrinsic signalling and we have been focusing on validating the platform for functional drug testing of targeted therapies

Experimental approach: More than 80 BC patient-derived explants were minced, digested overnight, dispersed in 2% ultrapure Ca^{2+} alginate and encapsulated by using an electrostatic bead generator. Explants were cultivated in a defined, serum-free medium, under orbital shaking, for up to 1 month. Tissue architecture, ERα expression and signalling were analysed by immunohistochemistry (IHC) and RT-qPCR of downstream targets upon oestrogen stimulus. The effect of standard of care targeted therapies upon viability was evaluated by LDH leakage, apoptosis detection and IHC

Results and discussion: Around 80% of BC explants kept high cell viability up to 1 month in culture, retaining the original cell architecture, including epithelial:stromal ratio and ERα protein . Upon oestradiol treatment, there was significant upregulation of ERα target genes, such as PS2, AREG and PR, indicating that ERα signalling was maintained in explants. Challenge with standard of care targeted therapies demonstrates the applicability of the platform for discovery and characterization of novel chemo, anti-endocrine drugs and therapeutic antibodies

P-305

IMPACT OF IGG AND FCΓ RECEPTORS N-GLYCOSYLATION UPON THEIR INTERACTION

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Background and novelty: The N-glycosylation profile of monoclonal antibodies (mAbs) is known to influence their effector functions and pharmacokinetic properties and is so considered as a critical quality attribute. Therapeutic agents with enhanced clinical efficacy have thus been developed via the selective enrichment of desired mAbs glycoforms. Of interest, the N-glycosylation of Fcγ receptors (FcγRs) can also affect immunoglobulin G (IgG) binding even though the mechanisms are still to be clearly determined. In this work, we investigated the influence of both IgG1 and FcγRs N-glycosylation on their interaction.

Experimental approach: To this end, we used a surface plasmon resonance (SPR) based assay aiming at measuring the interactions between IgGs and the extracellular domain of the FcγRs immobilized on the biosensor surface with our capture strategy based on coiled-coil interactions. Then, the affinity of eight well-characterized IgG1 glycoforms for FcγRs were determined while site-directed mutagenesis combined to glycoengineering were used to assess the FcγRs N-glycosylation impact on several IgG1 glycoforms binding.

Results and discussion: We first demonstrated that the IgG1 N-glycan profiles differently influenced the affinity for the FcγRs with an expected major effect on FcγRIIIa binding. We observed a marked impact in the absence of core fucose and a modest, yet noteworthy, impact of galactose and sialic acids regardless the fucosylation level on FcγRs binding. Moreover, affinities measured by SPR were perfectly echoed by an ADCC assay. On the other hand, both macro- and microheterogeneity of FcγRs N-glycosylation impacted to various extent their affinity for IgG glycoforms. Altogether, our results unravelled the complex and strong influence of N-glycosylation upon the FcγRs/IgG1 binding and will be instrumental to understand the impact of FcγRs N-glycosylation in their natural forms, while helping the development of next-generation mAbs.

P-306

HYBRID AGGLOMERATION OF INSULIN-PRODUCING BETA CELLS AND STEM CELLS

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Background and novelty: The standard insulin therapy for diabetics is associated with long-term damages and is a burden for the patient. Novel approaches replenish the destroyed beta cells with insulin-producing cells in order to restore the glucose homeostasis. A challenge are sources of functional insulin-producing cells and a manufacturing process, which generates enough high quality cells to satisfy the needs of cell therapies with 10^6-10^{10} cells per dose. Cell growth and functionality of beta cells decrease in standard monolayer/2D cultures. The cultivation as agglomerates and the co-cultivation with mesenchymal stem cells (hMSCs), can improve the survival and functionality of beta cell agglomerates. The positive hMSC effect is given either by direct cell-to-cell interaction and/or is based on secreted factors. Our aim is the development of a bioreactor-based co-cultivation process, which generates sufficient functional beta cell mass for cell therapy.

Experimental approach: We established the co-cultivation of beta cells and hMSCs from different sources as hybrid agglomerates and investigated the agglomeration process in static and dynamic systems.

Results and discussion: The agglomerate size is an important parameter to prevent mass transport limitations. Therefore, the determination of hypoxic regions and necrotic cells within the agglomerates was used to define the maximum agglomerate size. We also calculated the maximum agglomerate size based on the metabolite consumption rates. The distinction and arrangement during the agglomeration process of both cell types was investigated using two fluorescent long-term stains. We used the direct co-cultivation as hybrid agglomerates to investigate the optimal beta cell-to-hMSCs ratio, which was determined by the highest insulin stimulation. To conclude, our work gives an important insight into the cell behavior in hybrid agglomerates and serves as the basis for the planned bioreactor co-cultivation process.

P-307

RECEPTOR-MEDIATED CLEARANCE OF RECOMBINANT HUMAN DIAMINE OXIDASE

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Background and novelty: Human diamine oxidase (hDAO) is a key enzyme in the catabolism of histamine. Increased histamine concentrations contribute to the development of partially life-threatening symptoms in pathologies like anaphylaxis or mastocytosis. Histamine receptor antagonists are only partially effective. The use of recombinant hDAO (rhDAO) to rapidly degrade excess histamine is a possible treatment approach, but essential data on DAO are missing.

Experimental approach: rhDAO concentrations in the blood were measured via ELISA after intravenous administration to rats. A cellular in-vitro assay, based on flow cytometry, western blotting and fluorescence microscopy, was established to investigate potential receptor binding and internalization of rhDAO by various cell types. To study a potential N-glycan-mediated clearance via the asialoglycoprotein-receptor (ASGP-R), rhDAO mutants lacking N-glycans were tested in-vivo and in-vitro. Typical mannose-receptor (MR) or ASGP-R ligands were applied in excess to block the potential receptor-binding of rhDAO.

Results and discussion: The half-life of rhDAO in rats was less than 5 minutes. Neither the deletion of rhDAO N-glycans, nor the competition with MR- and ASGP-R ligands reduced the clearance of rhDAO. MR-expressing macrophages did not show cellular binding or uptake of rhDAO and ASGP-R-expressing hepatic epithelial HepG2 cells internalized only low amounts of the wildtype protein. A decrease in uptake was not observed with N-glycosylation mutants. Thus, glycan-mediated cellular uptake via the MR and/or the ASGP-R does not seem to be involved in the high clearance rate of rhDAO. A low-level uptake of rhDAO was also observed for other epithelial cell lines, while human fibroblasts and human endothelial cell lines displayed significantly higher internalization capacities.

These results demonstrate for the first time that DAO is likely cleared via a protein-specific, glycan-independent receptor-mediated internalization pathway.

P-308

GLYCOSYLATION VS RECEPTOR AFFINITY TO IMPROVE IFN4N ANTITUMOR ACTIVITY

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Background and novelty: The antiviral and antitumor activity of interferon-a2b (IFN-a2b) has inspired the development of several new IFN-based drugs. IFN4N is a hyperglycosylated variant developed in our laboratory by glycoengineering. Although this molecule exhibited reduced in vitro bioactivity compared to the non-glycosylated IFN, it showed improved pharmacokinetic properties and higher in vivo antitumor activity. Since R23N mutation has been identified as the main responsible of affecting IFN4N antiproliferative capacity, here we propose the design, production, purification and characterization of new highly glycosylated IFN muteins with better biological activity.

Experimental approach: Different groups of muteins were designed and produced in CHO-K1 cells: Group A, IFN variants with the same glycosydic content than IFN4N but higher in vitro antiproliferative activity (by restoring R23 to the molecule); Group B, muteins with higher glycosylation degree but lower in vitro activity (R23 not restored); Group C, muteins with both higher in vitro antiproliferative activity and higher glycosylation degree.

Results and discussion: Muteins with higher apparent molecular mass exhibited slower plasmatic clearance in rats. Experiments in nude mice implanted with prostate cancer-derived cells revealed that all new hyperglycosylated variants were able to reduce tumor volume (TV) compared the vehicle-treated control. Regarding IFN4N, muteins with the same glycosylation degree but with R23N mutation restored (group A) did not significantly decrease TV ($p>0.05$). Contrarily, muteins from groups B and C caused a reduction of TV in comparison with IFN4N ($p<0.001$). Thus, having or not restored the R23N mutation, their higher glycosylation degree improved pharmacokinetic properties and were responsible of the better in vivo bioactivity. Our results showed that properties conferred by hyperglycosylation would be more successful than receptor affinity to reduce TV of IFN-treated tumors.

P-309

THE EFFECT OF TELOMERE SEQUENCES ON CHROMOSOMAL TRANSLOCATIONS

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Background and novelty: Chinese hamster ovary (CHO) cell is widely used for the therapeutic antibodies production. Construction of a stable high producer from single CHO cell is time-consuming process and one of the bottleneck for cell line development (CLD) is the frequent chromosomal translocations. However, the cause or the loci of chromosomal translocations in CHO cells has not been fully identifed. In this study, we focused on the interstitial telomeric sequences (ITSs), and analyzed the relationship with the chromosomal translocations.

Experimental approach: To identify the position of chromosomal translocations and ITSs, multi-color fluorescence *in situ* hybridization (mFISH) and/or standard FISH were performed. Two differently-derived Chinese hamster cell lines were used for the analysis: Chinese hamster lung (CHL) cell, which was freshly established from lung tissue was used for long-term observation and CHO cell lines were used as to describe the effect of ITSs on chromosomal translocations. The number of chromosomal translocations and ITSs of each cell lines were analyzed and evaluated statistically.

Results and discussion: With increase of cultivation time, the number of chromosomal translocations and the ratio of ITSs at the chromosomal translocation sites of CHL increased dramatically. The ratio increased from 24% to 72% of the chromosomal translocation sites from day 96 to day 139, even though the total number of ITS in the cell stayed constant. From this result, ITS seems to be the loci where the chromosomal translocations most freqently occurs in CHL. However, the ratio of ITSs at chromosomal translocation sites varied among CHO cell lines. This indicates that the effect of the ITSs on the chromosomal translocations dpends on the cell lines. In summary, ITS might be the loci with high frequency of chromosomal translocations in Chinese hamster-dervied cells, which can be dependant on the cell lines.

P-310

IMPACT OF BEAD COLLISIONS ON HWJ-MSC EXPANSION PERFORMANCE

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Background and novelty: Mesenchymal stem cells isolated from the Wharton's jelly of human umbilical cords (MSC) are of increasing interest for cell therapies due to their reduced immunogenicity, high expansion capabilities, fast growth kinetics and various growth factors synthesis capabilities. To address the problem of cell confluence on microcarriers during their culture in mixed bioreactors, it was previously shown that the addition of fresh microcarriers could maintain cell growth and allow higher cell densities than without microcarriers feed [1]. However, the resulting increase in the bead shock frequency should also negatively impact cell quantity and quality. Until now, no quantitative study describing the impact of bead interactions on MSC death was reported. The aim of this study is to determine respective influence of microcarriers feed strategy and microcarriers mixing characteristics on cell viability and to propose robust culture conditions.

Experimental approach: Aiming at this, MSC were cultivated on Cytodex-1 microcarriers in HPL supplemented culture medium, in shaken flasks and in spinner vessels, below or above particle just-suspended states (Njs). DAPI nuclei and Live/dead cell staining were performed to determine cell viability and number of cells per microcarrier. Glucose, glutamine, ammonium, lactate and lactate dehydrogenase activity were monitored every day.

Results and discussion: Our results showed that, when particle mixing was below Njs, local increase of particle volume fraction promoted a significant cell death in both agitation modes: orbital and mechanical stirring. However, an increase in agitation rate above Njs was clearly beneficial to cell viability, growth and performance of MSC bead-to-bead transfer. These effects were magnified by microcarrier addition due to the increase of mean volume fraction of particles. Our study also revealed the critical influence of Njs and particle distributions within the bioreactor on MSC culture performances.

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P-311

A NEW DIMERIC BLOOD-BRAIN BARRIER PENETRATING TNFA INHIBITOR

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Background and novelty: In pathological conditions, microglia release a large amount of TNFα that play a pathogenic role in inflammatory neurological disorders. Biologic TNFα inhibitors cannot be developed for neurological disorders treatment since these large molecules do not cross the Blood-brain barrier (BBB). We report a novel TNFα inhibitor, a fusion protein composed by a BBB penetrating peptide, TNF receptor extracellular domain, and VEFG dimerization domain.

Experimental approach: The Angiopeptide was used to mediate transcytosis, and new BBB penetrating peptides were obtained by phage display biopanning. *In silico* systematic mutations on VEGF amino acid sequences were performed for identifying key residues, which mediate binding to its receptors. The fusion protein (MTH-TNFR2-VEGF*) coding sequence was cloned and expressed in stably transfected Chinese hamster ovary cells. Angiogenic activity was quantified by endothelial cell tube formation assay. Binding capacity and *in vitro* biological activity were determined by thermophoresis and inhibition of TNFα cytotoxicity on L929 cells assays, respectively. Immunofluorescence microscopy was used to evaluate the internalization of recombinant protein by ECV304 cell line.

Results and discussion: Two new mutations diminishing VEGF binding to both receptors were identified, Y21P and Y25G, and included in the recombinant molecule. Although purified MTH-TNFR2-VEGF* showed dimeric conformation, *in vitro* angiogenic activity was not observed. Our fusion protein binds TNFα with similar Kd value of Etanercept. Additionally, it inhibited the cytotoxic effect of TNFα *in vitro.* Internalization of MTH-TNFR2-VEGF on endothelial cells was also probed. These results make MTH-TNFR2-VEGF* a novel anti-TNFα candidate drug of systemic administration for neurological disorder treatment.

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HYPERGLYCOSYLATED EPO VARIANTS TO TREAT NEURODEGENERATIVE DISEASES

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Background and novelty: Neurodegenerative diseases affect the nervous system causing cognitive and behavior disorders. Since there is no effective therapy, new neuroprotective and neurotrophic agents are necessary. In this sense, human erythropoietin (EPO) has an important role considering its antiapoptotic, cytoprotective, angiogenic and antioxidant properties. Nevertheless, its hematological activity (HA) should be considered as a side effect. EPO molecular regions responsible of neuroprotective or neuroplastic activity are dissociated from those linked to HA. Thus, the incorporation of new potential N-glycosylation sites was carried out in EPO regions identified as essential for displaying the HA avoiding modification of residues needed to retain the neuroproperties

Experimental approach: EPO analogs were obtained by adding new extra N-glycosylation sites by site–directed mutagenesis and produced in transduced CHO.K1 cells. Muteins were purified by immunoafinity chromatography. Primary cultures from hippocampal neurons were used to measure apoptosis inhibition, neuritogenesis, filopodia density and synapsis formation. *In vitro* and *in vivo* HA assays were also carried out

Results and discussion: Three EPO variants were produced and purified with a purity level higher than 89% in only one step. They presented a molecular weight higher than EPO and a superior number of acid isoforms as result of the increased glycosylation degree. None of the variants evidenced HA evaluated both *in vitro and in vivo* ($p<0.001$). Nevertheless, all of them preserved the neuroprotective and neuroplastic activity as they prevented staurosporine-induced apoptosis ($p<0.001$) and they promoted neuritogenesis ($p<0.05$ and $p<0.001$), filopodia density ($p<0.05$ and $p<0.001$) and synapsis formation ($p<0.01$ and $p<0.01$). Thus, blocking the HA and retaining the neuroprotective and neuroplastic action in a context of long lasting/acting effects could potentiate these EPO variants as novel neurobiopharmaceutics

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ADVANCING MANUFACTURE OF HIPSC-HEP THROUGH BIOPROCESS UNDERSTANDING

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Background and novelty: Primary hyperoxaluria type 1 (PH1) is a rare metabolic disorder caused by mutations in the hepatic AGT. Defective AGT in PH1 patients is characterized by excessive oxalate synthesis, which leads to several kidney complications. Combined liver-kidney transplantation remains the most effective treatment but significant mortality and costs encouraged the development of advanced cell and gene therapies. Thus, our aim was to implement a novel strategy to generate hepatocytes from PH1 patient derived human induced pluripotent stem cells (hiPSC-Hep) for PH1 disease modelling application.

Experimental approach: We cultured hiPSC as 3D aggregates in stirred-tank bioreactors (STB) operated in perfusion and used a capacitance probe for *in situ* monitoring of cell growth/differentiation. After cell expansion, the hepatic differentiation step was integrated by addition of key soluble factors and controlling the dissolved oxygen concentration at various stages of the process to generate populations enriched for definitive endoderm, hepatocyte progenitors and hepatocytes.

Results and discussion: hiPSCs were able to grow as 3D aggregates showing an expansion factor of 6 after 4 days in culture while maintaining their pluripotent phenotype. The analyses of hepatic markers expression throughout the process confirmed that hepatocyte differentiation was improved in 3D spheroids when compared to 2D culture. Noteworthy, these hiPSC-Hep exhibited functional characteristics typical of hepatocytes (albumin production, glycogen storage and CYP450 activity). We also demonstrate the potential of dielectric spectroscopy to monitor cell expansion and differentiation in STB.

Overall, the strategy developed herein could provide wide applications in drug discovery, bio-artificial liver devices and regenerative medicine towards the treatment of PH1.

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3-D BLADDER TUMOR MODELS USING HYDROGELS AND SCAFFOLD-FREE SYSTEM

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Background and novelty: Three-dimensional (3D) cultures enable the maintenance of important conditions found in native tissues such as cell-cell and cell-ECM interaction and can be used in preclinical phase of drug development as a model able to recapitulate pathophysiological aspects of a tumor. Finding the best method to generate these cultures is still a challenge. This work compared the generation of 3D bladder tumor models with RT4 cell line using hydrogels and scaffold-free systems

Experimental approach: Scaffold-free system: forced floating (96-well Ultra-Low Attachment plates, Corning) and hanging-drop (96-well Perfecta3D plates, 3D Biomatrix) methods. Hydrogel cultures :0.25% HydroMatrix™ (Sigma), 2% v/v Alginate (Sigma) and 50% v/v Matrigel™ (BD Biosciences).

Results and discussion: Compact and uniform single spheroids with diameters raging from 300 to 500 µm were generated after 48 hours of culture in both scaffold-free methods. Cell proliferation was also observed in both methods (fold increase, FI, in the range of 3.0 to 7.0) as well as the presence of apoptotic cells (10%) in the end of 7-days culture. In hydrogel cultures many spheroids were formed per well with an aggregation time of more then 48 hours. The biggest spheroids were obtained in Matrigel™ cultures (100 to 200 µm) that also presented higher cell proliferation rate (FI=8,5). No apoptotic activity was observed in scaffold-based 3D cultures. Even with the best results regarding cell proliferation and viability, spheroids in Matrigel culture may not have the formation of hypoxic core (diameter higher than 200 µm) that influence drug resistance in solid tumors. Based in these preliminary results, we considered the scaffold-free a more suitable method to generate 3D bladder tumor spheroids. Future assays will focus in the comparison of drug sensitivity and gene expression profile of spheroids generated.

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DEVELOPMENT OF TYPE I ALLEGY THERAPEUTICS FROM GREEN ASPARAGUS

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Background and novelty: Type I allergic reactions, such as asthma and pollinosis, are major conditions that are common all over the world. It is well known that type I allergic reaction is provoked by the degranulation of immune granulocytes (mast cells, basophils). The bioactive components of green asparagus are thought to partly impact on these events. Although green asparagus (*Asparagus officinalis L*) consumption has increased world-wide, there are few reports concerning its anti-allergic effects. Here, we present evidence of novel degranulation inhibitors derived from asparagus and their mechanism of action using relevant allergy models.

Experimental approach: The anti-allergic activity of green asparagus was investigated using the calcium ionophore A23187-iduced degranulation of RBL-2H3 rat basophilic leukemia cells. The release of β-hexosaminidase was assessed as a marker of degranulation. The effect of asparagus components on an *in vivo* allergic response was evaluated using the atopic dermatitis NC/Nga mouse model. Mice were orally dosed with glycolipid or phospholipid fractions purified from green asparagus following periodic picryl chloride-induced atopic dermatitis.

Results and discussion: *In vitro* cell assays showed that green asparagus extract markedly inhibited β-hexosaminidase release by 45% without any cytotoxicity in A23187-stimulated RBL-2H3 cells. Treatment with both glycolipid and phospholipid fractions extracted from green asparagus resulted in the decrease of β-hexosaminidase release suggesting that these components could be responsible for the alleviation of type I allergy. Furthermore, the oral administration of glycolipid and phospholipid fractions markedly alleviated skin reactions in both back and ear atopic dermatitis model mice. In conclusion, *in vitro* and *in vivo* studies suggest that glycolipid and phospholipid components of green asparagus improve the atopic dermatitis response through the inhibition of degranulation reactions in granulocytes.

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GMOPM: AN HGM-CSF-DERIVED PEPTIDE AS A NOVEL O-GLYCOENGINEERING TOOL

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Background and novelty: One of the major concerns regarding administration of protein biotherapeutics for disease treatment lies in their low stability and short plasma half-life. To circumvent this problem, we have developed a 15-mer peptide tag named GMOPm. This sequence comprises the first 7 amino acids of the N-terminal region of human granulocyte-macrophage colony stimulating factor (hGM-CSF) together with 8 more residues that have been added in order to generate 6 potential O-glycosylation sites. The goal of this work was to study the ability of GMOPm to improve the pharmacokinetic of a widely used biotherapeutic, human interferon-α2b (hIFN-α2b), as a mean to increase its efficacy.

Experimental approach: Five chimeras were constructed by adding GMOPm to the N- and/or C-terminal ends of hIFN-2b in different proportions to obtain variants with 7 to 29 potential O-glycosylation sites as predicted in silico: GMOPm-IFN; (GMOPm)2-IFN; (GMOPm)3-IFN; (GMOPm)2-IFN-GMOPm and (GMOPm)3-IFN-GMOPm. The variants were purified, in vitro characterized, and analyzed regarding their pharmacokinetic parameters.

Results and discussion: CHO-K1 recombinant cell lines were cultured for IFN variants production and the chimeras were purified from culture supernatant by affinity chromatography, with yields ranging from 40 to 100%. SDS-PAGE and IEF analysis demonstrated that the more the number of GMOPm tags added, the higher the molecular mass and the lower the isoelectric point of the fusion protein. Interestingly, all IFN variants retained *in vitro* antiviral activity, although it decreased concomitantly with the number of fused tags. Pharmacokinetic experiments in rats demonstrated that the highest glycosylated variants, GMOPm2-IFN-GMOPm and GMOPm3-IFN-GMOPm, exhibited a plasma half-life 2and 4-fold higher and a clearance rate 3 and 5 fold-lower than the variant with a single GMOPm tag, respectively, demonstrating the success of this O-glycoengineering tool for improving IFN properties.

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CELL CULTURE EVALUATION OF EPO NEUROPROTECTION AND NEUROPLASTICITY

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Background and novelty: Neurodegenerative diseases affect the nervous system with an incidence increasing every year. In this sense, human erythropoietin (EPO) has a leading role because its antiapoptotic, antiinflammatory, antioxidant and antiproliferative effects have been observed in neural tissues. Thus, the recombinant form of EPO (rEPO) might be a potential tool for neuroprotection and neurogenesis of brain-damaged areas.

Experimental approach: The in vitro neuroprotective activity of rEPO was determined by measuring the capacity of the cytokine to reverse the staurosporine (STP)-induced apoptosis using a murine neuroblastoma cell line (N2a) and primary cultures from hippocampal neurons (HNPC). Neuroplasticity was determined by measuring neuritogenesis in N2a cells and filopodia and synapses formation in HNPC.

Results and discussion: The neuroprotection studies demonstrated that rEPO was capable to significantly reverse the STP-induced apoptosis in a dose-response way, both in N2a cells as in HNPC (between $p<0.01$ and $p<0.001$). Considering the evaluation of neuroplasticity, firstly N2a cell line was employed to measure neuritogenesis. Cells treated with 50 and 300 ng/ml of rEPO showed a significant increase in the neurite length ($p<0.05$ and $p<0.001$) and number ($p<0.05$ and $p<0.01$) compared with controls. Secondly, filopodia and synapses formation were studied in HNPC. These neurons treated with 50 and 300 ng/ml of rEPO showed a significant increase in filopodia density along 20 µm of dendrite length ($p<0.05$ and $p<0.001$) and in the number of synapses ($p<0.01$ and $p<0.01$) compared with control cells. Thus, N2a cell line and HNPC are useful and complementary platforms to evaluate the neuroprotective and neuroplastic role of rEPO and potential derivatives designed to display neuroprotection and neuroplasticity during the evolution and recovery of neurologic illnesses.

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NEUTRALIZING ACTIVITY OF A CHIMERIC ANTIBODY: GLYCOSYLATION IMPACT

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Background and novelty: Monoclonal antibodies are the main subset of marketedbiotherapeutics. Although they are considered bifunctional molecules, it was reported that constant regions of different IgGs with identical variable regions influence both their effector properties and their antigen-binding activity. While the glycosylation pattern strongly influences the effector functions, it was believed to have no effect on the antigen-binding ability. In this work, we evaluated the impact of the *N*glycosylation on the antigen-neutralizing ability of a chimeric antibody.

Experimental approach: A chimeric antihuman IFNα2b murine singlechain Fv fused to human Fcγ1 (scFvFc) was produced in CHOK1, HEK293 and NS0 cells (scFv-Fc_{CHO}, $scFvFc_{HEK}$ and $scFvFc_{NS0}$, respectively). A partially deglycosylated $scFvFc_{CHO}$ ($scFvFc_{DEG}$) was generated. The neutralization of the IFN biological activity was evaluated by three independent bioassays. *N*glycosylation structures were analyzed by hydrophilic chromatography.

Results and discussion: The scFvFc(s) showed differences in their IFNneutralizing ability. The molecule with the best neutralizing activity was $scFvFc_{CHO}$, which presented the highest *N*glycan site occupation mainly with G1F structures. The $scFvFc_{NS0}$ showed the lowest neutralizing activity with 70% of occupied sites (mainly with G2F structures) respect to the $scFvFc_{CHO}$. The $scFvFc_{HEK}$ showed a medium neutralizing activity with 30% of occupied sites mainly with G0F structures. Apparently, both the quality andthe quantity of *N*glycans influence the neutralizing ability of scFvFc(s). In accordance with this evidence, $scFvFc_{DEG}$ showed a considerably reduced neutralizing ability. Thus, glycosylation has to be considered in order to develop therapeutic antibodies, not only for its impact on antibody effector properties but also its influence on functional antigenbinding.

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CAN CELL CULTURE TECHNOLOGIES HELP WITH DIFFICULT TO EXPRESS PROTEINS?

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Background and novelty: Some engineered proteins are still difficult to express or/and have high level of microheterogeneity which can significantly impact the manufacturing costs. Moreover, the acceleration of biopharmaceutical process development is difficult when traditional experience-based sequential approaches are used. In this case study we will present the production challenges related to difficult to express antibodies and discuss the strategy and methodology taken at UCB to overcome low yielding processes while accelerating biopharmaceutical process development.

Experimental approach: In a first step, fed-batch platform modification, optimization of feeding strategy and optimization the media were assessed in a high-throughput system cell culture system and their impact on productivity and product quality were investigated. This screening work highlighted areas for potential productivity enhancement and the most promising conditions were further tested in conventional bioreactors. Those new information and data were then used in combination to platform knowledge to develop a toolbox for process optimization.

In a second step, an optimized process was developed and scaled up to 2000L bioreactor with a new clone producing the same therapeutic. In order to accelerate process development and reduce the number of experiment to test, the knowledge gained from the initial clone and the process intensification toolbox were applied in combination to an In Silico model [1] predicting cell culture performance.

Results and discussion: It was possible to double the final productivity compared to initial process and confirm it at 2000L scale with a minimum number of wet lab experiments. In summary, our modeling methodology combined to our process intensification toolbox, provide a much better insight into the impact of process parameters on production yields and product quality, thus improving process understanding and control as well as accelerating process development.

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TOWARDS MORE MATURE HPSC-CM VIA METABOLIC MODULATION IN 3D CULTURE

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Background and novelty: *In vitro* differentiation of human pluripotent stem cells into cardiomyocytes (hPSC-CM) is a crucial process for the use of these cells for therapy and drug discovery. However, cardiomyocytes generated are immature, reminiscent of fetal cardiomyocytes regarding structure, metabolism and function. Here we combined our expertise in metabolic modulation and 3D culture to further enhance maturation of hPSC-CM.

Experimental approach: Cardiac differentiation of hPSC was done in 3D culture using stirrred tank biorreactors yielding highly pure hPSC-CM cultures followed by metabolic maturation with a fatty acid enriched media (FAM)[1,2]. Whole-transcriptome and metabolic flux analyses, transmission electron microscopy, calcium fluxes and viability responses following exposure to cardiotoxic drugs were performed to assess maturation status.

Results and discussion: When compared to onset maturation, 3D cultures of hPSC-CMs matured with FAM displayed a down-regulation of glycolysis and lipid biosynthesis related genes and increased expression for tricarboxylic acid cycle, oxidative phosphorylation and mitochondrial genes. Structurally, no differences in sarcomere length or fiber alignment were seen between both groups, though a significant increase of mitochondrial density was evident in the FAM media matured cells. In both cultures, calcium fluxes were blocked by calcium channels inhibitors. Cell death was observed in a dose-response manner to doxorubicin, with higher sensitvity to toxicity in the FAM matured aggregates. This study highlights the relevance of both metabolism and structure for the maturation of hPSC-CM and their importance for sensitivity to cardiotoxic drugs.

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SCREENING FOR FOODS THAT ACTIVATE REGULATORY T CELLS

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Background and novelty: Immune response is an important mechanism for removing foreign antigens that might enter into our body. However excess immune responses might cause autoimmune diseases and allergic diseases. In the present study, we focused upon regulatory T cells (Treg) to suppress excess immune responses and maintain homeostasis. Treg cells are now known to be one of therapeutic target to suppress autoimmune diseases and allergenic diseases. Here, we focused on RALDH2 gene in dendritic cells, which function to induce Treg cells through producing retinoic acid, then we screened for foods that activate RALDH2 gene in differentiated THP-1 cells.

Experimental approach: We amplified human RALDH2 promoter by PCR using human genomic DNA as a template, and cloned it into the promoter-less EGFP expressing vector (pRALDH2p-EGFP). This vector express EGFP under the control of human RALDH2 promoter. pRALDH2p-EGFP was transduced into THP-1, human monocytic leukaemia cells, and established stable cell line (THP-1 (RALDHp-GEGFP)). After adding foods to this cell line and culturing for 2 days, changes in the EGFP fluorescence derived from pRALDH2p-EGFP were monitored by an IN Cell Analyzer 1000 (GE Healthcare, Amersham Place, UK), and Flow cytometer (EPICS XL System II-JK(BECKMAN COULTER, CA,USA).

Results and discussion: We identified several polyphenols that activate RALDH2 promoter in THP-1 cells. In the future study, we would like to evaluate the functionality of these polyphenols.

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IMPROVED IN-VITRO DETECTION OF TOXIC LEACHABLES IN SINGLE USE MATERIAL

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Background and novelty: The application of single-use (SU) bioprocessing material has increased especially in the field of regenerative medicine due to low cost and high process flexibility. Harmful substances, even at low concentrations, migrating from SU material into cell culture must be discovered to ensure safe medical products. For reducing the risk of contamination, cells are currently adapted to grow in xeno-free media. However, official biocompatibility tests as described in the ISO10993, still use serum. In this study we present new sensitive test systems for the detection of toxic leachables comprising of a human and a rodent cell line, both grown in suspension without animal derived supplements.

Experimental approach: For assessing test sensitivity, cell growth of a Human Embryo Kidney (HEK293T) and a Chinese Hamster Ovary (CHO DG44) cell line, was compared in the presence of *bis*(2,4-di-*tert*-butylphenyl)phosphate (bDtBPP), that is known to cause reduced cell growth [1, 2]. Results were compared with cell growth of the adherent L-929 cell line, which is recommended in the ISO 10993. Furthermore, the impact of serum on test sensitivity was examined. Therefore, CHO DG44 and HEK293T cells were cultured at different serum concentrations in the presence of bDtBPP.

Results and discussion: The effective concentration, at which cell growth is reduced to 50% (EC_{50}) was determined after three days of cultivation. Compared to the L-929 cell line, the EC_{50} of CHO DG44 and HEK293T are reduced by one log_{10} indicating increased sensitivity towards bDtBPP.

In addition, serum masks the toxic effect of bDtBPP even at low concentrations of 0.5%. These findings confirm the fact that serum decreases test sensitivity and thus should be avoided in biocompatibility testing. At present, cytotoxicity of further cell growth impacting compounds used in the manufacturing process of SU material are examined with both suspension cell lines to study potential cell-line specific differences.

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CULTIVATION OF LARGE 3D BIOPRINTED TISSUES IN PERFUSION BIOREACTORS

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Background and novelty: In the past 5 years, bioprinting became a highly promising scientific area for regenerative medicine applications. Nevertheless, production and *in-vitro* maturation of large living tissues (up to dm3 sizes) are still strongly limited by nutrient supply and later on *in-situ* neo-vascularization to sustain cell viability within the tissues. Thus, nutrient perfusion, optimized internal geometry and processes design have to be developed to reach customized large tissues production. Such scale-up will have to go through specific and controlled bioreactors developments and biological behavior modeling to better understand the parameters impacting the tissue maturation.

Experimental approach: A comparative study of static and dynamic culture of large bioprinted conjunctive tissues endothelized with human dermal microvascular endothelial (HDMEC) cells was performed. Tissues were printed and maturated within silicone 3d-printed perfusion bioreactor. Computational Fluid Dynamics (CFD) simulation were used to describe the heterogeneous perfusion flow path within the bioprinted tissue. Cell growth and tissue maturation were characterized by monitoring the cell density and viability, the metabolites consumption rates and by studying the tissue morphology and composition obtained through histological observations.

Results and discussion: Clear differences between static and dynamic culture conditions were observed on cell growth and tissue maturation. Culture perfusion at 300mL/h was chosen for its compromise between flow heterogeneity and limitation of stagnant zones and a well-established laminar regime. Dynamic maturation conditions at 300 mL/h culture medium flow rate had a positive impact on the extracellular matrix production while conserving bioprinted tissue internal geometry. Typical microvascular organization, composed of human dermal microvascular endothelial cells organized around an open lumen were found within the large bioprinted tissue.

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PROCESS TRANSFER FROM 250L SINGLE USE TO 5000L STAINLESS STEEL VESSEL

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Background and novelty: GBR XYZ is a monoclonal antibody for therapeutic use in human. To supply clinical advancement, the project was transferred to a CMO and scaled-up from 250L single-use to a 5kL stainless steel bioreactor. Glenmark's approach for this process transfer was based on an identification of scale-up associated risks. This triggered experimental studies allowing process fine-tuning and therefore, batch success first time at scale. This poster describes the methodology used for the transfer and adaptation of the upstream process.

Experimental approach: First, a gap analysis was performed in collaboration with the CMO to identify scale-up related risks and the ones associated with the fitting in the existing facility. This allowed to highlight multiple process steps requiring de-risking activities or process changes. For clarification of the cell culture fluid, a centrifugation had to be developed at scale from the first Engineering batch. In addition, the particle size distribution post-centrifugation indicated the need to adapt the subsequent depth filter steps, so a filtration study was performed at small scale. Other risks required a mitigation plan to reduce their criticality, for example a much higher power input per volume due to the bioreactor scale and geometry, and an expected higher CO_2 accumulation. Small scale runs were performed before the first campaign at the CMO site. P/V ranges and gassing strategies were evaluated in order to assess the impact on process performance and product quality.

Results and discussion: The gaps associated with this scale-up and process transfer were identified. If required, de-risking experiments at small scale were performed. As a result, the process at 5000L was found to be comparable with the initial process at 250L in terms of cell culture performance and product quality. Glenmark's methodology and efficient communication and cooperation between the teams allowed a successful transfer in very short time.

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RECAPITULATING DEREGULATED EXTRACELLULAR DYNAMICS IN CNS DISORDERS

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Background and novelty: Brain microenvironment is important in development and pathology. Neural cultures usually rely on exogenous matrices that poorly resemble the ECM. We developed a bioprocess with perfusion bioreactors to generate iPSC-derived neurospheroids with the 3 neural lineages. We hypothesized that if this strategy allow deposition of native neural ECM, would be possible to mimic cellular and ECM remodeling during neural differentiation, without exogenous matrices effects, and recapitulate pathological features of diseases in which alterations in cell-cell interactions and ECM are relevant.

Experimental approach: Microenvironment changes during differentiation were addressed by quantitative transcriptomic (NGS) and proteomic (SWATH-MS) analysis. Neurospheroids from healthy donors and a Mucopolysaccharidosis type VII (MPS VII) patient were characterized.

Results and discussion: NGS and SWATH-MS data showed recapitulation of neurogenic developmental pathways, an enrichment in structural proteoglycans typical of brain ECM, downregulation of basement membrane constituents and high expression of synaptic and ion transport machinery. MPS VII, a neuronopathic lysosomal storage disease, leads to glycosaminoglycan (GAG) accumulation in the brain. MPS VII neurospheroids recapitulated disease hallmarks, as accumulation of GAGs, and showed reduced neuronal activity and disturbances in network functionality. Analysis of MPS VII neurospheroids secretome and proteome is ongoing to elucidate molecular alterations underlying neuronal dysfunction. These data will provide insight into the interplay between GAG accumulation and neural network alterations, and its impact on MPS VII-associated cognitive defects. The model provides a platform to unveil the cellular alterations responsible for brain dysfunction in CNS diseases and to test new therapies.

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ESTABLISHMENT OF FAST-GROWING CELLS FROM CHINESE HAMSTER LUNG

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Background and novelty: The Chinese hamster ovary (CHO) cells are widely used as host cells for biopharmaceutical production, superior in terms of post-translational modification of proteins. Cells from Chinese hamster lung was established in this study to develop high-producing host cells that can substitute for CHO cell lines.

Experimental approach: Minced lung tissues from female Chinese hamsters were placed in IMDM (20% FBS). Expanded cells were adapted to chemically defined (CD) medium by gradual decreases in the serum concentration. Cells were subjected to biosafety testing (BioReliance). IgG1 expression vector was introduced into cells, and the IgG1 concentration of drug-selected hetero cell pools was determined by an Octet QKe (ForteBio).

Results and discussion: Primary cultured cells mainly showed a fibroblast-like morphology. Cells were immortalized by spontaneous transformation. The ratio of aneuploid cells increased in the process of acquiring immortalization. Some translocations with the same pattern as CHO-DG44 and CHO-K1 derived cells were observed in cells. The cell pool was named CHL-YN, in which safety was confirmed by an *in vitro* assay to detect viral contaminations, sterility testing by direct inoculation, and a mycoplasma detection test.

The doubling time of CHL-YN was 10.5 hours in CD medium. Based on the cell cycle assay, it was suggested that the CHL-YN have a shorter G0/G1 cell cycle phase compared with CHO-K1. The transfection efficiency in CHL-YN using polyethylenimine was more than 50%, the same as for CHO-K1. Between approximately half and the nearly same amount of IgG1 was produced in CHL-YN in a shorter period compared with CHO-K1. Estimated glycan profiles of IgG1 by LC-MS measurement exhibited the same high peaks between CHO-K1 and CHL-YN products. Selection of suitable clones and culture method optimization are expected to aid the development of new production of host cells.

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BONE-MARROW EXPANDED MESENCHYMAL STROMAL CELLS FOR BONE GENERATION

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Background and novelty: Treatment of bone pathologies require tissue deposition which is typically obtained from the patient's own iliac crest, although in some occasion previous surgical similar procedures have left the patient with limited treatment options. In addition, iliac crest extraction is frequently associated to morbidity of the donor area which may last longer than the original bone pathology.

The Blood and Tissue Bank of Catalonia together with attending traumatologists have developed an Advance Therapy Medicinal Product based on autologous bone marrow expanded mesenchymal stromal cells colonized in allogeneic cancellous bone (XCEL-MT-OSTEO-ALPHA) for bone regeneration.

Experimental approach: To date 3 clinical trialshave been conducted to test the safety and efficacy of XCEL-MT-OSTEO-ALPHA in 3 different indications: spinal fusion, osteonecrosis of the femoral head and pseudarthrosis.

Results and discussion: 108 patients have been enrolled in the trials being 52 of them treated with XCEL-MT-OSTEO-ALPHA. During the follow-up period 208 adverse events have been collected from the 52 patients treated with the test product, and remarkably, none of them have been determined as causally related to XCEL-MT-OSTEO-ALPHA, but mainly related to surgical procedure.

The safety of autologous BM-MSC is maintained when colonized in allogeneic cancellous bone. Therefore, based on safety information available so far for XCEL-MT-OSTEO-ALPHA, we could proceed with further clinical development.

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SCALE-UP OF CLINICAL GRADE MULTIPOTENT MESENCHYMAL STROMAL CELLS

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Background and novelty: the increasing demand of clinical-grade multipotent mesenchymal stromal cells (MSC) has prompted developers to reconsider productive designs, which were often limited to small productions within the autologous setting, thus leading to new challenges in the manufacture of cells for therapy that must consistently retain their critical quality attributes (CQA) from batch to batch. Our strategies to identify suitable donors, MSC derivation from source tissue, scale-up and banking in a cost- effective manner are presented, highlighting challenges and opportunities that are not restricted to the scale up of MSC, but common in the field of cell therapy manufacturing regardless of the cell type of interest.

Experimental approach: MSC were isolated from the Wharton's jelly (WJ) of donated umbilical cord tissue as reported elsewehere (1-2). Ex vivo expansion of MSC was performed manually for the creation of a GMP-compliant master cell bank. Further cell culture expansion was performed manually using 5-tiered cellstack (Corning) for the generation of drug products used in a phase I trial for the treatment of chronic spinal cord injury (eudract no. 2015-005786-23). Alternatively, WJ-MSC were expanded in bioreactors (Minibio, Applikon) on the surface of microcarriers. Identity, purity, karyotype, multipotency and immunopotency was assessed as described previously (1, 3).

Results and discussion: WJ-MSC were succesfully expanded both in planar and microcarrier-based culture strategies maintaining CQA within specifications (CD45-CD105+, CD31-CD73+, CD90+; viability above 80%; trilineage potential in vitro into the osteogenic, chondrogenic and adipogenic lineages; and capacity to inhibit the proliferation of stimulated lymphocytes), thus demonstrating the feasability of scaling up the production for banking for use in new treatment options using allogeneic, off-the-shelf cells.

Acknowledgements & funding: Spanish Cell Therapy Network (rd16/0011/0028) and Generalitat de Catalunya (2017sgr719).

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ENHANCING SCALABILITY AND OSTEOGENIC POTENTIAL OF WHARTON'S JELLY MSC

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Background and novelty: In the last years, the use of multipotent Mesenchymal Stromal Cells (MSC) has been reported safe and efficient for bone regeneration in increasingly prevalent bone-related conditions. Consequently, the development of procedures based on the use of suitable allogeneic MSC that could be used off-the-shelf is totally necessary. Among the tissue sources for MSC isolation, Wharton's jelly (WJ) constitutes an attractive option due to their accessibility and primitive feature. Nevertheless, scale-up methods to achieve clinically relevant cell numbers and osteogenic differentiation capabilities should be addressed to guarantee the successful generation of cell banks based on WJ-MSC for the cure of human diseases.

Experimental approach: In this work we investigated the possibility of using dextran positive-charged micro carriers (Cytodex 1®, Solo Hill) to increase the culture surface and hence, WJ-MSC production. Additionally, we also studied the osteogenic differentiation process of WJ-MSC *in vitro.*

Results and discussion: Data from crystal violet staining and ATP measurement evidenced that the use of Cytodex® 1 has no impact neither on cell growth nor viability. Regarding osteogenesis, alizarin red staining results and gene expression analysis revealed that WJ-MSC are less prone to differentiate into the osteogenic lineage than bone marrow (BM) MSC. However, we found that co-culture of both cell types as well as addition of extracellular vesicles or proteins purified from differentiating BM-MSC to WJ-MSC cultures promote WJ-MSC osteogenic differentiation. This suggests that bone microenvironment could induce WJ-MSC osteogenesis *in vivo.* To confirm this hypothesis, we treated NSG mice with intrabony injection of WJ-MSC in order to achieve bone regeneration. Although further results are required before clinical translation, the use of allogeneic WJ-MSC offers many advantages in the orthopaedic field compared to other cell types.

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STEM CELL MANUFACTURE IN A DISPOSABLE BIOREACTOR WITH A BIOMASS SENSOR

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Background and novelty: Stem cells in the bioreactors need to be monitored and controlled for both product quality and to satisfy GMP requirements and one of the most important parameters is the concentration of live cells. On-line monitoring of the live cell concentration can be used to monitor process irregularities, define when sufficient cells are available for the patient or it might be the trigger point for providing additional feed to the bioreactor.

Sampling for cell concentrations should be avoided to eliminate the risk of contamination. Moreover, with stem cell cultures once embryoid bodies form it is both difficult to take a representative sample and to get a true cell count using a trypsin pre-treatment. The same problems occur if the cells are grown on micro-carriers. In this work, we evaluated a rocking motion bioreactor fitted with multiple sensors for process control including an integral bio-capacitance probe for the measurement of the live cell concentration.

Experimental approach: A new design of disposable bio-capacitance probe to measure mesenchymal stem cells (MSC) grown on micro-carriers was integrated into an expanding rocking motion bag (Scinus, Holland). In addition to measuring the growth of cells in culture, the biomass probe was also used to identify changes in the bioreactor setup, such as addition of fresh medium, change in the rocking speed & acceleration.

Results and discussion: The bio-capacitance probe was able to follow the cell growth of MSC cells grown on micro-carriers from 50,000 cells/ml through to 300,000 cells/ml. The reference method based on trypsining the cells from the carriers and counting the nuclei with a Coulter Counter would only provide the total cells. A good correlation was found between the offline method and the online bio-capacitance method

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NOVEL BIOREACTOR SYSTEM FOR EXPANSION OF HUMAN MESENCHYMAL STEM CELLS

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Background and Novelty: There is a significant need for efficient systems that can be used to generate primary cells and stem cells that can be readily implemented in research laboratories to expedite process development studies and clinical testing. We have previously demonstrated efficient expansion of human mesenchymal stem/stromal cells (HMSC) in the PadReactor® single-use bioreactor system to the 40 L scale. Here we extend these finding by employing these components in a novel bioreactor system. The Allegro™ STR bioreactor is a new stirred tank, single-use bioreactor platform which is scalable, compact, ergonomic and designed to maximize usability and process assurance.

Experimental Approach: HMSC were thawed and expanded on flatware for a period of 3 days. Microcarriers were transferred into the Allegro STR50 bioreactor via the Allegro microcarrier delivery system (AMDS) which provides an efficient method for rapid delivery of microcarriers into bioreactors in a sterile fashion. Microcarriers were acclimated in the bioreactor for one hour and then cells were harvested from flatware and seeded onto microcarriers at a density of 3000 cells/cm2 in 30 L of media. Cells were then cultured for 6 days following a fed-batch process.

Results and Discussion: Excellent results were achieved propagating HMSC on microcarriers in the Allegro STR single-use bioreactor over a six day culture period. Cells harvested from microcarriers at the end of the culture reached a cell concentration of 0.79 B cells/L and were 97% viable. a total of 22 B cells were harvested. Importantly, the cells retained critical quality attributes after harvest when examined in standard cell characterization assays. Results achieved in this study were also comparable to those obtained previously in the PadReactor platform. In this study we demonstrated utilization of a novel, single-use platform for efficient generation of high quality cells for process development studies and clinical testing.

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EFFECT-BASED STUDY OF HUMAN PLATELET LYSATE IN VARIOUS CELL LINES

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Background and novelty: In this study, the growth promoting efficiency and functional metabolic influence of a human platelet lysate (hPL), GMP-manufactured from platelet apheresis, on various cell lines was investigated considering the postulate of a full replacement for Fetal Bovine Serum (FBS) in cell culture. Although FBS is still widely used as supplement in cell line cultivation, it is not suitable for future therapeutic purposes. For mesenchymal stem cells, hPL is recognized as growth supplement superior to FBS; however, it needs to be validated with respect to its metabolic activity in various cell types which may find applications in regenerative medicine or cell-based therapy.

Experimental approach: As model cells of primary origin, the HaCaT, human Gingival Fibroblasts (hGF) and human Dental Pulp Mesenchymal Stromal Cells (hDPSCs) were used. In addition, seven permanent cell lines from differing origins were cultured in media supplemented with either 5-10% FBS or 5% hPL. Cell morphologies and proliferation efficacies were analyzed using the WST-1 assay or manual cell counting. Enzymatic and immunofluorescent assays were performed to elucidate cell specific markers.

Results and discussion: Comparing the cell proliferation between media supplemented with hPL versus FBS showed active support of proliferation with minor differences. Cellular morphologies, however, revealed major differences in the case of HepG2: the cells lost adherence and grew as slightly attached spheres when cultured in the presence of hPL. In conclusion, growth curves and cell viabilities pointed to a growth-promoting effect of hPL in various permanent and primary cell lines, comparable to FBS or even better. Several cell lines responded unexpectedly to the hPL treatment whose mechanisms are elucidated by determination of cell specific markers. The present study shows the need of in-depth investigations for each cell type with respect to future applications in cell therapy.

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IMPACT OF THE IMPELLER DESIGN ON MICROCARRIER HWJ-MSC EXPANSION

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Background and novelty: A particular attention has been paid to mesenchymal stromal cells (MSC) for their application in regenerative medicine, with more than 800 completed or ongoing clinical trials. By concern of expansion process scale-up, these adherent-dependent cells may be cultivated on microcarriers, which are then suspended by an agitation. However, MSC are shear sensitive, making strategies of culture intensification in stirred bioreactors more attentive to the hydrodynamics and the turbulence generated by the agitation, and thus also to the impeller design [1].

Experimental approach: On this basis, a multi-objective optimization strategy was conducted by using CFD simulations (two phase Euler-Euler granular approach) to minimize the power per unit of volume received by the solid phase $(P/V)_{@p}$ and an energy dissipation / circulation function *EDC*. This method was applied with an Elephant Ear impeller in down-pumping mode in a 200 mL minibioreactor, and by varying (i) the blade slope angle *b*, (ii) the *D/T* ratio defining the impeller diameter on the vessel diameter, and (iii) the *C/T* ratio defining the impeller clearance off the base on the vessel diameter.

Results and discussion: The two objective functions, $(P/V)_{@p}$ and *EDC* did not lead to the same optimal configuration. hWJ-MSC cultures were thus performed in minibioreactors with 3 selected impeller designs, displaying similar $(P/V)_{@p}$ values but various *EDC* values. The lowest expansion performance was obtained for *EDC* = 420 J m^{-3} with over 25 % lower glucose consumption, in comparison with *EDC* = 329 J m^{-3}. However, the impeller displaying an *EDC* of 294 J m^{-3} led to 10 % less cells than using the impeller displaying *EDC* = 329 J m^{-3}. Complementary investigations are in progress to define potential $(P/V)_{@p}$ and *EDC* threshold values for MSC cultures in stirred bioreactor.

Acknowledgements & Funding: This work benefitted from the ANR 'Stemcreactor' project, and the FEDER INTERREG 'Improve-Stem' project.

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3D HIPSC-BASED CARDIAC TISSUES FOR PRECLINICAL RESEARCH

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Background and novelty: Development of complex in vitro cell-based models as well as the implementation of advanced tools that enable their characterization are of utmost importance for drug screening and disease modeling. Current in vitro cell-based models for cardiotoxicity assessment rely on homotypical cardiomyocyte cultures, which do not resemble the complexity of the human cardiac environment. The aim of this study was to develop a physiologically relevant in vitro cardiac microtissue that better mimics the human heart tissue and allows the prediction of potential cardiotoxic effects.

Experimental approach: Inter-cellular communication in hiPSC-based heterotypic cardiac tissue models was evaluated using co-cultures of hiPSC-derived cardiomyocytes (hiPSC-CM) and endothelial cells (hiPSC-EC). A toolbox of analytical techniques was applied to confirm the importance of the presence of hiPSC-EC on hiPSC-CM maturation. Afterwards, a novel 3D hiPSC-derived tri-culture cardiac microtissue was developed by combining hiPSC-CM aggregates with hiPSC-derived endothelial cells and mesenchymal cells (hiPSC-EC+MC) inside alginate microcapsules.

Results and discussion: A quantitative proteomic approach (SWATH-MS) for the analysis of the co-cultures provided new insights into the molecular mechanisms involved in the crosstalk between the two cell types. Moreover, the tri-culture microencapsulation strategy was successfully implemented showing evidences of structural maturation of the microtissue when compared to hiPSC-CM mono-cultures. Finally, exposure to cardiotoxic compounds has resulted in loss of metabolic activity and cell viability.

Overall, this study provides insights towards the establishment of biologically-relevant in vitro cardiac tissue models. Additionally, the characterization methods herein implemented were demonstrated to have an essential role in in vitro cardiac preclinical research for the understanding of in vivo microenvironment recapitulation.

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PERSONALIZED CELL LINES BY REPRODUCIBLE AND FUNCTIONAL IMMORTALIZATION

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Background and novelty: A major limitation of current research is the shortage of functional personalized cells. Especially cell systems which reflect a diseased phenotype are not available in sufficient numbers. To overcome this shortage, cell expansion or even cell immortalization is an attractive alternative. This can be achieved upon expression of immortalizing genes. however, this process of establishing novel immortalized cell lines is unpredictable and cumbersome. In addition, conventional immortalization regimens lead very often to a drastic alteration of the cell physiology.

Experimental approach: We developed a novel defined immortalization regimen based on a gene library allowing the efficient and reproducible establishment of novel cell lines. This regimen was employed to establish cell lines from primary cells from nine different species and from different individuals. Importantly, novel cell lines were generated from different primary cell types within two to three months (e.g. endothelial cells, astrocytes, smooth muscle cells, chondrocytes, fibroblasts, lung and intestinal epithelial cells).

Results and discussion: The resulting cell lines are immortalized as they can be cultivated for more than 100 cumulative population doublings, show a robust proliferation and can be frozen/thawn without any viability loss. Functional characterization of the resulting cell lines demonstrated that the established cell lines retained the expression of cell type specific marker proteins as well as their specific functions. This phenotype was stable throughout the whole cultivation period. Importantly, once an immortalization regimen is established it could be easily transferred to primary cells from healthy and diseased donors.

We envision this immortalization approach to provide personalized cell systems in sufficient numbers. Thereby facilitating personalized drug development approaches for precision medicine.

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NOVEL MURINE AND HUMAN INTESTINAL EPITHELIAL LINES

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Background and novelty: Most drugs are given orally and therefore they have to be absorbed in the intestine to enter the human body. Therefore, there is an urgent need for test systems that precisely mimic the in vivo conditions of human intestine in vitro. The epithelium of the intestine is composed of various cell types which significantly contribute to the barrier function of the intestine. These cell types set up the complex architecture of the intestine and are responsible for the absorption of drug candidates. Especially the enterocytes form a tight cellular barrier and in addition the Goblet cells produce mucus layer which is protecting the cellular barrier.

Experimental approach: With a recently developed immortalization technology (Lipps et al., 2018) we developed >50 novel immortalized cell lines. As starting material murine and human organoids from the intestine were used. These organoids have the advantage that they resemble the structure of the intestine and are composed of the abovementioned cell types. The organoids were randomly transduced with the CI-SCREEN gene library and the expanded to establish immortalized cell lines. Next, a process was established that assessed different parameters like morphology, gene expression, protein expression, protein localization and functional properties to identify a cell line that best reflects the in vivo situation.

Results and discussion: By this experimental approach we were able to generate murine and human cell lines that express intestinal epithelial specific markers on a RNA level as well as on the protein level. Importantly, this cell line is polarized as assessed by immunofluorescence and forms specific epithelial structures which could be visualized by electron microscopy. Furthermore, these cell lines are characterized by a tight barrier. These phenotypic characteristics were stable in culture for more than 40 population doublings, which renders this cell line an ideal tool for infection or drug absorption studies.

References: Lipps et al., Nat. commun 2018; doi: 10.1038/s41467-018-03408-4.

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A HIGH-THROUGHPUT SCREENING TOOL FOR MANUFACTURING OF T-CELL THERAPIES

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Background and novelty: Adoptive T-cell Therapy (ACT) is a promising area of medicine where the infusion of lymphocytes is used to induce antitumor effects in patients with haematological malignancies. Despite the recent commercial approval of chimeric antigen receptor (CAR) T-cells to treat B-cell acute lymphoblastic leukaemia and non-Hodgkin lymphoma, the design space for the manufacturing of T-cell therapies is yet to be fully characterised. The work presented assesses the feasibility of Applikon®'s micro-Matrix single-use platform for use as a high-throughput screening tool in optimising the perfusion culture of T-cells.

Experimental approach: A Design of Experiment (DoE) approach (a two-level three parameters full factorial) was used to evaluate the effect of different controlled conditions. Investigated parameters include different dissolved oxygen (dO_2) levels (25% and 90%), pH (6.9 and 7.4) and shaking speeds (100rpm and 200rpm). The response surfaces for different outputs were modelled by applying the least squares fit to the data gathered.

Results and discussion: Results have shown that significantly higher fold expansions were achieved in various conditions at 200rpm compared to 100rpm. Regarding the phenotypic composition of T-cells, our data revealed that different pH, dO_2 and shaking speed had no significant effect on the helper (CD4+) to cytotoxic (CD8+) T-cells ratio. Furthermore, we showed that CD8+ central memory T-cells favour low dO_2 and high pH conditions, suggesting a potential new operating condition for T-cell therapies manufacturing. Therefore, the micro-Matrix is a powerful tool for screening the expansion process parameters for the manufacture of T-cell therapies at the micro-scale.

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METABOLOMICS AS A QUALITY CONTROL TOOL FOR CHONDROGENIC MICROTISSUES

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Background and novelty: The use of 3D microtissues is becoming a standard for bone tissue engineering approaches, as this format allows cell-cell and cell-extracellular matrix interactions. Considering the role of metabolism as a key regulator of stem cell fate and the high sensitivity of metabolomics, this study aims to identify metabolic quality attributes indicative of a functional cartilage intermediate TE construct. To the best of our knowledge, this is the first metabolomic analysis of chondrogenic differentiation of an adult stem cell population cultured as spheroids.

Experimental approach: LC-MS (liquid chromatography-mass spectrometry) tracer analysis was conducted to investigate metabolic alterations during chondrogenic differentiation of spheroids of hPDCs (human periosteum derived stem cells). ^{13}C labeled glucose, glutamine but also serine and aspartate have been used, as these metabolites showed significant differences between the time points of interest in a prior exometabolomics study. Samples were analyzed at day 0, day 14 and day 21, as these time points capture the transition of hPDCs-derived chondrocyte phenotypes (proliferating, prehypertrophic, hypertrophic state).

Results and discussion: Our tracer analysis results showed progressive ^{13}C glucose enrichment in palmitate from 0% at day 0 to 8% at day 14 and 22% at day 21, suggesting activation of fatty acid synthesis. Furthermore, we observed ^{13}C glutamine enrichment in proline from 0 % at day 0 to 20 % at day 14 and 42,5 % at day 21 and a similar trend of ^{13}C glutamine contribution to hydroxyproline (from 0 % at day 0 to 35 % at day 14 and 38 % at day 21). These observations suggest that the consecutive stages of chondrogenic differentiation of hPDCs are characterized by specific metabolic adaptations and highlight the importance of quite unexplored metabolic pathways such as fatty acid and glutamine metabolism for the regulation of chondrogenic differentiation.

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EVALUATION OF HMSC-DERIVED EXTRACELLULAR VESICLES BY FTIR SPECTROSCOPY

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Background and novelty: In the last decade, the therapeutic effects of mesenchymal stem/stromal cells (MSCs) have been attributed to a paracrine activity exerted by extracellular vesicles (EVs) secreted by MSCs. Their properties as intercellular communication vehicles have led to an increase interest in their use for cell-free therapeutic applications.

Experimental approach: The present work aimed to evaluate how different culture conditions, as culture medium (xenogeneic -free (XFM) vs serum-containing medium (SCM)) and different MSC donors, affect the chemical characteristics of EVs. For that, purified MSC-derived EVs were characterized by Fourier-Transform InfraRed (FTIR) spectroscopy. The principal component analysis (PCA) of pre-processed FTIR spectra of purified EVs was conducted, enabling the evaluation of the replica variance of the EVs chemical fingerprint in a reduced dimensionality space.

Results and discussion: It was observed that the chemical fingerprint of EVs is more dependent of the medium used for MSCs cultivation than the donor. EVs secreted by MSCs cultured with SCM presented a more homogenous chemical fingerprint than EVs obtained with XFM. The regression vector of the PCA enabled to identified relevant spectral bands that enabled the separation of samples in the score-plot of the previous analysis. Ratios between these spectral bands were determined, since these attenuate artifacts due to cell quantity and baseline distortions underneath each band. Statistically inference analysis of the ratios of spectral bands were conducted and it was possible to define ratios that can be used as biomarkers, enabling the discrimination of EVs chemical fingerprint in function of the medium used for MSC grown and the MSC donor. This work is therefore a step forward into understanding how different culture conditions and donors affect MSC-derived EVs characteristics.

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OPTIMIZATION AND CULTIVATION OF OVARY STROMA AND MATURATED OOCYTES

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Background and novelty: Ovarian follicle culture system is suitable tools for advancing the folliculogenesis study and fertility preservation approaches progression. In vitro culture systems provide a controlled environment to study the follicle mechanism development. The ovarian stroma tissue consisted of interstitial – theca cells, neurons, blood vessels, macrophages and oocytes in different stages of oocyte. Stroma tissue is necessary for early stages follicles and it has a clear influence on follicle development. Also, it hypothesized that stroma cells could be differentiated to theca cells. In this study, culture and subculture of Sterlet stroma cells were done by explant method. Also, in this study, mature oocytes were maintained alive for more than 10 days in cultured flask. So, there is not any report about sturgeons stroma and mature oocytes as model.

Experimental approach: After anaesthetized the juvenile Sterlet (*Acipenser. Ruthenus*), ovary tissue was removed. After trypsinized and cut the stroma, it was cultured in flasks which contained L_{15}, FBS, Antibiotic and antifungal.

Results: After 48 hours, cells were started to proliferate. Also, after 4 days, mature oocytes were started to proliferate and growing. Proliferation cells were increased in next days.

Discussion: In the present study, the best method to isolate and cultivated stroma and mature oocytes derived Sterlet was carried out. Granulosa cell proliferation and apoptotic gene expression were associated with Ovarian stromal/ theca cells. So, stroma tissue culture optimizing is more important for reproduction system, especially, this study, we used explanations for culture which this technique is more similar in-vivo condition. Keeping alive of mature oocytes in medium is more suitable for next experiments and study about some drugs, chemical and toxic substances.

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DEVELOPMENT OF AN ANTI-HER2 PHAGE TO MONITOR UNTARGETED CELL PANNING

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Background and novelty: Phage display is a robust technology widely used in antibody discovery. This technology is based on highly diverse libraries of phages displaying antibody fragments on its surface, allowing the isolation of specific binders to virtually any target by an affinity selection process called panning. Many protocols have been proposed for targeted strategies. However, for untargeted approaches aiming at the identification of potential therapeutic antibodies from cells or tissues, only few successful cases have been reported, highlighting the need to develop new panning methods for this purpose. Therefore, our goal is to establish a protocol for cell-based untargeted phage display selections that allows the isolation of clinical-relevant antibody candidates.

Experimental approach: Development of a phage particle displaying the single chain variable fragment (scFv) of Herceptin, which targets the human epidermal growth factor receptor 2 (HER2). This phage will be used to spike-in the Tomlinson I+J phage library, as an internal control to assess selection efficiency when panning on $HER2^+$ cancer cells.

Results and discussion: The Tomlinson I+J phagemid, pIT2, was fully-sequenced and used as the vector for cloning the scFv sequence of Herceptin. Flow cytometry analysis on $HER2^+$ and $HER2^-$ breast cancer cell lines confirmed the specific binding of the anti-HER2 phage to $HER2^+$ cells. As a preliminary study, the phage library was spiked-in with the anti-HER2 phage particles and the selection was performed for four rounds on a $HER2^+$ cell line (BT474). The results showed an enrichment of the phage population towards $HER2^+$ cells after 3 to 4 rounds of selection and an increase in HER2 specific phages after 4 rounds of selection. The anti-HER2 phage allows monitoring phage display selections and it is an essential tool for the optimization of the cell-based phage display selection protocol.

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INDUSTRIALIZING IMMUNO-ONCOLOGY THERAPEUTIC DISCOVERY PLATFORMS

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Background and novelty: Novel classes of bio-molecules are currently evaluated for their use in cancer immunotherapy. Bi- and multi-specific antibodies, Ab-cytokine fusion proteins, non-Ig scaffolds, chimeric antigen receptors (CARs), engineered TCRs and TCR-based bispecific constructs promise significant advantages. These highly engineered molecules pose new challenges regarding their design, screening and production, either as dedicated molecules or in the context of cellular therapeutics (e.g., CAR T cells). A solution that provides an infrastructure that addresses these challenges and enables industrialization of these various novel therapeutic modalities is vital.

Experimental approach: We have designed a combined R&D workflow platform (Genedata Biologics® and Genedata Bioprocess®) representing a highly structured database that transforms the complexity of molecule design, screening and production processes into organized workflow steps. Novel molecule assembly mechanisms have been created to design large panels of novel-class biomolecules. Tools for pooled cloning deconvolution and chain pairing recovery were implemented. The platform was designed to support screening of the best molecule candidates and of the best producer cell lines with built-in tools for developability assessment.

Results and discussion: We present concrete use cases of multi-specific therapeutic modality screening and production process development. A special focus is on *in silico* molecule assembly, on mono- to multispecific activity comparisons and developability assessment and on the development and evaluation of CAR T cells. The platform shows unmatched flexibility in handling and exploring novel therapeutic modalities.

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HPL IMPROVES BONE FORMING POTENTIAL OF ADULT PROGENITORS IN BIOREACTOR

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Background and novelty: Xenogeneic free media are required for translating advanced therapeutic medicinal products to the clinics. In addition process efficiency is crucial for ensuring cost efficiency for large scale production of mesenchymal stem cells. Human platelet lysate (HPL) has been increasingly adopted as an alternative for Fetal Bovine Serum (FBS) for MSCs of different origin however its impact on their therapeutic potential and regenerative capacity in an in vivo setting is still largely unexplored.

Experimental approach: The effects of FBS and HPL supplementation was studied in microcarrier-based dynamic expansion of human periost derived cells. Subsequently their bone forming capacity was assessed by subcutaneous implantation in small animal models. We observed that the use of HPL resulted in 4 times faster cell proliferation, while cells maintained their differentiation potential with a suppression of adipogenic capacity. Differences in mRNA expression were also observed, suggesting lower expression of extracellular matrix related genes and lesser degree of differentiation when using HPL. When implanting these cells we observed a marked difference between the bone forming capacity of cells expanded in FBS and HPL. FBS expanded cells resulted in a fibrous tissue structure with very low amount of mineralisation. On the other hand, HPL supplementation resulted in extensive mineralised tissue formation which can be classified as newly formed bone verified by µCT and histological analysis.

Results and discussion: This work provides important data and support towards the use of platelet lysate as a viable alternative to FBS in a scalable suspension cell culture set-up. In this study, strikingly hPDCs were seen to be able to form significantly higher amount of bone when implanted together with CaP carriers in small animal models. This capacity was linked to activation of WNT and BMP pathways associated with the osteogenic capacity of several progenitor cells.

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OPTIMIZATION OF RABIES VIRUS PRODUCTION IN VEROS CELL

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Background and novelty: Vero cells are nowadays widely used in the production of human vaccines. They are considered as one of the most productive and flexible continuous cell lines available for vaccine manufacturing. We previously adapted Vero cells to suspension growth (Rourou et al., submitted to vaccine) to simplify upstream processing and process scale-up. The use of suspension adapted Vero cells, VeroS, may reduce costs of vaccine manufacturing to develop vaccines affordable worldwide. The current work describes the optimization of rabies virus (strain LP-2061) production in VeroS grown in animal free conditions.

Experimental approach: The experiments were carried out in shake flasks using IPT-AFM in-house medium next to two commercial chemically defined media: HycleII CHO and CD-U5. The cells were infected in various Multiplicity of Infection (MOI: 0.3, 0.1 and 0.05), different infection cell densities and times of infection.

Results and discussion: Cell density levels higher than $2x10^6$ cells/mL were obtained in the assessed conditions. The results were comparable to those obtained in spinner culture of adherent Vero cells. Cell infection with LP-2061 rabies virus strain at an MOI of 0.1 and a cell density of 10^6 cells/mL resulted in a virus titer higher than 10^7 FFU/mL in all the media tested. Further optimization assays are ongoing in order to improve virus yields. Our results demonstrate the suitability of the obtained VeroS cells to produce rabies virus at a high titer, and pave the way to develop VeroS cells bioreactor process for rabies vaccine production.

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VIRUS RISK MITIGATION FOR STEM CELL-BASED CELL THERAPY PRODUCTS

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Background: It is well known from the biopharma industry, that large scale cell cultures are at risk regarding virus contamination[i,ii]. In biopharma processes it is common practice to mitigate the risk of transmitting undetected virus contaminations from cell culture to final product by designing virus reduction into the downstream process. Such process steps can for obvious reasons not be applied to cell therapy products. For that reason, implementation of virus barrier steps on cell culture medium and solutions has been evaluated. 20 nm filtration was considered the best option and three different filters were assessed in virus reduction and growth promotion studies.

Experimental approach: Expansion of human embryonic stem cells (hESCs) cells was studied. The process comprises preparation of ready for use medium by spiking two stock solutions into the basal medium on the day of use, coating of cell culture plastic surface, seeding of single cells on the coated surface and incubation for three to four days. At confluence the cells are washed and detached with detachment agent.

Virus reduction study: The filters were challenged with basal medium spiked with a stock of Minute Virus of Mice (MVM). The filtrates were tested for MVM titer.

Virus reduction methods applied for preparation of basal medium and cell culture reagents: All reagents were 20 nm filtered or autoclaved

Chemical analysis to assess the impact of filtration: The filtered media and a non-filtered control sample were compared in LC-MS analyses.

Functional testing: The growth of the hESCs in the virus reduced culture systems were compared to growth in control medium in a serial passaging study over ten passages. Read-outs were cell counts, pluripotency and karyotype stability.

Results and discussion:
Virus reduction: All filters showed more than 4 log reduction of MVM.

LC-MS: No detectable differences between the filtered media and the control sample.
Growth promotion: No significant difference between filtered media and control.
Cell quality: No impact on pluripotency and karyotype.

The results of the study show that any of the filters could be implemented in the cell expansion process without impacting the cell growth or quality. Implementation of such virus reduction steps will improve the virus safety of stem cellbased cell therapy products.

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CHO PRODUCED RFIX PTMS DIFFERENCES ON FED-BATCH & PERFUSION PROCESSES

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Background and novelty: Human coagulation factor IX (FIX) is a protein that relies on an extensive spectrum of posttranslational modifications that enable it to function correctly and efficiently in the coagulation pathway[1, 2]. These consist of 7 disulfide bridges, 2 N-glycans, 6 O-linked glycans, 1 sulfation site, 1 phosphorylation site, 12 γ-carboxylation (GLA) sites as well as 1 β-hydroxylation sites[3-9]. This study aims to investigate the differences in the PTMs of recombinant FIX (rFIX) produced in CHO fed-batch and perfusion cultures, compared with plasma-derived FIX (PDFIX).

Experimental approach: The cell line used was CHO-K1SV expressing rFIX.Two fed-batch bioreactors were conducted using commercial CD-CHO media and EfficientFeed A and B respectively. Perfusion cultures were conducted in the same base medium using an Applisens Biosep acoustic perfusion unit at a dilution rate of one reactor volume a day. The bioreactors were sampled daily for off-line measurements to track cell growth, metabolism and productivity. These samples were also used for SWATH-MS analysis[10]. In parallel, purified rFIX from these cultures were analyzed after an in-gel digestion using LC/ESI-MS/MS to characterize the PTMs.

Results and discussion: The fed-batch cultures responded differently to each of the feeds despite achieving similar peak cell densities of 15x10^{6}cells/mL. Almost all the PTMs of PDFIX were observed in rFIX of both fed-batch cultures, although they showed partial occupancy and higher heterogeneity. Preliminary qualitative analysis suggested that -carboxylation in the rFIX GLA domain is more complete in one fed-batch compared to the other. As a comparison, pseudo steady-states were established in the perfusion cultures at 15x10^{6}cells/mL via bleeding of the cultures under the control of an online turbidity probe. Samples were collected from these steady-states and purified for PTM analysis to establish comparison across the different modes of cultures and PD-FIX.

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VIRUS RISK MITIGATION FOR STEM CELL-BASED CELL THERAPY PRODUCTS

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NEW WAYS TO DEPICT MULTIVARIATE DATA FROM GLYCO PROFILES ACROSS SCALES

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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.

Experimental approach: One approach to control glycosylation attributes is to supplement cell culture media with additives known to influence glycosylation patterns. Typically, media additives are tested in combination, using a design-of-experiments approach. Depending on the number of factors, y-variables, and study design the resulting dataset can be highly complex. Here, tested ten media additives in univariate and in combination.

Results and discussion: We present a novel way of depicting multivariate data that shows effect type, estimate, robustness, and model terms in a single graph, and allows for easy interpretation of highly complex data. Guided by this dataset, we designed targeted experiments to manipulate certain aspects of the glycosylation profile. We show proof of concept across different lab scale models, including deep wells, shake flasks, and 2L single-use bioreactors. Further, we characterized the extent of product quality modulation across CHO cell lines with different genetic backgrounds producing the same IgG1 molecule, and we characterized differences between a canonical IgG1 vs. an isotype-matched mAb with engineered cysteine residues. Taken together, this study provides novel insights into glycosylation product quality control through media engineering, and introduces a new tool for the display of multivariate data, applicable to any complex dataset.

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INVESTIGATING CHO SCALABILITY

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Background and novelty: Traditional scaling of bioreactors involves selecting the basis for scaling and then verifying the comparability at different scales; however, whilst this approach has proven effective from 250mL to 1kL, it may be less effective when scaling from 15 mL to 50 L. The ambr 15 is a workhorse of the BioPharm industry but the performance of CHO cell lines within ambr 15 in terms of cell growth and productivity is variable with respect to larger scale bioreactors. To investigate this discrepancy, GSK is collaborating with Sartorius during the development of Scale Conversion Tool software. This software combines the single time point scaling approaches traditionally applied to these challenges with cell culture trajectory analysis to integrate engineering characterisation and heuristics when formulating operating conditions to scale-up/-down.

Experimental approach: Analysis of historical cell culture data and engineering characterisation was used to enable the design of complex wet work in ambr 250 to investigate the relative sensitivity of a cell line to combinations of controllable parameters including seeding concentration, volumetric impeller power input and gas flow rate. The Scale Conversion Tool was used to predict the oxygen transfer rate under various configurations, allowing independent levels of volumetric impeller power input and sparge gassing to be sustained over the course of fed batch cell culture, while maintaining characteristic gassing profiles.

Results and discussion: It was found that certain extreme combinations of factors had a significant effect on cell growth and productivitiy. These combinations were then analysed within the Scale Conversion Tool to account for the deviation in terms of fundamental aspects of the culture time course. The results from these analyses were then re-applied experimentally to the ambr 15 to determine if they resulted in improved process outcomes.

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CHEMOMETRICS FOR ETANERCEPT BIOPROCESS MONITORING IN THE PAT CONTEXT

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Background and novelty: In the last years, regulatory agencies in biopharmaceutical industry have promoted the design and implementation of Process Analytical Technology (PAT) in order to improve the quality monitoring of bioprocesses. In this context, spectroscopic techniques for data generation in combination with chemometrics represent alternative analytical methods for on-line critical process variables prediction. In this work, a novel multivariate calibration strategy for Etanercept (Et) at-line concentration prediction in CHO cells perfusion processes is presented.

Experimental approach: Sterile samples from standard Et processes were daily obtained for the determination of viable cells and Et concentration in supernatant by using standard off-line univariate methods. Additionally, fluorescence excitation-emission matrices (EEM) were obtained (second-order data), whose were then modelled by different chemometric algorithms. Firstly, multilinear decomposition methods such as Parallel Factor Analysis (PARAFAC) and Multivariate Curve Resolution (MCR) were utilized to explore the qualitative information of the system. Then, two multivariate regression strategies for Et prediction based on Unfolded Partial Least Squares (U-PLS) and Back Propagation Neural Network (BP-ANN) models were implemented and compared.

Results and discussion: The PARAFAC profiles were related to the biological fluorophores present in the culture media during fermentation of CHO cells (aromatic aminoacids, pyridoxine, flavin, folic acid and NAD). In quantitative terms, the prediction performance of BP-ANN-based model was superior to U-PLS, since the first is able to cope with non-linearity in calibration models, reaching a mean relative error of about 10%. This strategy represent a fast and inexpensive approach for Et monitoring, which conforms the principles of PAT.

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BIOSENSORS FOR QUANTIFICATION OF INFECTIOUS LABEL-FREE VIRUSES

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Background and novelty: Basic virology research and biotechnological applications making use of wild type or recombinant viruses require reliable and fast detection and quantification analytics. However, current methods are time-consuming, lack high-throughput or make use of reporter genes (labelled-virus), inadmissible in clinical context.

Previously, we developed a cell-based sensor requiring virus labelling – the Single-Step Cloning-Screening method[1]. We now report the development of genetically encoded switch-on fluorescent biosensors for quantification of label-free infectious viruses – VISENSORS.

Experimental approach: Protein structural distortion maintains VISENSORS in a non-fluorescent state. Upon cell infection, viral protease activity – thus a label-free system – relieves the distortion and fluorescence is triggered. Different designs – using GFP or split-GFP fragments – were optimized in terms of amino acid composition and fully characterized by fluorescence microscopy and flow cytometry.

Results and discussion: VISENSORS were initially designed for detection of Adenovirus (AdV), one of the main vectors for oncolytic and gene therapy applications. After design optimization and confirmation of specific activation by viral proteolysis, 293 cells stably expressing VISENSORS were established. Sensor cells supported efficient virus amplification and showed correlation between increase in viral load and fluorescence activation, demonstrating its potential in real-time monitoring of AdV infection. A cost-effective flow cytometry-based protocol using these sensor cells was established, allowing quantification of label-free infectious AdV 48 hours post-infection – faster than standard assays taking up to 14 days. Detection of other viruses and additional sensor designs are under evaluation.

VISENSORS can be used to establish new cellular platforms enabling to reduce timelines and costs of production, titration, and characterization of clinically relevant label-free viruses.

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SPR QUANTITATIVE ASSAY FOR INFLUENZA VACCINE PRODUCTION MONITORING

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Background and novelty: Today, influenza vaccine formulation and lot release rely on single-radial immunodiffusion (SRID) assay. SRID requires annually updated reference sera reagents for seasonal vaccine lot release and is not implementable for process development as it cannot handle non-purified influenza virus material[1]. Therefore, to speed up the vaccine development in case of new pandemic outbreaks, WHO and regulatory agencies strongly support the development of more efficient influenza vaccine potency assays[2]. In the present work, we propose a rapid and label-free quantitative assay for both influenza hemagglutinin (HA) antigen and whole influenza viruses based on surface plasmon resonance (SPR).

Experimental approach: The method is based on affinity capture of hemagglutinin antigen by glycans at the surface of the fetuin-functionalized sensor. In-process reference materials generated at different process stages were used for assay evaluation. Commercial trivalent inactivated vaccine ("TIV") has been used for the determination of optimal analytical conditions. Additionally, influenza H1N1 virus samples were processed onto the sensor to evaluate viral production kinetics and establish correlations with infectious and total viral particles quantification techniques.

Results and discussion: The developed assay offers a quantification of HA within minutes with a wide dynamic range (30 ng-20 µg HA/mL) while offering 100 times higher sensitivity and better reproducibility than SRID and its potential alternatives recently proposed (3% RSD vs 6-15%)[2,3]. In-process monitoring of cell-based influenza vaccine production has also been validated by the determination of influenza viral production kinetics in good correlation with total particles quantification (Limit of detection $<10^6$ Infectious Viral Particles/mL).

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ANALYTICAL METHODS TO ASSESS PRODUCT QUALITY OF LENTIVIRAL VECTORS

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Background and novelty: Lentiviral vectors (LV) are a popular gene delivery tool for a broad range of research applications. Extensive characterization is required in GMP manufacturing and virus-based gene vectors tend to have greater variability between batches, especially for potency (ratio of total to functional vector particles). It has been an on-going challenge to make accurate assessment of LV's product quality and standardized methods do not exist. The aims of this project are to optimize the gene transfer assay (GTA) by adapting the method to suspension cells and to develop a droplet digital polymerase chain reaction (ddPCR) method, ultimately to generate detailed protocols for general use in the gene therapy field.

Experimental approach: Vector functionality in terms of transduction and expression can be determined by performing GTA, a cell-based assay that measures the integration of the transgene, followed by flow cytometry. Adherence cells are traditionally used for this method; however, adapting the method to suspension cells will improve assay throughput. Total vector particles can be determined by ddPCR, a more robust and higher throughput method as compared to the gold standard quantitative PCR, as it provides absolute quantification with more data points and omits the use of a standard curve.

Results and discussion: GTA was successfully adapted to and optimized for human embryonic kidney suspension (HEK293SF) cells. Results show assay reproducibility for the same LV sample across different plates with ≤5% CV and an average of 9.81E6 transducing units per mL. However, there is a 2-fold difference between different LV dilutions, which may be addressed with further assay optimization. ddPCR method was successfully established with assay reproducibility of ≤10% CV and an average of 8.42E7 vector genome per mL. In addition, in-house primers were compared with primers from a collaborator and showed comparable results, which further indicate assay robustness.

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ONLINE AND OFFLINE MONITORING OF CELL CULTURE TO EXAMINE CELL DEATH

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Background and novelty: Monitoring and maintenance of Chinese hamster ovary (CHO) cell health in a bioprocess is crucial with regards to both the productivity of the cells and to the overall quality of the final product. Traditional approaches involve sampling and offline testing of cell health (e.g. viable cell count using dye exclusion) at fixed points throughout the process. Novel methods of online monitoring by digital holography and impedance flow cytometry were compared to conventional flow cytometry and dye exclusion. The online methods have the advantage of providing a continuous picture of cell health during a bioreactor run, allowing for informed intervention at potential critical points in the process should it be required. These online methods not only provide an in depth evaluation of the state of the cell population, but also can analyse the population on a single cell basis.

Experimental approach: Online analysis methods including digital holography (Ovizio Imaging, Belgium) and capacitance (Aber Instruments, Wales) were used to analyse batch CHO cell cultures in a 5L benchtop bioreactor. Each culture was sampled daily until the measured cell viability decreased below 30%. Offline cell samples were analysed by trypan blue assay as well as annexin V and mixed caspase assays with an Accuri c6 flow cytometer. Impedance flow cytometry (Amphasys, Switzerland) was also used to examine offline samples.

Results and discussion: The commonly used dye exclusion method (trypan blue) does not provide a full picture of the health of a population of cells. Loss of membrane integrity is a late-stage, irreversible apoptotic event whereas ionic dysregulation occurs earlier. Online cell capacitance data from the Aber probe was shown to be an earlier indicator cell viability loss compared to the other methods studied. Furthermore, the on-line methods provided real-time information on the metabolic and morphological changes of individual cells during apoptosis.

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PREDICTING INDUSTRIAL CELL CULTURE SEED TRAINS – A BAYESIAN APPROACH

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Background and novelty: For the production of biopharmaceuticals in suspension cell culture, seed trains are required to increase the cell number from cell thawing up to production scale. Since they are time- and cost-intensive and have a significant impact on cell performance in production scale, seed train design, monitoring and optimization are important techniques.

Experimental approach: Model-assisted prediction methods can lead to advanced seed train design, monitoring and development of optimization strategies. The performance depends on the prediction accuracy which can be improved by inclusion and quantification of prior process knowledge, especially when only few high-quality data is available, and description of inference uncertainty, providing, in addition to a 'best fit'-prediction, information about the probable deviation in form of a prediction interval.

Results and discussion: This contribution illustrates the application of a Bayesian approach for seed train prediction to an industrial cell culture process, enabling inclusion of prior knowledge as well as expression of predictive uncertainty. It was implemented using the Markov Chain Monte Carlo (MCMC)-method. In order to integrate information from new data for updating the prediction of the current seed train cultivation, the sequential Bayes method was applied and the impact on prediction accuracy was investigated.

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DYNAMIC BIOMASS-BASED FEEDING IN AN INDUSTRIAL CHO FEDBATCH BIOPROCESS

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Background and novelty: For production of protein-based therapeutics the predominant culture mode is still the fedbatch. On the one hand, fedbatch processes circumvent limitations of classical batch cultivations and concomitantly lead to increased cell and product concentration. On the other, handling remains easy, when being compared to more complex continuous or intensified bioprocesses.

For the sake of simplicity and reliability, it is worthwhile to put additional energy into further development of the old fashioned fedbatch process. Batch-wise feed additions may stress cells by milieu changes and overfeeding, especially during early growth phases, whereas in later stages the feed amount might not be sufficient to support all cells' productivity and longevity. An optimal nutrient supply of each cell can solely be guaranteed by implementation of a feeding strategy based on the integral of viable cell concentration [IVCC] or growth rate dependent calculated feeding.

Experimental approach: Assuming each cell has the same nutritional needs for survival and the same capacity to produce a certain product over a defined time-period, we first operated dynamic feed flows with manual day-to-day tuning. The adjustment was done, either (i) calculating the IVCC in combination with a certain feedfactor, or (ii) by determination of the expected viable cell concentration using a retrospective calculation based on growth rates. In parallel we implemented a biomass probe to the bioprocess.

Results and discussion: IVCC feeding may lead to a reduction of total feed medium needed. During transition from late log and stationary phase, retrospective calculation based feeding leads to overfeeding. Permittivity of our cells correlates well with offline cell counting. Therefore, as a further step we intend to combine the feeding strategies with the online signal of the biomass probe, yielding a real-time adjusted dynamic feeding of our cultures by an automated biomass triggered feedback loop.

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CHEMOMETRICS TO MONITOR CHO CELLS CULTURES QUALITY BY IN SITU NIRS

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Background and novelty: There is a recognized need to develop online monitoring tools to evaluate the evolution of cell cultures since monoclonal antibodies-mAb are quite sensitive to the manufacturing process. Attempts have been made to build prediction models based on spectroscopic data. However most studies have only focus on some culture media compounds while physiological state of cells and mAb characteristics such as glycosylation have received little attention. Consequently, mAb glycosylation and physiological state of cells remain usually being monitored using off-line techniques, which limit the establishment of retro-control processes based on Process Analytical Technology-PAT to target specific mAb properties.

Experimental approach: This study aimed to evaluate the relationships between NIR spectra captured *in situ* within numerous CHO cell cultures (2 L bioreactors) and culture compounds through regression techniques so that more accurate and precise prediction models could be developed.

Results and discussion: We first noticed the presence of effects that limited the widely used linear regression methods (PLSR, PCR), particularly for mAb properties. Then we addressed them by the novel use of other regression techniques as local regressions-LR and supported-vector regressions-SVR. Results showed that LR enhanced prediction ability of models so that the physiological state of cells may be better characterised in terms of metabolic rates using real-time data. Moreover, the complicated relationships between spectra and mAb properties were successfully handled using SVR such that mAb quality, in terms of glycosylation profiles, was real-time monitored accurately during a whole batch culture. These are encouraging results to go further in PAT retro-control systems so that the impact of cell culture parameters on mAb glycosylation during CHO cell culture processes may be better understood and controlled.

P-411

TEMPERATURE DEPENDENT CELL NUMBER CONTROL IN BIOREACTORS

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Background and novelty: A typical challenge of upsteam processes are variations in the growth behaviour of cultures. This can result in batch to batch variations of the cell density at the time of subculture. These variations can cause problems with further scheduling of the upsteam cascade.

As a solution for operational practice, a cell number control system was developed on the basis of the temperature dependence of the growth rate. The system can readily be implemented in existing production units. To our knowledge, this is the first report of such a controller for mammalian cell cultures.

Experimental approach: In a first step, the temperature dependence of the growth rate was experimentally determined for a typical CHO cell (CHO-DP12_1934). In a second step, a mathematical model was derived on the basis of this data and implemented in a custom made control algorithm. In a third step and with the help of an in line cell density sensor (Incyte, Hamilton), predicted end-of-cultivation cell densities are calculated during the process and, in case of deviations from the target cell density, adjusted temperature setpoints fed into the bioreactor controller to modulate the growth rate.

Results and discussion: It now is possible to define the exact cultivation time and biologically achievable target cell concentration upfront. These parameters can also be changed during the cultivation process. The optimised algorithm successfully uses current batch temperature and cell density to calculate the necessary process temperature, needed to adjust the growth rate, in order to achieve the target cell concentration in the defined process time.

A possible influence of temperature changes on the biology of the cells, essential for the quantity and the quality of the product, is momentarily under investigation and will also be discussed.

Acknowledgements & Funding: We thank Hamilton (Bonaduz, Switzerland) for providing the Incyte cell density sensor.

P-412

HOW CELL CULTURE AUTOMATION TAKES USP DEVELOPMENT TO THE NEXT LEVEL

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Background and novelty: In this talk it will be presented how our in-house developed, fully automated multiwell plate (MWP) based screening system for suspension cell culture takes upstream process development to the next level. The development, the applications and the benefits of this cell culture system will be demonstrated.

Experimental approach: The system itself is characterized by a fully automated process workflow with integrated analytical instrumentation. It uses shaken 24 well plates as bioreactors which can be run in batch and fed-batch mode with a capacity of up to 576 reactors in parallel. Because the results of the multiwell plates are predictive for the bioreactor scale, the system was established as a routine instrument in upstream process development. The cell culture automation system is completed by an in-house developed and unique Process Control System (PCS). It enables the planning and evaluation of the screening experiments as well as the fully automated data handling.

Results and discussion: In this talk an overview about several applications in late stage process development of using cell culture automation in contrast to standard bioreactor systems will be presented. In addition, the future trends to increase efficiency will be shown.

All examples will show the potential of cell culture automation as a routine tool in process development.

P-413

EVALUATION OF KLA AS SCALE-UP PARAMETER FOR CHO CELL CULTURES

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Background and novelty: Monoclonal antibodies (mAbs) are the basis of many drugs for the treatment of high-impact diseases such as asthma, rheumatoid arthritis, and various types of cancer. Therefore, mAb global sales are projected to $ 125 billion by 2020. CHO cells are the most employed cell line for producing mAbs, because of their efficient posttranslational processing of complex proteins. At up-scaling CHO cultures, Oxygen delivery is considered a large limitation. As culture volume increases, more forceful operation conditions are required for achieving an adequate Oxygen Transfer Rate (OTR). OTR is defined as the contribution of two terms, the volumetric mass transport coefficient (k_La) and the difference of concentrations between liquid surface and the average in liquid bulk. Since CHO cultures are usually performed in stirred tanks, increasing either stirring speed or bubbling flow enhances k_La, and thus, OTR. However, these alternatives would boost cell death rate, reducing culture productivity.

Experimental approach: In this work, a parental CHO cell line was cultured at different values of k_La to evaluate the pertinence of k_La as scale-up parameter. Cultures were performed in 100ml Spinner Flasks adapted for bubbling and online dissolved oxygen measurement. Response variables were cell, glucose, lactate, and ammonia concentration, osmolality and pH as well. Additionally, free lactate dehydrogenase (LDH) concentration was quantified as necrosis death indicator.

Results and discussion: Results show the effect of k_La on CHO metabolic behavior. Oxygen limitation was presented in cultures with the lowest k_La value at higher cell concentration than 1×10^6 cell ml^{-1}, whereas at higher k_La, Carbon source limitation was first evidenced. At highest k_La values, LDH was rapidly liberated, and higher cell concentrations were achieved, though. This strategy may be used for further screening and scale-down studies.

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P-414

EFFECTIVE BIOREACTOR PH CONTROL USING ONLY SPARGING GASES

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Background and novelty: pH control is critical in bioreactor operations, typically realized through a two-sided control loop, where CO_2 sparging and base addition are used in bicarbonate-buffered media. Though a common approach, base addition could compromise culture performance due to the potential impact from pH excursions and osmolality increase in large-scale bioreactors.

Experimental approach: To reduce or eliminate the use of base addition in pH control, we propose to modulate sparge gas composition to achieve precise pH control. With the incorporation of sparge air in the pH control loop, when pH at the lower limit of the deadband, air sparging can be automatically increased to improve CO_2 removal, hence increase culture pH, and vice versa.

We compared the air sparge-based pH control approach with the traditional base addition method in Chinese hamster ovary (CHO) fed-batch cultures. Tight pH control was evaluated in small-scale ambr®250 bioreactors for multiple processes at a range of pH set-points (6.95, 6.90, 6.85, 6.80) with a pH deadband of 0.05.

Results and discussion: The results demonstrated that the air sparge control strategy could successfully maintain desired culture pH setpoints, and yield comparable cell culture performance compared with the traditional pH control method using a base pump. This novel air sparge-based pH control was also applied in a 200 L single-use bioreactor (SUB) and exhibited the same effectiveness as in ambr®250, demonstrating scalability of the proposed methodology.

Acknowledgements & Funding: We acknowledge helpful comments from Henry Lin.

P-415

AN INTEGRATED PAT SOLUTION FOR MONITORING AND CONTROL

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Background and novelty: Generation of process understanding is the key task of process analytical technology (PAT) as "a system for designing, analyzing and controlling" bioprocesses and enables quality by design (QbD) [1]. Hence, PAT tools must facilitate timely measurements as well as data management, which can be realized by automation and digitalization of the process and process environment [2]. An integrated PAT solution is demonstrated that enables deeper process understanding and the possibility of science-based process design.

Experimental approach: CHO cultivations were performed with an advanced PAT setup, comparing PAT tools using direct or indirect measurements. The signal of an in-line probe (dielectric spectroscopy) was applied for feed control, i.e. the control of specific substrate uptake rates. An automated sampling system (Numera) enabled direct measurements for on-line and real-time monitoring using HPLC for amino acids, vitamins and product quantification and a biochemical analyser for substrates, metabolites and product quantification.

Results and discussion: It could be shown that indirect measurements are valuable PAT tools but still require manual intervention, e.g. for recalibration, especially in changing conditions at process development. Direct measurements on the other hand directly deliver the desired information. Automated sampling combined with on-line analytics lead to generation of process understanding, process transparency, an increase of the acquired information content as well as an increase in control quality. In addition, the need of an integrated solution is demonstrated to unite measurement and data management. This is the basis for improved process robustness and reduced time to market.

Summarizing, it could be shown, that automated liquid handling in combination with reliable data management is a hard-to-replace integrated PAT tool for current and future challenges in bioprocessing.

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P-416

CHEMSTRESS®: A NOVEL METHOD OF QUALITY CONTROL FOR CELL CULTURE MEDIA

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Background and novelty: Cell culture media is a complex chemical mixture that is an essential component of bioprocessing. Given the complexity of the formulation and potential instability of the chemicals, lot-to-lot variation can occur with a serious impact on the growth and productivity of producer cells. Despite this lot-to-lot variation, there is a considerable lack of efficient, cost-effective, quality control assays which offer biologically relevant information on the performance of cells in a given manufactured lot. ChemStress® aims to offer users the ability to QC media prior to release or use, by generating informative biological data on cells response to media.

Experimental approach: The ChemStress®assay is a 96-well microtiter plate, coated with specific chemical stressors. These stressors are designed to simulate toxic environments or stimulate specific stress response pathways which cells may experience during the manufacturing process. The biological response to these stressors is taken as a measure of growth and titer for CHO cells producing monoclonal antibodies. A specific CHO cell line is grown on ChemStress®plates in the presence of a specific media lot. Poor growth and titer performances highlight lots which do not pass QC.

Results and discussion: N=5 lots of the same media were evaluated using ChemStress®plates. A standard mAb-producing CHO cell line was used. Variations in the growth and titer response of cells when grown in different media lots was observed. These data indicate a variability in the performance of cells when grown in different media lots.The ChemStress®assay can therefore act as an informative QC assay which determines lots with poor biological performance which should fail release by media manufacturers or be discarded by users before use in bioreactors.

P-417

A NOVEL DATA ANALYSIS TOOL FOR CELL CULTURE MEDIUM DEVELOPMENT

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Background and novelty: As the most important raw material, culture medium plays an indispensable role in the cell culture processes. As the composition of cell culture medium is complicated, most of current analysis tools for medium optimization use PLS (Partial Least Square) method which is based on linear model. The accuracy of PLS model is not desirable to achieve optimization objective when the relationship is non-linear between components. Developing a novel statistical model which can handle high-dimensional data with remarkable accuracy on small data-set would bring great value. Therefore, this study is to establish a new tool combining statistical method with machine learning model for the first time with superior performance for medium optimization.

Experimental approach: Based on statistical learning methods, this tool integrated LOESS (Local Polynomial Regression Fitting) and RRF (Repeated Random Forest) analysis to perform component importance evaluation. To improve the accuracy and effectiveness of the key components analysis, both nonlinear and linear relationships between response value and component were applied in the model.

Results and discussion: In the case of a medium development to support the growth of CIK (Cytokine-Induced Killer) cells, five key variables affecting cell growth were accurately identified among 55 components with a small number of experimental groups using this method described (LOESS + RRF). Then we tweaked the model by adjusting only important components and choose data points in high cell density area. Hereby, a complete serum-free medium suitable for CIK cells was established. The results showed that the serum-free medium successfully supported the CIK cells to 300-1500 times expansion which was comparable to the parallel culture in the popular medium supplemented with 5% serum. This study provides an efficient analysis tool for high-dimensional data analysis and optimization on the overall compositions of cell culture medium.

P-419

NOVEL AT-LINE SENSOR DESIGNS FOR LIVE CELL DENSITY

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Background and novelty: Many of the new processes developed, particualry in regenerative medicine, now have a "cell expansion" module and perfusion culture is often needed to reach the desired target cell number.There are benefits in increasing the level of online monitoring and process control in any perfusion process and the real time measurement of the viable cell density (VCD) is seen as a key measurement if the bioreactor is run automatically without the risk of contamination from sampling.The measurement of bio-capacitance using Radio Frequency Impedance (RFI) spectroscopy is a very promising tool for this application. In some cases the bioreactor design might be unsuitable for incorporation of an online sensor and there is usually an option to have small recirculation loop where several "at-line" sensors including VCD can be housed.

Experimental approach: In order to assess any new design of RFI probe it is important to compare the performance of the at line probe in terms of accuracy and precision with conventional online probes.The at-line sensors ideally should remain stable after gamma irradiation, show minimal variation within a large batch of single use sensors and should not impede the flow or allow cells to build up within the chamber.

Results and discussion: Initial results have shown that there are two new RFI probe designs that can allow an unimpeded flow without allowing cells within the chamber connected to tubing with internal diameters of less than 6mm.As each design of sensor was calibrated to take into account the restriction of the electrical field within the small chamber, the VCD measurements estimated by the at-line sensor in the recirculation loop and the online sensor in the conventional stirred tank bioreactor were less than 2% different. The results confirm that VCD can be accurately measured within a recirculation loop using radio frequency impedance and the at at–line sensor will be suitable for use in disposable bioreactors.

P-420

DEVELOPMENT OF A SMALL ONLINE BIOMASS MONITORING TOOL FOR MICROPLATES

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Background and novelty: Strain selection and media composition screening is one of the essential prework for development of recombinant protein production processes. Increasing the throughput of performed experiment, makes sense in terms of time saving and R&D costs. Therefore, cultivation of GMO in microtiter plates evolved to a versatile screening method in process development a scale up [1,2]. Online monitoring in 24-wellplates is still a challenge since only few online monitoring systems are commercially available. These are usually bulky, expensive and cannot be used with custom incubators. Here, we report on the development of a small, online biomass sensing device. It is compatible with virtually all orbitally shaken incubators.

Experimental approach: The biomass sensor is based on light-scattering of cells through the transparent bottom of the wells at a dominance wavelength of 625 nm and measures 24 well deep well plates. The fully non-invasive measurement can be performed at a high measuring frequency of up to 4 s for each well. The biomass where calibrated against common growth scales like OD_{600} or cell dry weight.

Results and discussion: In the context of this work a prototype was applied to monitor the growth of GFP-GST and hLIF-protein producing *E. coli* BL21. As a future perspective the given sensor data, could also be used in order to screen for optimal media composition and precisely determine a process specific moment of induction at a certain optical density.

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P-421

OXYGEN UPTAKE RATE SOFT-SENSING FOR BIOMASS AND METABOLIC TRANSITIONS

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Background and novelty: Mammalian cell culture bioprocesses are nowadays the system of choice for the production of complex therapeutic proteins such as monoclonal antibodies in biopharmaceutical industry. In aerobic bioprocesses dissolved oxygen is a key nutrient and optimal oxygen supply has to be ensured for proper process performance. To achieve optimal growth and/or product formation the rate of oxygen transfer from the gas to the liquid phase has to be in right balance to the consumption rate by the cells.

Experimental approach: In this work, a 15L lab-scale mammalian cell culture bioreactor was characterized in respect of k_La under varying process conditions in a rational experimental setup. Within the chosen process space, it was found that distinctive physicochemical properties in culture medium, temperature and superficial gas velocities had the strongest impact on k_La behavior. The dynamic k_La description combined with functions for the calculation of oxygen concentrations under prevailing process conditions, led to an easy-applicable model, which allows for real-time calculation of the oxygen uptake rate (OUR) during the bioprocess. In a further step, the established OUR software-sensor was correlated to the viable biomass in the reactor.

Results and discussion: This approach delivers robust OUR profiles without the use of additional off-gas sensors. As a result, precise observation of oxygen uptake alterations based on present cell volumes has enabled the detection of metabolic shifts. Moreover, evaluation of amino acid profiles led to a deeper understanding of these metabolic alterations in a recombinant Chinese Hamster Ovary (CHO) fed-batch process. The awareness of these underlying causes empowers process optimization.

P-422

ONLINE BIOPROCESS MONITORING BASED ON 2D-FLUORESCENCE SPECTROSCOPY

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Background and novelty: The initiation of the Process Analytical Technology guidance led to re-evaluate how bioprocesses should be monitored and controlled to ensure product quality. Although, in the biopharmaceutical industry a positive trend towards a more dynamic Quality by Design approach is evident, still important process variables are monitored by time-consuming offline measurements. Therefore, often spectroscopic methods are considered due to their non-invasive character. For the purpose of advanced process control and monitoring, 2D-fluorescence measurements in combination with multivariate data analysis methods were used to establish soft-sensors, which allowed the estimation of specific process variables.

Experimental approach: An intensified experimental setting was performed to capture and understand the variations of the process. Therefore, an online real-time 2D-fluorescence spectrometer and a broad analytical platform were applied on two different recombinant Chinese hamster ovary cell lines in a 15 L fed-batch scale. The development of the soft-sensors was achieved after pre-processing the data and splitting it into 9 training and 2 test runs. Besides the common linear Partial-least square regression other non-linear methods were used (such as Random Forest, Support Vector Machine). The generated soft-sensor models based on the first clone were then transferred and tested on the second cell line.

Results and discussion: We successfully established soft-sensors for 16 different variables such as viability, titer and amino acids. Most of these components do not fluorescent. Furthermore, 10 out of these 16 variables had a less than 10% error of prediction. In addition, some of these built-up soft-sensor models could very well be applied for the second clone. To conclude, these models not only allowed us to estimate process relevant concentrations but also helped in providing enhanced process knowledge and build the basis for advanced bioprocess control.

P-423

IMPACT OF PROCESS IMPURITIES ON THE DETERMINATION OF HARVEST YIELD

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Background and novelty: High performing cell culture processes enable now to reach high cell culture densities and also high final product titer. But the intensification of process performance also increases the levels of impurities released during the production. These impurities can then impact primary recovery, downstream process but also the performance of in-process analytical methods.

For the successful development of cell culture harvest process, it is essential to monitor the concentration of the product of interest to calculate process yield. It is typically measured by HPLC using affinity resin coupled to an absorbance detector. It is well known that process related impurities such as DNA, or HCP may interact with monoclonal antibody and therefore may interfere in the quantification assay.

Since the population of impurities is changing from one clarification step to another, their impact on the analytical quantification method should be assessed to calculate accurate clarification yield.

Experimental approach: To explore the impact of impurities on quantification accuracy, we spiked in-process samples with purified product at different stage of the clarification process. We also assessed the impact of flocculating agent known to remove negatively charged impurities at neutral pH.

Results and discussion: Preliminary results showed that the addition of flocculating agent induced a diminution of the product titer measured with the HPLC method. Further work will be done to determine if this reduction of titer results from the removal of impurities interfering in the quantification assay, by the loss of product or by the removal of product variant such as aggregates.

P-424

HT QUANTITATION OF IGG BY FLUORESCENCE POLARISATION 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 Immunoglobulins, host cell proteins 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 and <5 min per 384 samples), automation friendly, 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 over a wide functional range (0-2g/L). The solution-phase FP assay is based on the detection of immunoglobulin Fc domain containing analyte binding to FITC-conjugated recombinant Protein G binding domain-1 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 <2%, and a between-plate standard deviation below 1%.

P-425

SCALE UP, SCALE DOWN: CHARACTERIZATION OF REDUCED SCALE MODELS

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Background and novelty: Process development according to FDA guidance [1] often requires the use of Design of Experiments (DoE). While DoEs will provide the most information from the data, they may still require practically unreasonable amount of bioreactor runs, if full scale process (FSP) runs are used.

Reduced Scale Models (RSM) may be developed and used to circumvent the issue, but scale equivalence and limits of the RSM should be shown.

We propose an approach defining the multivariate combination of process parameters (PPs) that ensure representativity of the RSM. The result is a tool for further process development or optimization at reduced scale.

Experimental approach: The methodology presented uses of historical data to establish expected performances of the FSP Results from analytical method validation allows a sound definition of limits distinguishability. All those combined with the DoE results fully characterize the RSM.

All data are used in a series of statistical models, ranging from the simplest models to multivariate Bayesian models to provide the relevant results.

Results and discussion: The experimental and statistical approaches can be divided into three stages:

– Definition of PP set points and of suitable quality attributes
– Qualification of the RSM for the selected set points values
– Defining and running the proper DOE.

The main results consist in multivariate maps, representing the multivariate region of the experimental space in which:

– The two studied scale provide similar values.
– The two different scale provide results within specifications.

The intersection of those map allows the definition of the region of space in which:

– RSM is representative of FSP
– FSP meets specifications.

Which finally defines the region in which the RSM is useful for process development and optimization.
The approach is illustrated using a cell culture case study.

References:

[1] *FDA Guidance for Industry on Process Validation: General Principles and Practices*

P-426

CLONE STABILITY ASSESSMENT USING CHEMSTRESS® FUNCTION PROFILING ARRAYS

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Background and novelty: Clone instability remains a serious bioprocess concern. We identified that the focus upon titer in most stability trials means clones often misleadingly appear stable despite significant underlying change in growth and productivity, indicating the need to look at cell function in greater detail. A deep analysis of cell function was conducted using ChemStress® function profiling arrays and Valita®PROFILE data analytics. ChemStress® is a multiwell plate system that monitors growth and titer in response to a battery of chemicals selected to mimic real bioprocess stresses. This gives a rich, bioprocessing-relevant profile of cell function. Valita®PROFILE's time series tools were used to quantify change in these profiles as the basis of a novel metric for assessing clone stability.

Experimental approach: Stability trial data were collated from >100 clones. Titer change was compared to changes in growth and cell-specific productivity. This demonstrated that apparently stable clones are, in fact, often highly unstable. ChemStress® function profiles were collected for these clones throughout stability trials. Valita®PROFILE's time series algorithms were applied to quantify rate of function profile change. A simple heuristic was applied to these rates to rank clones by function stability.

Results and discussion: We demonstrate that current stability trials, which are numerously flawed, give a misleading account of clone stability because titer masks underlying cellular change. Understanding stability properly requires a deeper assessment of function. ChemStress® function profiling arrays are a fast, affordable way to create this deeper functional insight. Valita®PROFILE analytics were used to quantify the rate of profile change to provide a more principled basis for selecting stable production clones.

P-427

STABILITY OF PARA-AMINOBENZOIC ACID IN CELL CULTURE MEDIA

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Background and novelty: Para-Amino benzoic acid (PABA) is a compound commonly added to chemically defined (CD) cell culture media as the source of folate synthesis by cells. PABA is a very stable molecule and difficult to degrade. However, when PABA was measured in CD formulations, unexpected low recoveries were observed. The objective of this study is to identify possible PABA reaction product(s) and the underlying reaction mechanism.

Experimental approach: PABA in a complex CD formulation or a mixture of PABA and D-glucose was incubated at accelerated stability conditions (35.5°C/55%RH). To mimic manufacturing conditions, PABA was milled with D-Glucose. After incubation for 7-30 days, the powders were rehydrated in water and analyzed by HPLC and HPLC-MS. PABA recovery was calculated by HPLC analysis and the reaction product was proposed based on reaction mechanism which was confirmed by HPLC-MS analysis.

Results and discussion: CD media stored at 4°C for 30 months showed PABA dropped to <20% of the initial levels. When an aqueous mixture of D-glucose and PABA was heated at 100°C for 4 hours, 16.4% of PABA was recovered. Similarly, the reaction was observed when PABA and D-glucose were milled and stored under accelerated stability conditions for four weeks. It was proposed PABA may react with D-glucose to give 4-carboxyphenyl-D-glucosamine in above storage or forced degradation conditions. The proposed reaction product was confirmed by HPLC-MS analysis via comparison with an authentic sample synthesized in the lab. This study demonstrates that PABA reacts with D-glucose in cell culture media to result in a Mailard reaction-type product.

P-428

IMPACT OF WHEAT HYDROLYSATE VARIABILITY IN CELL CULTURE PERFORMANCE

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Background and novelty: Hydrolysates are a complex, chemically undefined raw materials that cause variability in cell culture media. Understanding and characterizing this variability is important to make a consistent, high-quality product. Towards this goal, different omics tools like LC-MS, NMR, Fluorescence, Raman and Differential Scanning Calorimetry (DSC) were used to fingerprint wheat hydrolysate profiles for chemical composition and cell culture performance. This presentation will highlight our findings on the differences between good and poor performing hydrolysate lots and an attempt to identify this variability as potential markers for future screening of wheat hydrolysates.

Experimental approach: Different lots of wheat hydrolysates were categorized as the good and poor performers based on viable cell density VCD between days 4-6 during a growth promotion assay. A PLS-DA model with S-plot (coefficients plot) and variable data plot (VIP) was used to correlate the peak components to cell culture performance. Further, peaks with more than 1.5X fold difference ($p<0.05$) were bucketed and m/z were vigorously searched through different databases using their MS/MS spectra and other parameters for identification and characterization.

Results and discussion: Our omics data showed that 99 components were significantly increased in the poor performing hydrolysates. Upon identification, certain components like dipeptides and Maillard Reaction Products (MRPs) were found to result in poor cell growth whereas the presence of specific amino acids like ornithine and sugars like maltose improved cell growth. Further quantitative studies of these hydrolysates also showed notable differences in sugars and trace element profiles affecting the cell culture. These results indicate that the first step of hydrolysate manufacturing i.e. the hydrolysis of raw gluten can be improvised as a potential source of controlling the lot-to-lot variability.

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AUTOMATION OF HIGH-THROUGHPUT TITER ASSAYS FOR CELL LINE DEVELOPMENT

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Background and novelty: High-throughput methods for screening and automation in bioprocess development have been introduced into most industrial labs in the last ten years and paved the way for screening larger sample numbers, resulting in higher product yields as well as shorter development time lines and reduced costs. Depending on the specific needs, automation may integrate several parts of the workflow, e.g. clone imaging, hit picking, titering and different liquid handling operations associated with these tasks.

The automation solutions can therefore reach from partly or semi-automated to fully automated, which may eventually require capital investments exceeding one million Euros.

PAIA titer assays are easy to automate because they all share the exact same workflow and the same volumes of liquid have to be dispensed, so that the same protocol can be used for each of the PAIA assay, be it for for IgGs, Fc fusion proteins or IgG fragments.

In the poster we lay out different ways to automate titer measurements using PAIA titer assays. Among those we highlight the use of two low-cost pipetting solutions in combination with the automated cell imager Cellavista, which can, at the same time, be used for proof of monoclonality and cell growth measurements.

Experimental approach: We describe options for automation of PAIA titer assay with different liquid handling hardware, perform different titer assay on them and compare results with other titering methods. We also assess the precision and accuracy of the liquid handling systems.

Results and discussion: We present the assay results obtained with the different automation solutions and discuss advantages and disadvantages of each approach.

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P-430

IGG GLYCAN SCREENING ASSAYS IN CRUDE CELL CULTURE SUPERNATANTS

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Background and novelty: Glycosylation of antibodies is a critical quality attribute (CQA) which affects stability, aggregation, serum half-life and immunogenicity of the drug substance.

The control of glycosylation during cell line development receives increasing attention because different studies have shown that small scale fed-batch cultures (e.g. in deep well plates) are not only predictive for product titers but also for glycosylation at larger scales. Since modifications of the cell culture process, e.g. by media supplements, can only change product glycosylation within the capacity of the cell line, it is critical to include glycosylation screening when selecting cell lines, especially if a defined glycosylation profile needs to be achieved (e.g. in biosimilars).

We present a novel technology that overcomes current analytical bottlenecks and allows for fast and reliable screening of non-purified cell culture samples using bead-based assays.

Experimental approach: We used the PAIA assay technology with affinity capture beads for IgG and fluorescence labeled lectins that detect different types of glycosylation. The method measures intact glycoproteins in a one-hour, high throughput and completely plate-based assay which does not require purification of the IgG.

Results and discussion: We present data examining the differences in glycosylation between biosimilars and originators and case studies from screening campaigns in early cell line development and compare the results with orthogonal methods.

PAIA glycosylation assays provide a fast method to screen cell culture supernatant samples and determine relevant differences in product quality that allow cell line selection as early as in 96 deep well plates. We believe that this technology will close an important analytical gap in cell line development.

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ONLINE MONITORING OF VIABLE CELL CONCENTRATIONS IN SMALL BIOREACTORS.

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Background and novelty: Viable cell concentration (VCC) is one of the most important key performance indicator during mammalian cell cultivation mainly measured offline. According to FDA´s PAT initiative, process monitoring and control should be applied to gain process understanding and improve control of process parameters leading to high product quality. Thus, the implementation of an online capacitance probe in a small scale bioreactor is demonstrated. Capacitance sensors using one frequency are frequently used to monitor biomass. However, the capacitance signal is dependent on cell diameter changes and correlates with the viable cell volume instead of the VCC. In this work a multivariate model based on frequency scans is used to investigate its ability to directly predict the online VCC based on capacitance measurements at different frequencies.

Experimental approach: A CHO cell culture fed-batch process producing a monoclonal antibody was used for the experiments. Cultivations were conducted in a single-use, small scale bioreactor (0.25 L). A capacitance sensor was integrated into the bioreactor. Based on the frequency scans the multivariate model was created using the orthogonal partial least square regression method. Multiple standard cultivations were carried out to validate the model. Furthermore, model robustness was investigated by inducing different process deviations.

Results and discussion: A multivariate model was successfully implemented to monitor directly online VCCs using a small number of cultivations. The model provided a prediction of the VCC with a coefficient of determination above 95 %. This approach leads to robust results superior to single-frequency measurements and comparable to the offline reference. Process deviations were detected immediately in the online prediction of the VCC. Thus, the presented method is proposed to be used for process monitoring and control that can save batches, time and costs.

P-432

DEFINE THE UNDEFINED – TOWARDS REALLY CHEMICALLY DEFINED MEDIA

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Background and novelty: Reproducible and stable cell cultivations have been achieved by changing from complex to chemical defined media. To further increase precision of prediction models for the quality of bioprocesses, there is a need for detailed insights into media formulations. Characterization and monitoring of raw materials used in media production becomes mandatory to achieve next level for media consistency. As quality standards today are high, the amount of possible impurities that impact cell growth, product quality, productivity and medium stability are manageable. Since influences of impurities on biopharma productions are foremost process and cell line depending, the cell specific response has to be checked individually, as well as influence of single impurity components on media stability, regarding unwanted reactivities and degradation products.

Lot-to-lot divergencies caused by impurities through raw materials can hardly be avoided, as production methods and sources of supply can lead to quality deviations. Rational media design starting from minimal component sets of necessary ingredients will have the advantage of better performance prediction and reduced risk of lot-to-lot divergencies.

Experimental approach: During investigation, a broad spectrum of different raw materials, quality standards and suppliers with measurable impurities were evaluated to control quality standards of media formulations.

Results and discussion: We could observe differences in impurity patterns and dynamic concentration ranges for all proteinogenic amino acids from up to four different suppliers, the highest trace element contamination values could be measured for cystine and tyrosine. In addition, three different quality standards from selected amino acids were measured to evaluate the increase of the dynamic range with changing the quality standards. Further raw materials (e.g. salts) were investigated to access their impact on cell culture media consistency.

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AUTOMATING AT-LINE METABOLITE ANALYSIS FOR HIGH THROUGHPUT BIOREACTORS

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Background and novelty: Automated small-scale bioreactor systems like the ambr 15 and ambr 250 have been proven to increase the speed and throughput of cell line and process development. Combining the ambr systems with integrated analytics like pH modules, cell counters, and metabolite readers including Nova FLEX2 not only ensures accuracy and consistency of the measurements but also allows for automatic data transfer and advanced control strategies.

Experimental approach: This poster highlights the work carried out to evaluate these integrated systems, including academic and industrial development partners. Data presented includes comparing manual versus automated samples and implementing automated feedback control using both cell count and glucose measurements for a CHO fed-batch process.

Results and discussion: Key takeaways from the case study to be presented include identifying glucose control strategies that led to increased peak cell densities and prolonged culture duration as well as showing that automated sampling and data transfer allows for walk-away control of glucose concentrations in CHO cell culture, including feedback and feedforward approaches.

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IN SITU FLUORESCENCE MONITORING IN INSECT CELL BIOPROCESSES

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Background and novelty: The process analytical technology (PAT) initiative shifted the bioprocess development mindset towards real-time monitoring and control tools to measure relevant process variables online, and acting accordingly when undesirable deviations occur. Online monitoring is especially important in lytic production systems in which released proteases and changes in cell physiology are likely to affect product quality attributes, as is the case in the insect cell baculovirus expression vector system (IC-BEVS), a well-established system for production of viral vectors and vaccines.

Experimental approach: Here, we applied fluorescence spectroscopy as a real time monitoring tool for recombinant adeno-associated virus (rAAV) production in the IC-BEVS. Fluorescence spectroscopy is a simple, yet sensitive and informative tool for bioprocess monitoring. To overcome the strong fluorescence background of the culture medium and improve predictive ability, we combined artificial neural network models with a genetic algorithm-based approach to optimize spectra pre-processing.

Results and discussion: We obtained predictive models for rAAV titer, cell viability and cell concentration with normalized root mean squared errors of 7%, 4%, and 7%, respectively, using leave-one-batch-out cross-validation. Our approach shows fluorescence spectroscopy allows real-time determination of the best time of harvest in order to maintain rAAV infectivity, an important quality attribute, and detection of deviations from the golden batch profile. This methodology can be applied to other biopharmaceuticals produced in the IC-BEVS, supporting the use of fluorescence spectroscopy as a versatile PAT tool.

P-435

PROCESS-INDUCED CELL-CYCLE SYNCHRONIZATION

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Background and novelty: The influence of process parameters on cell population heterogeneities is still not fully understood. We recently found that the progression of cells through the cell-cycle leads to strong metabolic regulations with variable productivities in antibody-producing CHO cells [1]. However, it is unknown how the process conditions themselves influence the cell-cycle. In this study, process-induced cell-cycle-synchronization was assessed with antibody-producing CHO cells in repeated-batch and fed-batch cultures.

Experimental approach: Interleukin-8 producing CHO DP-12 cells were transfected with the FUCCI (Fluorescent Ubiquitination-based Cell Cycle Indicator) system [2]. In brief, two fluorescent markers (representing either the G1 phase or the S/G2/M phases) are expressed and degraded according to the cell cycle. An automated flow cytometry set-up was developed to measure the cell-cycle distribution online. Firstly, a model-based study was performed to identify suitable process parameters. Secondly, the simulated cultivation concepts were evaluated.

Results and discussion: Stable cell-cycle oscillations were measured in repeated-batch cultures. The G1 phase distribution oscillating between 41% and 72% and cell-cycle-dependent metabolic regulations were identified. Furthermore, cell-cycle oscillations were determined in (bolus) fed-batch processes with up to $25 \cdot 10^6$ cells ml^{-1}. Interestingly, the cell-cycle-synchronizations were based on the pulse feeding solely. In summary, we demonstrate how combined methods enable to assess the effects of cultivation conditions on the dynamics of population heterogeneities. This provides a novel approach to improve process stability and efficacy.

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P-436

STRATEGIES TO CONTROL A PROCESS USING CELL DENSITY ONLINE MEASUREMENT

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Background and novelty: Control strategies are needed to run a bioprocess and to achieve consistent cell culture productions across sites and scales. Typically, this includes the measurement and control of physicochemical parameters such as temperature, pH, dissolved oxygen and stirrer speed, which can be monitored online. However, cell growth is possible the most important parameter of a bioprocess and yet is commonly measured at discrete time points only. The availability of reliable online measurement systems for cell density can help to process understanding and increasing productivity saving time, efforts and errors.

The present work is focused on the application of the online cell density measurement for monitoring of cell growth in 2L and 50L bioreactors to scale up the process and to avoid manual steps.

Experimental approach: Cell cultures were conducted in single use bioreactors. Cultures samples were regularly collected offline to determine cell concentration.

Online measurement of viable cell density and total cell density was carried out using a biomass monitoring system.

Results and discussion: Regarding cell density and viability comparable results were obtained between 2L and 50L, demonstrating the excellent scalability of the process.

The comparison between online and offline data of viable and total cells showed similar results. Therefore, online measurement is a robust method to control cell growth and physiological status of the cells in batch cultures. Moreover, it is a promising tool to reduce laboratory work and to minimize the risk of sample manipulation.

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IN SILICO CHO MODEL GUIDES CELL CULTURE PROCESS DEVELOPMENT

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Background and novelty: Chinese hamster ovary (CHO) cells are the most prevalent cell factories for producing recombinant therapeutic proteins. Although the product yields have dramatically increased over last few decades, such achievements are largely accomplished based on empirical approaches.

Experimental approach: Now, using the CHO genome sequence available, we previously developed genome-scale model (GEM) which allowed us to examine the metabolic signatures of CHO cells upon varying bioprocess conditions in a systematic manner. Here, we presented the most comprehensive and biochemically consistent CHO GEM to-date, iCHO2166, by including the necessary kinetome information to enable its ready use during the simulations, thereby improving the phenotype predictions significantly.

Results and discussion: To fully leverage on the model, we implemented a new constraint-based flux analysis framework that is based on minimal enzyme utilization, energetic efficiency and cellular maintenance. As a result, the intracellular flux predictions are highly consistent with 13C-MFA measurements, outperforming basic flux balance analysis (FBA), and parsimonious FBA (pFBA). In the sequent analysis via case studies, we first explained overflow metabolism and redox balancing. We also examined the effect of aeration on cellular metabolism and showed how a higher aeration rate negatively impacts cellular metabolism by invoking certain wasteful and stress coping mechanisms. In summary, this study outlines the importance of accounting enzyme capacity constraints in GEM to achieve realistic flux solutions, and thereby effectively utilizing the *in silico* modeling approaches to guide bioprocess optimizations and complex cell engineering efforts.

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DATA RICH CELL LINE SCREENING: COMBINING AMBR15 WITH BIOPROFILE FLEX2

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Background and novelty: Study details a cell line screening campaign from research cell bank screening to phenotypic stability evaluation for a panel of stable CHO cell lines expressing a monoclonal antibody fusion protein by implementing the use of an integrated, low volume, automated nutrient analyser the BioProfile FLEX2 with the ambr15 micro bioreactor system to generate a rich data set of offline process parameters to improve cell line selection. Additionally, the lead cell line was successfully scale-up to bench scale (3L) and mini-pilot scale (30L) bioreactors by using the cell line screening data.

Experimental approach: Bioreactor experiments were carried out following the cell line construction campaign to enable the selection of the final manufacturing cell line by screening of multiple RCBs using the ambr15 micro bioreactor system followed by bench scale (3L) and mini-pilot scale (30L) single-use bioreactor evaluations. ambr15 based studies implemented automated pH correction, VCD based nutrient feeds and fully automated glucose feedback loop by leveraging the hands-off two-way data transfer between the two systems.

Results and discussion: Initially RCB clones were evaluated in duplicate using the ambr15 system and a full panel of offline data such as cell counts, viability, pH, pCO2, glucose, lactate and ammonia was generated.

Subset of cell lines were progressed to evaluate cell line suitability for long term manufacturing. The availability of a full panel of offline parameters from the FLEX2 enabled the tracking of changes not only in cell culture parameters but also the metabolic profiles of the RCB clones at different cell generations and aid selection of the final cell line.

Based on pCO_2 and gassing data obtained during the screening campaign an optimised gassing strategy was developed to enable successful scale-up to 3L and 30L bioreactors with cell culture and mAb fusion product quality attributes matching the 15mL ambr15 micro bioreactor systems.

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IDENTIFICATION OF AN EXTRA BAND IN TLC TEST OF YEAST EXTRACT

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Background and novelty: Yeast extract (YE) has been widely used in cell culture media formulations to supply vitamins, amino acids, peptides, carbohydrates and some micronutrients. Thin Layer Chromatography (TLC) has been internally established as one release test for YE. In a recent testing, an extra TLC band was observed in one lot of YE when compared to the control YE. Identification of the extra band would provide a better understanding of the YE fermentation process to improve the lot–to-lot consistency.

Experimental approach: Two TLC plates were run in parallel: the position of the extra band was visualized and identified by spraying the first plate with ninhydrin solution, and the same region where the extra band appeared was excised from the unsprayed plate. The excised material was extracted with methanol or IPA/EtOAc/NH_4OH and analyzed by Gas Chromatography-Mass Spectrometry (GC-MS) and Ultra High Performance Liquid Chromatography-Quadrupole Time-of-Flight Mass Spectrometry (UHPLC-QTOF MS).

Results and discussion: Tyramine and phenylethylamine were identified by GC-MS and confirmed in UHPLC-QTOF analysis by comparison with authentic samples via the retention time match and mass spectrum match. The major component in the extra band, tyramine, was quantified by GC-MS to be at 0.1% of the original YE sample. It was proposed that Aromatic Amino Acid Decarboxylases (AADCs), which are found in various organisms, catalyzed the formation of tyramine and phenylethylamine from tyrosine and phenylalanine, supported by the fact that YE with the extra band had a reduced level of tyrosine and phenylalanine, as compared to the control YE. This study demonstrates the importance for the control of microbial bioburden in YE manufacturing.

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ENHANCED METHOD TO MONITOR CELL CULTURES BY DIELECTRIC SPECTROSCOPY

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Background and novelty: Physiological state of cells has a strong impact on post-translational modifications of biopharmaceuticals. Therefore its accurate monitoring and control is mandatory to guarantee medicines properties and safety of patients. The specific cell growth rate (μ) may globally be used to depict the cells state during cultures. Using *in-situ* dielectric spectroscopy, μ can be used to control the feeding strategy of cell cultures so that glycosylation quality of monoclonal antibody (mAb) remained under proper levels (1). However, the widely used simple linear regression (SLR) of measured permittivities to real-time estimate the viable cell density (VCD) and then calculate μ, can led to a lack of accuracy and precision. To avoid limitations, this study aimed to evaluate the novel implementation of Supported vector regression (SVR) on dielectrics.

Experimental approach: Capacitance spectra were collected every 12 min by using Biomass Evo 200 during several mAb producing CHO cells cultures in bioreactors (2 L). Off-line measurements of VCD were related to measured permittivities by both regression methods (SLR and SVR) to build prediction models. Then the on-line estimated VCD and the calculated μ, were compared to off-line values during culture.

Results and discussion: Clearly, the prediction of VCD based on the classical SLR method was affected by the evolution of the cellular properties (size, membrane, conductivity), which mainly occurred during the VCD decrease phase. On the contrary, the SVR allowed to accurately real-time calculate VCD and μ during the whole batch culture. Based on these results, the use of this chemometric regression method could enhance the cell culture monitoring by dielectric spectroscopy particularly when cells are subject to strong physiological changes.

References:

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P-441

SUFFER YOUR MEDIA FROM BURN-OUT? – ANALYZING STRESSED MEDIA

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Background and novelty: Typically cell culture media contain a variety of different nutrients including vitamins and amino acids that are crucial for cellular growth and therefore for the outcome of a process. However, preparation and surrounding conditions might play a crucial role related to the performance of a medium. Here we present a study in which, among others, vitamins and amino acids were monitored after exposure to different conditions other than the cellular influence. The aim was to determine whether the induced, yet not unusual, conditions applied to cell culture media result in molecular, time independent, changes which may have an effect on the media performance.

Experimental approach: We tested different experimental set ups in order to gain more insights regarding the stability of common nutrients in cell culture media. Therefore, media was exposed to different conditions, such as a cultivation environment (without cells) in a bioreactor at 37°C and varying storage approaches. Samples were taken at different time points and quantitative analyses of media components including 9 different vitamins and 25 amino acids were performed. For each experiment suitable controls were chosen in order to validate the given hypothesis.

Results and discussion: We were able to monitor instabilities for different molecules during several experimental approaches. Furthermore, our results indicate that reactions leading to the loss of desired nutrients can occur, at least at an accelerated rate, due to the exposure conditions. Amongst others, this is true for the previously described thiamine and ascorbic acid.

Increased knowledge about the media components, their stability and reactivity is necessary to get the best performance out of your bioprocess and in our case the results lead to more robust media developments.

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MIMICKING INDUSTRIAL SCALE CO_2 PROFILES IN CHO SMALL SCALE PROCESSES

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Background and novelty: CO_2 is a critical process parameter, which is known to affect various process performance parameters (pH, lactate, osmolality) and even product quality. During scale-up, it is not feasible to keep the CO_2 concentrations constant between the development scale and the final production scale. The different CO_2 removal rates between the scales will result in process specific increased CO_2 concentrations in the final scales. To our knowledge, a direct investigation in small scale bioreactors addressing the influence of large scale CO_2 concentration on the process or the product was not published jet. In order to address these limitations, a CO_2 control loop was established in 3 L bioreactors. This provides the possibility to adjust and program a CO_2 set point that follows the historical large scale data in an automated way. At the same time, it was possible to prove that the approach did not negatively interact with the pH control and the bicarbonate buffer system was working as expected. As consequence, representative small-scale studies mimicking large-scale CO_2 profiles can be performed using any given process.

Experimental approach: CO_2 probes and a CO_2 control loop using additional submerse Air/CO_2 flows were established in 3L bioreactors. The CO_2 control was applied in current cell culture production processes with diverse process characteristics (pH/osmo. shift). Furthermore, the CO_2 influence in distinct growth phases of a production process was tested.

Results and discussion: The study showed, that pH, lactate and osmolality profiles of the SDM were optimized in case large-scale CO_2 concentration profiles were mimicked. Although the CO_2 control was active, further modifications to the process or the control system (DO, pH, N) were not needed although pH and osmolality shifts were performed. Additionally, the influence of CO_2 concentrations in distinct growth phases on product quality, especially glycosylation, will be evaluated and discussed.

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COMPARING DIFFERENT AT-LINE ANALYTICS FOR ONLINE RAMAN SPECTROSCOPY

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Background and novelty: The use of PAT (process analytical technology) at-line and in-line tools is becoming increasingly important in biopharmaceutical development and production. The monitoring of metabolites and cell parameters currently relies on at-line analytical technologies including sampling, transfer to instrument and time-delayed acquisition of results. Therefore, in-line measurements are particularly important during cost-intensive manufacturing of biopharmaceuticals in order to facilitate early process fault detection, mitigation of contamination risk and real time product release. Spectroscopic sensors enable simultaneous in-line bioprocess monitoring of various critical process parameters (CPPs) during the cell cultivation process.

Raman spectroscopy provides well-resolved vibrational fingerprints of various chemical constituents and is relatively insensitive to water. Chemometrics enable the quantitative analysis of multiple cell culture constituents in the complex measurement matrix in a bioprocess.

Experimental approach: In the work presented herein, partial-least squares (PLS) regression with the reference analytical panel generated quantitative predictions of several process parameters such as glucose, lactate and glutamine concentration. For reference analytics, we will report a comparison of different at-line methods and their analytical performance for bioprocess monitoring. Different metabolite and cell counters were compared and evaluated.

Results and discussion: The results were used for a subsequent evaluation of an optimal reference analytics for chemometric model. Upon model optimization using n=8 different cell cultivations, model quality was evaluated and compared to results obtained by the initial reference analytics panel. The transferability of the PLS models was evaluated for different cell culture products and different process strategies.

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Background and novelty: Lentiviral Vector manufacture is characterised by expensive development and therefore high treatment costs of up to $500,000 per patient. Monitoring of critical quality attributes is vital in viral vector manufacture with the fundamental attribute being infectivity. Infectivity assays are the gold standard for viral vector titering, allowing for an estimation of the number of infectious particles contained in a sample through the infection of cells. Typically these assays are carried out in 24 well plates with limited automation and only end-point monitoring after multiple days. As gene therapy treatments become more personalized and manufacture more decentralized, there is a need for increased sensitivity and speed in analysis and a reduction in sample volume. Here we present combined microfluidic and image analysis approach for low volume, non-invasive, rapid, cell based infectivity analysis of Lentiviral vectors.

Experimental approach: A microfluidic device was designed and manufactured using micromilling. HEK293T cells were cultured in the device for 72 h and automated injection of Lentivirus containing sample was carried out. Infection of cells was monitored continuously using FLUOTAST software.

Results and discussion: A Cyclic Olefin Polymer (COP) perfusion microfluidic chip was manufactured. HEK293T cells growth was monitored through confluency measurements and an increase in cell infection was observed FLUOTAST image analysis and validated against FACS analysis (end-point analysis). This system promises to provide a fast, low volume alternative to current viral vector infectious titering methods.

Acknowledgements & Funding: I would like to thank Professor Nicolas Szita, the Szitalab Group and CPI.

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EXPLORING THE CELL CULTURE DESIGN SPACE BY PREDICTIVE DIGITAL TWINS

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Background and novelty: Commercial manufacturing of drugs mandates a well characterized cell culture process that can be reliably controlled within the normal operating ranges. However, characterizing cell culture processes that are inherently complex and dynamic poses a difficult challenge. It is particularly difficult to characterize the impact of variability in complex parameters such as cell culture media which consist of more than 50 components. Here, we present the Insilico Digital Twin that can effortlessly navigate the high-dimensional design space by predicting the performance of cell cultures in a multitude of experimental scenarios. Nonlinear interaction terms between two or more parameters can be easily determined and analyzed with respect to product quality and/or process performance.

Experimental approach: The Insilico Digital Twin mimics the dynamics of a cell culture process by combining a model of the fed-batch/perfusion process and a model of the genome-scale metabolic network of CHO cells. This novel approach uses artificial intelligence (AI) to determine the dynamics between the extracellular metabolite concentrations and the intracellular flux distributions which results in breakthrough predictive power. Therefore, it can be used for predicting process performance and product quality in a multitude of experimental scenarios.

Results and discussion: In this presentation, we demonstrate the application of the Insilico Digital Twin for simulating the cell culture performance under variable media component concentrations and the determination of their acceptable ranges. In conclusion, the Insilico Digital Twin is able to effortlessly and systematically explore the high-dimensional design space of cell culture processes and thoroughly characterize the impact of media variability, something that was hereto impossible with traditional methods.

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DEVELOPING AN AMBIC CHO REFERENCE PLATFORM FOR THE BIOTECH COMMUNITY

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Background and novelty: Chinese hamster ovary (CHO) cells are a major industrial cell culture platform for biomanufacturing of therapeutic proteins including monoclonal antibodies. Variability in host cell lines, process parameters, basal media and feed formulations limits comparison of observations across laboratories.

Experimental approach: To address this challenge, the National Science Foundation-funded academic-industrial collaborative research consortium AMBIC(Advanced Mammalian Biomanufacturing Innovation Center) has invested efforts toward the development of a standardized, shareable, CHO platform to facilitate research advances which should be representative of industrial platforms in terms of cell performance and media. The AMBIC Reference Cell, Media and Feed project aims to establish an industrially relevant CHO platform as an AMBIC and a CHO community-wide reference standard.

Results and discussion: Two IgG producing cell lines, CHO-K1 and GS-CHO were chosen as the model reference cell lines considering the prevalence of these lineages in the industry today. The consortium is able to demonstrate performance consistency, in shake-flask and bioreactor fed-batch studies, across multiple sites using the same methods. To identify a formulation for chemically-defined cell culture media and feeds that can ultimately be used by the community, AMBIC began to develop a baseline, non-streamlined, chemically-defined medium and feed that was not proprietary to any given organization. An in-person crowdsourcing workshop held by AMBIC companies further streamlined this medium and feed formulation. Through this partnership, a universal CHO platform is being established for consistent and reproducible studies across the CHO community worldwide.

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CASE STUDY: UNEXPECTED IMPACT OF SHEAR STRESS ON INTENSIFIED PROCESS

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Background and novelty: Process intensification by high cell density perfusion seed culture has been rapidly embraced in recent years by biopharma to boost volumetric productivity1. At Merck Serono, a CHO cell line-based platform process was intensified using an (N-1) perfusion seed culture. The TFF-based system enabled seeding the production bioreactor at high cell density, and thereafter achieved high yields with comparable product quality. This case study presents the challenges and learnings from the scale-up of this process to manufacturing scale.

Experimental approach: When scaling up a high seeding fed-batch process, unexpected differences in the cellular performances may be observed, such as cells' physical characteristics and behavior in culture. Differences in shear stress environment at the (N-1) seed bioreactor stage generated by the recirculation of the cell culture in the external perfusion loop (TFF unit) may induce changes in cell characteristics and cell culture behavior. A scale-down model was developed to evaluate the impact of high shear stress levels. A CFD model was also used to simulate several components of the large scale (N-1) seed bioreactor perfusion loop to identify high shear elements.

Results and discussion: The scale-down model was able to mimic some of the features observed at large scale. Based on the CFD model and further studies from the shear model, special attention should be taken into consideration when designing a large scale (N-1) seed bioreactor perfusion loop. This study is expected to provide a perspective on tackling challenges in scaling-up of new technologies for biomanufacturing.

Acknowledgements & Funding: The authors would like to thank Masimo Morbidelli group at ETH, Zurich for performing the CFD simulations.

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HIGH THROUGHPUT GLYCOSYLATION ASSAYS FOR GLYCOPROTEINS USING LECTINS

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Background and novelty: Glycosylation of therapeutic glycoproteins is a critical quality attribute (CQA) which affects different properties of the drug such as stability, aggregation and serum half-life.

It is critical to control glycosylation during cell line and bioprocess development because culture conditions have a great impact on product glycosylation and because they allow optimization of glycosylation properties, e.g. sialylation. Non-Mab glycoproteins can have very complex glycosylation patterns, making the analysis of many samples in parallel a difficult and time-consuming task. In addition to that, purification of glycoproteins is necessary for analytical methods using released glycans. We present a bead-based assay that does not require the release of glycans and uses lectins to detect differences in glycosylation.

Experimental approach: The PAIA assay uses capture beads carrying a glycoprotein that binds the fluorescent lectins for the detection of different types of glycosylation. The glycoproteins in the sample release the lectin from the beads depending on its degree of glycosylation. The release of the fluorescence labeled lectins is measured directly in an entirely plate-based no-wash assay in 384-well plates.

Results and discussion: We present data for assays testing sialylation, mannosylation, galactosylation and fucosylation with different glycoproteins, discuss the influence of cell culture media and supernatants on the assay and the applicability of the assays in supernatant screenings.

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NEW: HIGH THROUGHPUT AUTOMATION FOR ASSESSMENT OF CLONE ATTRIBUTES

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Background and novelty: Viable cell counting, along with the assessment of other clone attributes, is an essential is part of early cell culture, cell line development and upstream process development. Counting using a haemocytometer is the gold standard for many laboratories but is low throughput and can show inter-user variability. Other automated methods are available but typically have drawbacks, due to their requirement for large sample volumes per read, the sequential nature of sample processing and the expensive consumables that must be purchased to operate the system.

Providing users with an automated method of viable cell counting whilst improving the throughput and maintaining or improving accuracy is essential. Being able to generate this information earlier in the process would also be attractive.

In this poster we present a new Solentim platform with its first available application of high throughput, low-volume viable cell number (VCN) assessment.

Experimental approach: This VCN application uses the on-board automated, liquid handling to perform cell counts for up to 96 cell samples in parallel in just over an hour. The application is designed to process and accurately count cells, targeting a range between 0.1 x10^6 cells/mL – 5x10^7 cells/mL using three automated protocols.

Results and discussion: Initial data for testing of a range between 0.1 – 2x10^6 cell/mL (sample #: 288 samples throughout the range) gives CV values of 7-11%. Testing of higher concentrations up to 1x10^7 cells/mL is yielding similar accuracy.We will outline further data sets obtained using the VCN application's accuracy in viability assessment for the top end of the range.

Finally, we will outline the features and benefits of this application over gold standard methods, as well as discuss the range of further applications under development which will be offered on the platform for earlier assessment of clone attributes.

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POTATO: APPLICATION FOR CELL CULTURE PROCESS DEVELOPMENT,AND CONTROL

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Background and novelty: It is quite common to evaluate cell growth curves, in terms of Total Cell density (TCD) and Viable Cell Density (VCD). Viabilities and population doubling times (PDT) are also often considered.

Those four attributes are strongly related to each other. While TCDs and VCDs can commonly be considered log normally distributed, the distributions of the viabilities and PDT are not clearly identified, calling for the use of Bayesian statistics.

Based on predictive modeling, the application provides a tool that allows risk-based decision making in process development, and the definition of time-dependent control limits for process control and capability analysis.

Experimental approach: The four attributes mentioned are strongly related to each other. While TCDs and VCDs can commonly be considered log-normally distributed, the distributions of the viabilities and PDT are not clearly identified, calling for the use of Bayesian statistics.

Based on TCD and VCD values, a Multivariate Bayesian mixed model with correlated random effects is adjusted.

From the model, new TCD and VCD profiles are predicted, and those are used to predict new viability profiles and PDT profiles. Once those predictions are available, the output of the application can be defined.

This means that the data required for the application consists in TCD and VCD, at multiple timepoints, for multiple runs of the process.

Results and discussion: Main outputs of the application are:

In term of process control: Time-dependent prediction regions for the cell growth parameters being monitored.

In process development and capability analyses: Given some targets, the probability for the process to meet those targets at all considered culture timepoints, individually and simultaneously allowing the definition of a minimum culture time

In process change assessment: Graphical representation of the comparison is provided.

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INTEGRATED SAMPLE AND DATA MANAGEMENT FOR MICRO-BIOREACTOR EXPERIMENTS

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Background and novelty: Cell line and process development groups increasingly apply miniaturized and automated cell culturing and analytical test methods. These include micro-bioreactor systems, Process Analytical Techniques (PATs) and several methods for early assessment of product quality. Therefore, the amount of data which needs to be analyzed and reviewed, has grown significantly. Thus, data structuring and curation represents a serious bottleneck to proper data analysis and valid decision making.

Experimental approach: We have designed a highly integrated data management system (Genedata Bioprocess®), which supports automated workflows and provides the foundation for an increase in throughput in cell line and process development. For scale-down bioreactor-like experiments performed either in microtiter plates, shake flasks or micro-bioreactors, such as the ambr® systems, we implemented barcoded sampling and automated data capture workflows. All online, at-line and offline data are automatically processed, aggregated, and visualized, enabling multi-parametric assessment of any type of bioreactor data in the context of experimental settings. In addition, the applied raw materials and their quality attributes are associated to the experiments.

Results and discussion: We present concrete use cases demonstrating how the platform supports screening in scale-down models. The integration with product quality and molecule data enables a comprehensive assessment of best-producer cell lines and processes. In addition, we demonstrate how the system's tracking capabilities for raw material lineages (e. g., media and media components) enables the monitoring of raw material batch-to-batch variation and correlation of raw material lots with process performance. We show how the platform enables the correlation of process parameters with key performance indicators of the processes (e.g., Titer, Qp) and product quality attributes (e.g., aggregation, glycosylation profiles).

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A PLATFORM APPROACH TO ASSESS DEVELOPABILITY RISKS OF BIOLOGICS

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Background and novelty: Larger panels of molecules are being subjected to more intense characterization associated with developability assessments being performed earlier in the biologics R&D process, which creates a barrier to the already significant challenges of large-molecule characterization. The need to have well-defined, high quality products for development and CMC is vital. A solution that supports integrated developability studies is critical to ensure the technical success of the chosen lead molecule.

Experimental approach: We present a scalable, off-the-self enterprise workflow system that enables systematic developability and manufacturability assessments from the very early stage to the later stages of the biopharma R&D process. It uses both *in silico* methods and high throughput analytical confirmatory methods. Moreover, it enables clear diagnosis of developability issues using genealogy management.

Results and discussion: We present concrete use cases not only for mAbs, but also for complex multi/bispecific formats, as well as engineered therapeutic cell lines (e.g. CAR T cells). We start with a peptide mapping use case where we compile a large number of processed MS results (MW, PMF coverage, PTMs – e.g. deamidation, oxidation, glycosylation). We complement this by a second use case showing the N-linked glycan analytics results for various antibody candidates from stable cell lines in which automated workflows make it possible to interpret the data sooner. A third focus is on the bispecific heterodimerization where we show automatic, accurate identification and reporting of MW and abundance of homodimeric and heterodimeric species. This integrated biopharma platform forms a solid basis for building predictive models for developability.

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OFF-GAS METABOLITE ANALYSIS FOR QUALITY CONTROL AND PROCESS CONTROL OF MAMMALIAN CELL CULTURE

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Background and novelty: The use of cell culture production methods for biological therapies is a growing trend that leads to a high demand of novel quality control methods. To improve quality and productivity, these methods should therefore address appropriate online monitoring and control. At the present especially for personalized products there is a lack of an established process control strategy. Innovative analysis methods are aimed at non-invasive procedures that are able to extract a large data volume out of one measuring point.
The goal of this study was to establish a non-invasive measurement for personalized cell production procedures.

Experimental approach: The measurement set-up was exemplary established with adherent and suspension adapted HEK293 andCHO cells. Different sized culture vessels (T- and Erlenmeyer-flasks, bioreactor) should be used to examinethe scalability of the system. The off-gas of the culture was sampled and measured via GC-IMS.Additionally metabolite analysis was carried out with standard enzyme kits.

Results and discussion: The new sampling and measurement set-up leads to better process understanding through continuous monitoring of the off-gas. The non-invasive sampling does not interfere with the production process and owing to the gained big data-set, specific parameters can be derived for the process. That can be a huge potential to reduce time-consuming multi-experimental analysis and costs in the personalized drug production.

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HYDROCYLONE FOR MAB PRODUCTION IN A PERFUSION SINGLE-USE BIOREACTOR

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Background and novelty: Several publications explore hydrocyclones (HCs) as cell retention devices for perfusion cultures. These low-cost, high-capacity devices are compact and free from clogging issues. also, they can be produced by 3D printing. these features make them well suited for cell retention in single-use perfusion processes. However, previous reports on HC use for mammalian cell perfusion were limited to non-disposable lab-scale bioreactors and relatively low cell densities (up to ~ 10 million cells/mL). Thus, the aim of the present work was to evaluate the HC as a cell retention device coupled to a 50 L single-use bioreactor in an animal-derived component-free cho process. furthermore, steady-state operation at cell densities in the 50 million cells/mL range would be tested.

Experimental approach: an HC prototype was connected to a single-use bioreactor bag (XDR-50, GE Healthcare). Three perfusion runs were performed at 40 L working volume, evaluating different ways to connect the HC underflow port (concentrated stream) to the bag. Due to its high processing capacity, the HC was operated intermittently by easily connecting a simple timer to the peristaltic pump used for cell recirculation.

Results and discussion: Perfusion runs reached steady states of up to 50 million cells/mL using an HC as cell retention device. To our knowledge this is the highest reported steady state concentration. The perfusion runs were operated at high cell viabilities, using cell-specific perfusion rates of 50 down to 15 pL/cell/d for 20 to 25 days. Pressure drops in the HC of 2 bar provided total separation efficiencies up to 96% and allowed natural cell bleed through the diluted overflow orifice. Perfusion run comparisons with this cell line and medium using other cell retention units (ATF and inclined settler) are ongoing.

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STABLE TRANSFECTION OF SF9 CELLS FOR THE CONTINUOUS PRODUCTION OF AMPS

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Background and novelty: Since the discovery of antibiotics and their wide field of application, humanity faces the development of microbiological resistance. Arising incidence of resistant pathogens combined with a decreasing number of newly developed antibiotics necessitates the development of novel drugs. Antimicrobial peptides (AMPs) are active substances with the potential to answer this need. The research project addresses the development of a platform process for the production of an AMP, derived from *Lucilia sericata* using *Spodoptera frugiperda (Sf9)* cells.

Experimental approach: The establishment of the process is based on continuous perfusion to enable high cell densities and, thus, an effective AMP-production. As the commonly used baculovirus expression vector system leads to cell death after a prolonged process-time, stably transfected cell lines are necessary for long term production. Therefore, plasmids and the transfection itself are key factors for the success of this project as well as the selection of suitable process equipment.

Results and discussion: An expression construct was designed and synthesized. It encodes a fusion protein consisting of a His_6-tag, the fluorescent marker tdTomato and the actual AMP. A well-positioned thrombin cleavage site enables the final recovery of a tag-free AMP. During the first 48 h post transfection, protein expression can already be observed via fluorescence measurement. Furthermore, the fusion protein was successfully detected in the supernatant by His_6-specific western blotting, indicating the successful secretion of the fusion protein into the medium.

The next step is the generation of a monoclonal, stable production cell line. In parallel, first experiments with the reactor setup are intended to demonstrate long-term viability for *Sf9* wild-type cells using acoustic separation. Based on these results, suitable solid-liquid separation (SLS) settings will be identified.

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APPLICATION OF 2-COMPARTMENT SYSTEM TO STUDY LARGE-SCALE HETEROGENEITY

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Background and novelty: With an increasing demand for biopharmaceuticals more facilities containing large scale bioreactors up to 20 kL are being built to supply this demand. Process development is however mainly performed in lab scale bioreactors, or at even smaller scales to achieve high throughput. The major difference of large production bioreactors is their increased mixing time, which leads to inhomogeneities in the reactor. 2-compartment systems are widely used to study the influence of these gradients, since they allow the exposure of only a fraction of the cells to a different environment, rather than of the whole cell population. While these systems are routinely used to investigate effects on microbial cells, the application for mammalian cells is scarce.

Experimental approach: For this reason, a 2-compartment system was developed to investigate the effect, which the addition of base from the top of a large-scale vessel has. The 2-compartment system consists of a stirred tank reactor (STR) and a plug flow reactor (PFR). The system was characterized with tracer pulse experiments (Leavenspiel 2011) to determine the pump rate necessary for the simulation of the mixing time of a 12 kL bioreactor. To investigate pH inhomogeneities, base was added in the PFR to regulate the pH in the STR.

Results and discussion: Therefore, a zone with a higher pH was generated in the PFR, which mimics the part of a large-scale vessel, where base is added from the top. Exposure of the cells to an intermittently higher pH resulted in changes to the process performance, showing that inhomogeneities occurring at large scale need to be considered when scaling up a process. The presented 2-comparatment system could furthermore be an advantageous tool to screen cell lines for their robustness to large-scale inhomogeneities and therefore predict process performance at larger scales.

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POST-LAUNCH REMOVAL OF ASM IN AN NS0-PROCESS

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Background and novelty: NS0 myeloma cells are cholesterol auxotrophs and are grown in serum-free medium supplemented with an animal-derived lipoprotein. It is desirable to move away from animal-sourced material (ASM) for several reasons including a reduced risk of prion related contaminations and batch-to-batch variability. The NS0 cell line presented here not only uses an ASM but also relies on a complex plant-origin nutrient supplement which leads to process variability and significant impacts on charge variants. This work describes how 1) ASM was successfully removed, 2) the resulting product quality was modulated by incorporation of a specific and tailored feeding strategy and 3) process variability was controlled by introduction of specific chemically defined nutrients.

Experimental approach: These studies incorporated a statistical study design whereby a series of shake flasks and bench-scale bioreactor studies were completed to develop the process in ASM-free medium. The removal of ASM also necessitated specific cholesterol feeding strategy and optimisation of the process to maintain comparable product quality and ensure process robustness. An optimal feeding strategy to modulate critical quality attributes and reduce process variability was developed based on parametric trend analysis, NMR (nuclear magnetic resonance) spectroscopy and ICP-MS (inductively coupled plasma mass spectrometry). The process was scaled to 300L pilot scale bioreactors.

Results and discussion: Effects of ASM-removal were ameliorated through process parameter adjustments and incorporation of a chemically-defined supplement to the media. Comparable process performance and product quality attributes to the current process were achieved. A robust "next generation" process was developed. This new process delivered an ASM-free process with less process variability, higher titre output and comparable product quality.

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HIGH CELL DENSITY CULTIVATION TO IMPROVE INFLUENZA A VIRUS PRODUCTION

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Background and novelty: Manufacturing of human influenza vaccines using animal cell culture technology is one pillar of todays supply chain. Different cell lines have been described as possible producer cell lines. Adherent MDCK cells still hold the record of highest cell-specific virus yield (CSVY) of up to 20 000 virions/mL. High cell density cultivation (HCD) of suspension cells could be one approach to increase the overall virus titers by process intensification. Here, we present a perfusion process using alternating tangential flow (ATF) filtration using one selected suspension MDCK cell line.

Experimental approach: Two MDCK cell lines (from ECACC & ATCC) adapted to two chemically defined media (Smif8 & Xeno-CDM) were evaluated for influenza A virus production. The best candidate was cultivated subsequently in semi-perfusion mode to evaluate process options for scale-up in stirred tank bioreactors. A lab-scale perfusion process was established in a 1 L DASGIP system connected to an ATF unit using a constant cell-specific perfusion rate (CSPR) for media feeding. All infections were carried out with influenza A virus (A/PR/8/34 H1N1 RKI) and addition of trypsin.

Results and discussion: Among the four cell lines, $MDCK_{ATCC}$ in Xeno-CDM medium showed superior performance not only for cell growth (t_D<22 h) but also for virus production (CSVY >12000 virions/cell). With this cell line, cell concentrations above 4.2×10^7 cells/ml were obtained in semi-perfusion in 6 days. High HA titer of 4.00 $\log_{10}$(HAU/100 µl) was measured in multiple harvests, which accumulated over harvest time to an HA titer of 4.20 $\log_{10}$(HAU/100 µl). Cultivation in perfusion mode resulted in higher cell concentration of 5.6×10^7 cells/ml. Compared to semi-perfusion, virus titers above 4.00 $\log_{10}$(HAU/100 µl) were achieved with comparable CSVY of 7500 virions/ml for both systems. Overall, this MDCK-based HCD process is a very promising option for increasing the capacity in influenza vaccine production.

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ACOUSTIC BIOPROCESSING FOR PERFUSION APPLICATIONS

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Background and novelty: Advances in perfusion cell culture have led to cell densities in excess of 100 million cells/mL, with product titers similar to fed batch (3-5 g/L). This progress drives improvements in yield and efficiency of the cell harvest and clarification stages to generate a stream of Harvested Cell Culture Fluid (HCCF) for capture chromatography and downstream processing.

Experimental approach: In the present work, we report on a novel disruptive and scalable single-use technology for cell retention during perfusion cell culture based on an acoustophoretic separation technology.

Results and discussion: We investigate the continuous cell retention during a perfusion culture of a CHO cell line expressing a mAb. At process development scale, we continuously process CHO cell culture and retain cells at densities of up to 100 million cells/mL, at flow rates of up to 2 bioreactor volumes per day. Since the clarification technology does not involve the use of hollow fiber tangential flow filtration (TFF), we measured 100% transmission of the mAb through the AWS device for over 60 days without affecting the quality of the HCCF, the product itself, or the viability of the returning perfusion cell culture. The post-AWS HCCF is 99% clarified. Residual cellular material can be removed using a small gamma stable membrane filter or directly loaded onto a 0.2 micron filter prior to chromatography.

AWS technology enables the continuous cell retention from perfusion bioreactors in a single-use operation. AWS technology has been shown to perform well at cell densities of up to 100 million cells/mL. This novel cell retention approach offers economic benefits in terms of yield improvement by eliminating the hollow fiber TFF operation.

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DOE PROCESS OPTIMIZATION TO REDUCE IL-2 FRAGMENTATION

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Background and novelty: Interleukin-2 (IL-2) is a potent molecule in cancer therapy. Clinical application, however, is limited due to its strong side-effects during treatment. Roche Glycart AG has developed an IL-2 variant (IL-2v) immunocytokine to circumvent the drawbacks of the current IL-2 therapy. This molecule targets a tumor marker (TM) and, hence, realizes a local effect of IL-2v in TM bearing tumor tissue. However, during early process development for the TM-IL-2v molecule, antibodies with fragmented IL-2v moieties were detected. Initial experiments indicated that temperature, pH value, duration of fermentation, and inoculation cell density have an impact on fragmentation, but also affect the titer.

Experimental approach: To find specific process conditions to decrease fragmentation without decreasing the effective titer, a Design of Experiment (DoE) approach was used after a working hypothesis was formulated. An ambr15 was inoculated with different cell densities. Temperature and pH value were shifted after the growth phase on day 8. To assess optimal cultivation duration, samples were taken during the last days of the process.

Results and discussion: We could show that fragmentation increases with high temperature, high pH value, high start cell density, and later harvest. To optimize the effective titer, a multivariable linear regression model was generated using JMP software. We found that the optimal output can be obtained by inoculating with a higher cell density, shifting the temperature to a lower temperature and the pH value to a higher value. Additionally, the optimal day of harvest was predicted to be day 14. These findings were confirmed by cultivations in 2 L scale bioreactors showing a reduction of fragmentation by 66 % while keeping the effective titer comparable to the standard process. In summary, these findings will help to control fragmentation in CHO production processes of different IL-2v or IL-2 containing therapeutic proteins.

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BIOREACTOR PRODUCTION CONDITIONS ON GLYCOSYLATION

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Background and novelty: Investigation of the impact of process conditions on product quality of mAb produced in a GS CHO cell line and two hybridoma cell lines in different production systems.

Experimental approach: Different cell lines – one GS CHO and two hybridomas were used to produce different mAbs in different production systems to analyse for the changes in glycan and titer analyses.

Results and discussion: Results of analyses on metabolite consumption and antibody production indicated significant differences under the different process conditions. In a GS CHO cell line, production in stirred tank bioreactor with temperature shift and use of supplement yielded a peak mAb yield of 4g/L against the control run of 0.9g/L. Importantly, results indicated comparable N-linked glycan profile and binding kinetics in all the process conditions. Conversely, mAbs produced in the two hybridomas aside from the differences in production performances, results of protein quality assessment indicated consistent and significant differences in N-linked glycan profile. In general, significant increase in N-linked glycan site occupancies were observed for mAbs produced in stirred tank bioreactors and perfusion systems during production as compared to the traditional shake flask system.

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HIGH CELL DENSITY CLARIFICATION USING SINGLE-USE TECHNOLOGIES

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Background and novelty: Process intensification in biomanufacturing of therapeutic proteins enables increased productivities with simultaneous reduction of production volumes. Promising upstream scenarios are high cell density (HCD) cultivations where mammalian cell concentrations of more than 100 million cells/mL (> 30 % wet cell weight) have already been reached. This development increases the pressure on downstream processing to develop scalable, robust and cost-effective single-use (SU) based clarification solutions. In this study, different SU technologies, including a novel SU centrifuge system, were examined regarding their ability to harvest such processes.

Experimental approach: A HCD process for the production of an FDA-approved antibody provided a CHO cell concentration of at least 100 million cells/mL at harvest point. Clarification experiments of this cell suspension were performed on a 1-10 liter scale using depth filters, dynamic body feed filtration as well as a SU centrifuge system including testing of subsequent post-centrifugal filters. The approaches were characterized regarding their process performance based on processability, scalability, biomass reduction, turbidity, filter capacity and product yield. In addition, analyses were done to evaluate product quality, like DNA and host cell protein content.

Results and discussion: The tested filtration technologies showed limited applicability for HCD clarification due to their relatively low biomass loading capacities. However, SU centrifugation facilitates an almost complete removal of the biomass. An enhanced product yield was achieved by an integrated cyclic washing of the cell pellets. By implementing a post-centrifugal filtration a biomass free and sterile solution was obtained for further purification. This concept provides a promising approach for the development of a scalable and robust HCD clarification process solution.

P-509

HIGH ZINC SUPPLEMENTATION IN CHO CELLS INCREASES MAB AND EPO TITER

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Background and novelty: Biopharmaceutical production requires the use of serum-free (SF) media formulations, due to safety concerns associated with the use of animal-derived serum. To offset the loss of serum, many media additives have been tested with a goal of replicating bioreactor performance in serum-containing media. In this study, we aimed to investigate the effects of copper and zinc in modulating viable cell density (VCD), viability and titer effects in two producer CHO cell lines in an in-house chemically-defined media (CDM).

Experimental approach: CHO-DP12 (IgG-producer) and rCHO-K1 (SK15) (recombinant erythropoietin (EPO) producer) cell lines were adapted to two in-house CDM (CHO-91b-A and CHO-91b) supplemented with increasing concentrations of either zinc (1x, 10x, 15x, 25x and 30x) or copper (1x, 2.5x, 7.5x, 13.8x, 20x). VCD, viability and titer effects were analysed in triplicate 5ml suspension cultures. Supernatant samples were collected for IgG and EPO titer determination by ELISA assay. The Oxygen Consumption Rate (OCR) and Extracellular Acidification Rate (ECAR) levels were analysed using XF96 Seahorse.

Results and discussion: Supplementation with 25x Zn enhanced DP12 and SK15 titers in both media, displaying an 80% and 20% increase respectively, of IgG and EPO in CHO-91b-A, and 70% greater EPO and 2.6-fold greater IgG titers in CHO-91b. VCD profiles revealed a 24% – 31% lower peak in DP12 (vs both control media) and SK15 (vs $3.2x10^6$cells/ml in CHO-91b-A control) with viabilities above 80% observed throughout the culture. In contrast, exposure to copper at 13.8x and 20x resulted in cell line/product-type dependent effects. The energetic phenotype of both cell lines in 25x Zn-supplemented CHO-91b media revealed a 2-fold increase in OCR compared to non-supplemented cells. Together, these data suggest that high zinc supplementations in CDM may induce an increase in oxidative respiration metabolism that results in increased product titer in CHO cultures.

P-510

EFFECTS OF ALTERNATIVE SUGARS AND LACTATE ON THE GLYCOPROFILE OF IGG

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Background and novelty: The glycosylation profile of therapeutic monoclonal antibodies (mAbs) is a crucial quality parameter for industrial Immunoglobulin G (IgG) production. Several alternative carbon sources, which function as glycosylation precursors, have been reported to impact the glycosylation pattern. Since the cells give priority to glucose uptake, the presence of this substrate can lower the effects of alternative sugars on the glycosylation.

Experimental approach: In order to get a better understanding of the influence of alternative sugars on the glycosylation and to investigate how they impact each other, combinations of mannose, fructose, galactose and fucose were fed to Chinese hamster ovary (CHO) cells in batch culture when the glucose became depleted and the lactate, accumulated in the culture, was used as carbon source.

Results and discussion: Feeding with a feed containing mannose or glucose decreased by 3 to 7% the percentage of high mannose glycans compared to a feed without mannose or glucose. Feeding with a feed containing galactose led to 8 to 20% increase of monogalactoglycans (G1) glycans and 2 to 6% rise of digalactoglycans (G2) glycans compared to feeding without galactose or glucose. The cells fed with fucose exhibited a significantly higher concentration of intracellular GDP-Fucose. This work indicates that a feeding strategy based on non-glucose sugars and potentially lactate, could be adopted to obtain a targeted glycosylation profile.

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P-511

GREBA:A NOVEL MODEL FOR THE GLYCOSYLATION OF IGG PRODUCED BY CHO CELLS

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Background and novelty: The structure of N-linked glycosylation is a very important quality attribute for therapeutic monoclonal antibodies. Different carbon sources in cell cultures, such as mannose and galactose, have been reported to have different influences on the glycosylation patterns [1]. Accurate prediction and control of the glycosylation profile are important for the process development of mammalian cellcultures.

Experimental approach: In this study, a mathematical model, named Glycan Residues Balance Analysis (GReBA), was developed based on the concept of Elementary Flux Mode (EFM), and used to predict the glycosylation profile for the steady state cell cultures. Spintubes experiments were carried out in pseudo-perfusion cultivation of antibody producing Chinese Hamster Ovary (CHO) cells with various concentrations and combinations of glucose, mannose and galactose.

Results and discussion: Cultivation of CHO cells with mannose or the combinations of mannose and galactose resulted in decreased lactate and ammonium production, and more matured glycosylation patterns compared to the culture with glucose. Furthermore, the growth rate and IgG productivity were similar in all the conditions. When the cells were cultured with galactose, lactate was fed to be used as the complementary carbon source, leading to comparable cell growth rate and IgG productivity as feeding with the other sugars. The data of the glycoprofiles were used for training the model, and then to simulate the glycosylation changing with the concentration of mannose and galactose. In this study we showed that the GReBA model had a good predictive capacity of the N-linked glycosylation. The GReBA can be used as a guidance for development of glycoproteins cultivation process.

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P-512

MEDIA ADDITIVES AFFECT ANTIBODY QUALITY PROFILES IN PERFUSION CULTURE

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Background and novelty: G1/G0-phase cell cycle arrest in recombinant cell cultures is often associated with increase in protein specific productivity, but the effects on product and downstream process should be assessed because culture conditions could influence quality profile.

This work evaluated not only cell growth and productivity, but also quality attributes during initial stages of process development, to map molecule quality as early as possible. Previous reports to determine the effect of medium supplements on protein quality have focused on physicochemical properties but impact on biological activity is not usually investigated at this stage. This report also indicates that ligand–binding surrogate assays could serve as screening step to narrow media choices as critical variable from the very early preclinical development.

Experimental approach: A two factor complete factorial design in parallel cultures at 125 mL scale assessed the effect of G1/G0-phase cell cycle inhibitors (sodium acetate (NaAc) and a commercial feed (CF)) on productivity of an IgG4 recombinant CHO cell line. Protein A purified antibodies were characterized by gel filtration (GF) HPLC, SDS – PAGE and Western Blot. Antibody functionality was evaluated by kinetic interaction of samples for PD1 antigen using a ligand capture technique on Biacore system.

Results and discussion: Results indicated that the increase of both substances caused a decrease on viable cells density, cell growth rate, cell cycle arrest with a diminishing in S phase, but production rate wasn´t enhanced in any case.

Media additives induced aggregation of the monoclonal antibody detected both in SDS-PAGE and GF-HPLC. Monomer content diminishing to values between 66 and 83,07% was deeply increased in connection with the presence of CF on culture media, and this quality parameter had a significantly inverse correlation with antibody binding affinity to the ligand into a narrow nanomolar range (91,02 to 166,25 nM).

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EXPRESSION OF FULL-LENGTH SHARK-DERIVED ANTIBODY BY CHO CELL

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Background and novelty: Shark-derived immunoglobulin new antigen receptor (IgNAR) is gathering attention recently as a new antibody candidate. Unlike mammalian IgG, IgNAR antibody is a homodimer of heavy chains and are reported to be highly stable against urea. However, the structure of the full-length IgNAR (full IgNAR) has not been determined, because the artificially expressed constant domain C5 tends to be misfolded [1]. In this study, we attempted to generate IgNAR producing cell lines using Chinese hamster ovary (CHO) cells, which are widely used for the expression of IgG and purified it for structural analysis of produced IgNAR antibody.

Experimental approach: CHO-K1 cells, transiently transfected with IgNAR expression vectors were cultivated in BalanCD CHO Growth A medium. The expression vectors were designed to form C5 single domain and two types of modified IgNAR: full IgNAR including C5 single domain fused with the Human IgG Fc region and full IgNAR labeled with His-tag. The Fc-fused full IgNAR and C5 single domain were purified by Protein A. The His-tagged IgNAR was purified by Ni^{2+} resin batch purification. The expression of IgNAR was confirmed by Western Blotting and SDS-PAGE analysis.

Results and discussion: Clear single bands were observed in the result of SDS-PAGE for the Fc-fused full IgNAR and C5 single domain. Also, the band corresponding to molecular weight of IgNAR labeled with His-tag was observed in the result of Western Blotting for His-tagged IgNAR. However, other contaminated bands were observed in the result of Western Blotting for His-tagged IgNAR. It seems that Ni^{2+} resin batch purification was not effective for purification. In summary, we demonstrated for the first time that full-length IgNAR was expressed and purified using CHO cells as the host. We are attempting to obtain the full IgNAR using enzymatic cleavage and analyze the structure of these products.

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P-514

DISPOSABLE HARVEST STRATEGIES FOR TOMORROW'S INTENSIFIED PROCESSES

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Background and novelty: Despite the current trend towards perfusion processing, fed-batch and intensified fed-batch processes are expected to be around for some time to come. Large stainless steel facilities are usually quite well equipped to handle increasing biomass concentrations in the cell culture fluid as primary separation is done using disk-stack centrifuges. This is normally not the case in smaller single-use facilities with bioreactor volumes up to 2000 L, where depth filtration is the method of choice. One alternative to this could be disposable centrifugation to align the processes without introducing stainless steel equipment into single-use facilities.

Experimental approach: A variety of single-use systems have been evaluated to identify methods suitable for future harvest processing. These methods include single-use centrifugation, novel depth filtration membranes and hollow fiber membranes. Harvest efficiency was evaluated based on particulate reduction, host cell impurities and protein quality in the clarified cell culture fluid.

Results and discussion: The efficiency of the tested systems varies between vendors and clarification principles, and it is difficult to judge efficiency by principle alone. Primary clarification efficiency varies between 80% and 100%, with a tendency of more complex processes yielding higher efficiencies.

The data shows that most methods are applicable for intensified fed-batch harvest. One feature of a large fraction of these methods is the relative high cost of the disposables involved, especially when compared to standard depth filters. These higher costs tend to come with a potential benefit in product quality, and thus it should to be evaluated from case to case, whether the higher cost is justified for any given molecule.

P-515

CONTROL OF PROTEIN SIALYLATION IN CHO CELL CULTURE PROCESS

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Background and novelty: Glycosylation plays important roles in biopharmaceuticals. It is still challenging to control protein glycosylation in bioprocesses with limited understanding of the relationship between glycosylation and culture process. Here, we report researches on sialylation process in an antibody fusion protein-producing CHO cell process. Quantitative analysis was introduced for the first time to understand the effect of incomplete intracellular processing and extracellular degradation on the loss of protein sialylation (in N- and O-glycans) during culture. This study lays a solid foundation for better understanding and control of protein glycosylation during cell culture processes.

Experimental approach: Through cell-free incubation and quantitative calculations, the contributions of glycan extension and degradation process were evaluated. Then, the effects of individual medium supplements and process parameters on the two processes were explored. In the end, combinatorial process control methods were validated by combining the effectors of enhanced intracellular processing and decreased extracellular degradation to achieve a desired final protein sialylation level.

Results and discussion: Significant loss of sialic acids in the antibody fusion protein during the production phase was observed. The quantitative calculations showed the contribution of incomplete intracellular processing and extracellular degradation was almost 1:1. Intracellular processing was hampered because of limited nucleotide-sugars supply and down-regulation of some glycosyltransferases. Extracellular sialic acid degradation was caused by extracellular host cell sialidase. Then, hormones and glycan precursors were found to enhance both sialylation levels of N- and O-glycans in combination with process parameter optimization leading to a lower level of sialic acid degradation. Finally, an optimized cell culture process was established to keep consistent protein sialylation level during culture.

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CO2 ISSUES INDUCE METABOLIC DYSFUNCTION IN CHO CELL CULTURE PROCESSES

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Background and novelty: With the development of animal cell culture techniques, the adverse effects of CO_2 in large-scale high-density cultivation have gained increasing attention. Operational strategies have been adopted to ease such undesired impacts on cell culture processes. However, intrinsic effects of high or low CO_2 partial pressure (pCO_2) on cells remain unclear, causing limitations in rational design of CO_2 control strategies to meet Quality by Design(QbD) practices. Therefore, this work, for the first time, revealed the mechanism of CO_2 affecting cell growth, productivity and metabolism, laying the groundwork for the design of robust and stable processes.

Experimental approach: The characteristics of CHO cell growth, metabolism and protein production was investigated under different pCO_2 conditions. Details of intracellular status were unveiled by transcriptome and metabolome analysis using RNA-sequencing and LC-MS/MS, combined with key enzyme activity detections. Moreover, experiments on the supplement of intermediate metabolites were performed to examine the compensational effects on the metabolic deficiency induced by CO_2.

Results and discussion: The results indicated that insufficient central carbon metabolism was triggered by either high or low pCO_2 conditions. For high pCO_2 the CHO cell mitochondrial functions were hammered due to the product inhibition of isocitrate dehydrogenase. As for low pCO_2 conditions, we discovered that the supply of intracellular pyruvate was inhibited by the expression of lactate dehydrogenase A and the increase of the intracellular pH in the CO_2 insufficient environment, hence the decreased flux of the TCA cycle. Furthermore, strategies of key intermediate supplement were successfully applied to relieve the detrimental effects of CO_2. This study provides new insights into the relation between pCO_2 and metabolic responses of CHO cell lines, which will benefit the establishment of robust and stable processes.

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ACCOUNTING ENZYME REGULATION IN PROTEIN GLYCOSYLATION MODELS

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Background and novelty: Quality of pharmaceutical proteins, including monoclonal antibodies (mAbs), is greatly influenced by N-linked glycosylation. While several experimental strategies have been implemented to control protein glycosylation, mathematical models have been also widely used to describe and, ultimately, control the produced glycofrom *in silico*. Among mathematical models, kinetic models, although often detailed and mechanistic, are usually limited to specific operating conditions because of the inability to predict *a priori* regulation over enzyme expression in response to changes in the cellular environment. In the present work, a refined holistic modelling framework describing (i) Chinese hamster ovary (CHO) cell metabolism, (ii) Nucleotide Sugar Donor synthesis and (iii) N-linked glycosylation is proposed, in order to account for enzyme regulation events and improve model predictions.

Experimental approach: A previously developed software (GLYMMER) is used to estimate the concentration of glycosylation enzymes that result from different galactose and uridine feeding strategies in fed-batch CHO cell cultures. The obtained enzyme profile data is then used to refine the model.

Results and discussion: The modelling framework includes a refined glycosylation model that describes both the ER and Golgi apparatus, introducing in this way the variability of the initial glycan structures to the processing steps in the Golgi. The model uses five continuous stirred tank reactors to describe and segregate the reactions, including four ER-localized and thirteen Golgi-localized enzymes. In addition, the glycosylation model can be easily adapted to broader reaction networks yielding highly complex glycan structures that have been reported to exist on certain host cell proteins by automatically generating the reaction network and minimizing the simulation time by 90%.

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COMPARISON OF ENZYMATIC IGG DEFUCOSYLATION IN SOLID AND LIQUID PHASE

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Background and novelty: Non-fucosylated glycoforms of IgGs exhibit dramatically enhanced ADCC and therapeutic efficacy[1]. Approaches such as cell engineering and liquid phase enzymatic modification have been explored as an endeavour to obtain non-fucosylated antibodies. However, the industrially applicable production of therapeutic antibodies fully lacking core fucose is still a major challenge. We present a method of cleaving N-glycan fucose enzymatically while antibody is trapped to a Protein A column during downstream step. This method is promising compared to liquid phase modification because it enables easier access of fucosidase to core fucose and eliminates the need for intermediate purification.

Experimental approach: A high yield of rituximab was produced by a novel cumate-inducible CHO cell platform[2] cultivated in shake flask fed-batch mode using BioGro media. Rituximab was purified by affinity chromatography. Unmodified rituximab glycan was cleaved enzymatically in solid phase and labelled according to protocol [3]. Intact IgG was loaded in either a 2mL Eppendorf tube or a protein A column and incubated overnight at 37°C with fucosidase, glycobuffer and water. Using the same protocol [3] glycan was released by PNGase F and labelled with 2-AB solution. Rituximab glycans of unmodified, defucosylated in solid phase and in liquid phase were analized on an Acquity UPLC (Waters), Empower software was used to calculate the GU values for each peak and assign structure based on reference GU values from Glycobase.

Results and discussion: After 17 days of cell culture 587 mg/L of Rituximab was obtained. The glycan profile of fucosidase treated rituximab was dramatically different from that of the unmodified control, suggesting the defucosylation of IgG using solid-phase approach was successful. Results of further optimization of the process will be presented.

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P-519

CFD MODELLING FOR CHARACTERIZATION OF GROWTH AND PRODUCTION PARAMETERS

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Background and novelty: The linkage between hydrodynamics, cell growth and antibody productivity is not fully understood[1]. Computational fluid dynamics (CFD) methods are an established approach for the determination of local and averaged hydrodynamical parameters in cell culture processes. Results from CFD modelling can only predict the hydrodynamical behavior of a system. Hence, they are coupled with cultivation experiments showing the response of a cell line such as changing productivity or glycosylation. By combining simulation and targeted experiments as a process development strategy, a deeper understanding of the process is generated, leading to a high potential of the proposed approach.

Experimental approach: As a proof-of-concept firstly the fluid flow in a lab-scale bioreactor was modelled. Cultivations of IL-8 antibody producing CHO DP-12 cells were performed at different volumetric power inputs. Cell growth was monitored as well as viability, substrate consumption, metabolite and product formation. Statistical correlations between hydrodynamics and growth or production parameters were made. Drawn conclusions will be transferred to a scaled-up or scaled-down reactor, as the latter allows a higher experimental throughput. Further, the influence of the energy distribution in cultivation systems on the cells shall be investigated to gain a deeper understanding of the link between hydrodynamics and biological phenomena.

Results and discussion: CFD models of the stirred tank reactor Medorex Vario 1000 and orbitally shaken cultivation systems, namely baffled shake flasks and deep well plates were evaluated. An established CHO cell process was routinely run at an averaged energy dissipation rate of 2 W m-3. The results showed an optimum concerning growth and antibody productivity between 15 W m-3 and 106 W m-3. This revealed that the cell line is more robust than expected since cell growth and viability did not decrease up to a power input of 198 W m-3.

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P-520

DESIGN OF EXPANSION PROCESSES USING A COMPUTER-AIDED METHOD

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Background and novelty: Rising demands of biopharmaceuticals and the need to reduce costs increase the pressure to develop productive and efficient processes. At present, Design of Experiments (DoE) methods are used, which can result in a large number of time-consuming and costly experiments. Furthermore, heuristic limitations of boundaries and the high number of factors as well as the subjective determination of responses and factors lead to stepwise iterations with several runs. In this study, a combination of mathematical models with DoE was used to reduce the experimental effort by statistically planning and simulating of experiments.

Experimental approach: A mathematical process model can be used to describe the growth, the substrate (e.g. glutamine and glucose) and metabolite (e.g. ammonia and lactate) concentrations as well as the productivity (e.g. antibody) of the specific cell line. Therefore, the model is adapted to first cultivation data, e.g. based on literature and existing knowledge. The model is then used to simulate responses so that the experimental space can be reduced as well as different experimental designs can be compared regarding their suitability for the given purpose. Different boundary conditions, factor and response combinations as well as uncertainties can be tested. This offers a new approach, which provides new insights in the process development.

Results and discussion: This method is exemplary shown for the optimization of a fed-batch process, e.g. feed concentration, start point of feeding and feeding rate, for antibody producing Chinese Hamster Ovary cells and the process design of a Mouse Fibroblast cell. Based on a few experiments the experimental space of experimental designs can be reduced using the mathematical model. The stepwise iteration with several runs can be performed using simulations, which make the process more and more knowledge-based. This leads to a reduction in time and costs required for the development of bioprocesses.

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BIOACTIVE COMPONENTS FROM HYDROLYSATES FOR MEDIA SUPPLEMENTS

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Background and novelty: There is a need for a diversity of chemically defined media (CDM) formulations required for different cell lines or for different modes of culture. Active components of these formulations can be identified from the growth promoting properties of protein hydrolysates from plant and microbial sources. Media has traditionally been supplemented with chemically undefined sources of bovine serum or hydrolysates, which are a source of hormones, growth factors, and trace elements. These components show variability between batches and also presents a high risk for product contamination. Hydrolysates are an alternative to bovine serum for supplementation, although they are not chemically defined. It has been shown that compounds isolated from hydrolysates are responsible for increased cell growth. The isolation and identification of these compounds can lead to the development of a CDM, which would reduce batch variability and eliminate product contamination.

Experimental approach: This work characterised compounds found in protein hydrolysates using a variety of techniques: molecular weight cut off filters (50 kDa, 10 kDa, 3 kDa), strong anion and cation exchange columns, trace metal analysis, peptide and amino acid analysis. Fractions generated by MWCO filters and ion exchange were monitored by HPLC-UV. Trace metal analysis was performed using ICP-MS. Peptide and amino acid analysis was performed by HPLC-QTOF.

Results and discussion: MWCO filters showed that the complexity of the chromatograms resulted from compounds with a molecular weight of less than 3 kDa. Sub-fractionation of the 3 kDa filtrate by anion and cation exchange columns determined that compounds present in hydrolysates interacted with the stationary phase to varying degrees. The amino acid sequence of peptides was determined for each detected peptide. Analysis by ICP-MS resulted in significant differences in metal content between hydrolysates which could impact cell growth and productivity.

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UNDERSTANDING CHO CELL CULTURE PROGRESSION THROUGH PHOSPHOPROTEOMICS

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Background and novelty: Antibodies have continued to dominate biopharmaceutical product approvals, and 85% (57 out of 68 mAB) are expressed using a Chinese hamster ovary (CHO) cell-based system [1]. Growth control strategies are often used to prolong culture duration and increase specific productivity; however, the regulatory pathways underlying growth strategies are poorly understood in CHO cells. Protein modifications such as phosphorylation are dynamic and respond to changes in culture conditions; this may reflect the status of the cells with respect to growth and viability of the culture. Little work has been carried out on the phosphoproteomic characterization of CHO cells in bioprocess-relevant conditions.

Experimental approach: In this study, we performed phosphopeptide enrichment in conjunction with LC-MS/MS to carry out a large-scale differential phosphoproteomic and proteomic analysis of IgG producing CHO DP12 cells at various phases of growth in serum-free suspension batch culture to characterize changes to the phosphoproteome with changing culture conditions.

Results and discussion: At various growth phases, 3777 differentially expressed phosphopeptides are identified from 1415 phosphoproteins. Total proteomic data revealed the differential expression of 834 proteins, with an overlap of 188 proteins between the proteomic and phosphoproteomic analyses [2]. Phosphoproteomic data improves proteome coverage and gives insights into the post-translational level of regulation during cellular growth of CHO cells.

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P-523

CHANGES IN FATTY ACID CONTENT OF CHO CELLS SWITCHING GROWTH CONDITIONS

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Background and novelty: Very little attention has been given to lipidomics in CHO cells despite the importance of lipids in many aspects of cell structure and function, including secretory activity. Indeed, their involvement in recombinant protein production systems is crucial to energy metabolism, vesicular transport, membrane structure, dynamics, and signalling. In this study a combination of lipidomics, transcriptomics and proteomics analysis were performed in order to investigate the impact of different culture conditions on CHO cells.

Experimental approach: Two cells lines were chosen, CHO-K1 and CHO-DG44. CHO-K1 was cultured in adherent culture for several passages and then moved to suspension culture. The reverse was applied to CHO-DG44, i.e. starting in suspension culture and moving to adherent. Lipid profiling was carried out by Gas Chromatography (GC) analysis on the corresponding fatty acid methyl esters extracted. Additionally, proteomics and transcriptomic analysis were performed to identify protein and genes differentially expressed between the different growth conditions and the associated pathways.

Results and discussion: The lipid profile, measured as the ratio between polyunsaturated fatty acids (PUFA), monounsaturated fatty acids (MUFA) and saturated fatty acids (SFA), revealed a fast remodelling with replacement of almost the whole PUFA fraction with MUFA when moved from adherent to suspension culture format with the reverse pattern in the opposite conditions. The SFA fraction was stable in both conditions before and after the culture transition. Furthermore, the full proteome combined with the transcriptomic data provide more accurate understanding of the possible relationship between different growth environments, cell membrane arrangement and lipid metabolism.

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DE-RISKING SCALE UP THROUGH EFFECTIVE BIOREACTOR CHARACTERISATION

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Background and novelty: Scale up and tech transfer of cell culture processes has many inherent challenges. How the bioreactor is designed and operated influences the environment to which the cells are exposed which can potentially influence the performance and product quality profile of the culture.

At UCB, a range of bioreactors are used across different sites and scales of operation for process development and for the production of clinical and commercial material. Detailed understanding of these bioreactors was needed to de-risk scale up and tech transfer variability between sites.

Experimental approach: Bioreactors were characterised through a combination of methods. A detailed geometrical analysis of each bioreactor allowed the calculation of key engineering parameters as defined from literature. In addition, a number of empirical tests were completed to map characteristics such as kLa and mixing time. Furthermore, modelling approaches were employed to better understand the fluid dynamics of each system.

Results and discussion: The bioreactor characterisation has supported the tech transfer of cell culture processes to 15kl scale through improved understanding which can be used to guide the selection of operating parameter ranges to de-risk process variability and to aid comparability studies.

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INTENSIFICATION OF A PLATFORM FED-BATCH PROCESS- A CDMO PERSPECTIVE

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Background and novelty: In recent years CHO-based biopharmaceutical mAb process designs have converged and today rely on similar infrastructures and protocols. Typically, cells are expanded in a series of batch cultivations in shake flasks, rocking-motion and stirred-tank bioreactors to generate sufficient cell counts for the inoculation of a production bioreactor. Production bioreactors are usually operated in fed-batch mode employing basal media complemented by static addition of feed solution(s). Respective processes commonly reach a maximum of 15-25 $MVCmL^{-1}$ and 3-5 gL^{-1} of mAb in 12-14 days [1,2,3]. Lately, increasing demand for biopharmaceuticals has led to the advent of new intensified process designs [1]. These intensified processes enable higher VCCs yielding mAb titers beyond 5 gL^{-1} in 8-12 days but often integrate new technologies that require changes to the existing manufacturing infrastructure and thus may be difficult to implement [1,2,3]. This work demonstrates intensification of a CHO-based mAb process leveraging existing fed-batch manufacturing facilities by implementing an intensified seed train and a dynamic feeding strategy based on commercial fed-batch media and feed solutions.

Experimental approach: First, an intensified seed train was established involving HD cell banks and a rocking-motion bioreactor. Cells were grown in perfusion mode utilizing a custom-made perfusion medium based on a blend of commercial fed-batch medium and feed solutions. Cells inoculated an intensified fed-batch process with 1.2 $MVCmL^{-1}$. Continuous and dynamic cell-specific feeding was employed to support optimal cell growth and productivities.

Results and discussion: The intensified seed train made use of HD cell banks and reached up to 145 $MVCmL^{-1}$ in 10 days for direct inoculation of a 1-3 m^3 production bioreactor. The intensified fed-batch process reached respective mAb titers 3-4 days earlier. Concluding, time from vial to drug substance could effectively be reduced by 40%.

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Abbreviations:

HO: Chinese hamster ovary
HD: high-density
mAb: Monoclonal antibody
$MVCmL^{-1}$: Million viable cells per mL
VCC: Viable cell concentration

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AUTOMATION AND DIGITALIZATION IN CELL LINE AND UPSTREAM DEVELOPMENT

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Background and novelty: Nowadays, automated fermentation systems such as ambr®15 and ambr®250 (Sartorius) increase the throughput of cell line and upstream process development. Consequently, more in process control samples need to be analyzed and more data need to be evaluated.

Experimental approach: We established high throughput ambr15/ambr250 core facilities with automated sample processing and analytics via a Fluent® pipetting robot (Tecan) and the Cedex Bio HT Analyzer (Roche Diagnostics), and last but not least an automated data processing that integrates all devices.

Results and discussion: We use ambr15 for early clone selection or process development due to its higher throughput. In addition, we use the ambr250 system for the final clone selection process and to validate results obtained by the ambr15, since ambr250 showed a better fit to larger scale bioreactors. For both systems core facilities were built to harbor automation of fermentations, sample processing and analytics in close proximity. Therein, sample plates from ambr systems are manually transferred to Tecan Fluent pipetting robots that prepare cell-free supernatants by centrifugation and distribute the samples directly into racks and tubes for the subsequent analysis. Thus, 48 samples are processed in 30 min while the operator has to spend only 5 min instead of 60 min for manual processing. Moreover, all devices are integrated into an IT system using the software Sm@rtLine Data Cockpit (AGU) to enable the complete sample tracking and initiation of analytical measurements as well as an automated data feedback loop to the ambr systems. Thus, no manual handling of sample IDs or data files are required to consolidate all information in our data warehouse.

Hence, we are now able to run about three times more cultivations with the same manpower and were able to demonstrate that small-scale bioreactors are more predictive to identify cell clones with high titer and high product quality.

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VARIABILITY IN TRACE METAL LEVELS AND ITS IMPACT ON PRODUCT QUALITY

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Background and novelty: Despite the improvements seen in the upstream process from shifting to chemically defined cell culture media and feed, we are still faced with challenges when it comes to impact of low trace metal levels on the upstream process. Variability in product quality especially during early technology transfer across labs or scale up were found to be caused in some cases by changes of certain trace elements. Changes at Parts per billion levels can impact different product quality attribute, thus the need for significant investigation to better understand the impact of single element as well as the interaction between different elements on recombinant protein product quality.

Experimental approach: The process we looked at uses Chinese hamster ovary (CHO) cells in a bi-phasic fed-batch process. Samples from the process at different stages showed variability in product quality with changes in trace elements concentrations with some of these elements not regularly analysed for their low possible impact on the process. In this study we tested the impact of specific element (Cobalt) and its interaction with other metals in relation to impact on product quality.

Results and discussion: The results provide better understanding of variability introduced by not commonly analysed trace elements and the possibility of identifying concertation ranges that impact product quality.

P-528

DEVELOPING A GLUCOSE-LIMITED GALACTOSE-SUPPLEMENTED FED-BATCH STRATEGY

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Background and novelty: In cell culture production processes the lactate concentration is commonly monitored since high levels of lactate have a clear negative impact on cell growth and productivity. Therefore, reduction of lactate accumulation and/or induction of lactate consumption is a matter of particular interest.

In this work, in order to decrease the production of lactate, the availability of glucose was limited by continous feeding in a controlled bioreactor system (1 L scale, DASGIP system). As a result, the cell growth and productivity of a monoclonal antibody increased significantly. Even though the cell concentration was higher under glucose limitation, the viability of the cells decreased earlier indicating cellular stress under these conditions.

In order to negotiate this shortcoming the culture medium was supplemented with galactose as an alternative carbon source. Thus, a galactose-supplemented, glucose-limited fed-batch strategy was developed and succesfully tested.

Experimental approach: CHO cell cultures were cultivated under glucose limitation by continuos feeding using the EXCELL Advanced CHO Feed 1 (SAFC) . Moreover, the Fraunhofer in-house culture medium was supplemented with galactose to maintain the energy supply of the culture when glucose (and lactate) were depleted.

The glucose limitation was realized by daily calculating the glucose consumption of the cultures in retrospect for the previous 24 h and then adjust the continous feeding rate for the next 24 h.

Results and discussion: The use of galactose as a equivalent of the energy supply in glucose limited cultures resulted in a prolonged growth and a higher viability aswell as in a higher productivity and product titer, which was elevated by 32 %.

Thus, a process was developed synergizing the benefits of a glucose-limited cultivation with the assets of a cultivation with reliable energy supply.

The study improved the understanding and the predictability of the glucose-limited culture process.

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DEMONSTRATING PROCESS SCALABILITY WITH A COMPLETE UPSTREAM PLATFORM

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Background and novelty: Recombinant cell line development and scale-up of small scale processes for production are time-consuming and resource-intensive steps in Biotherapeutic production process. Along with achieving and maintaining multi gram/L titers throughout this process, it is further expected to maintain consistent protein quality attributes. Utilizing an optimized platform of materials (DNA vector, cGMP-banked cells, cGMP cell culture media and a scalable set of bioreactors) and protocols will improve the success rate to meet these challenges while reducing resource requirements and timelines.

Experimental approach: We utilized the CHOZN® GS cell line, to rapidly isolate highly productive and stable recombinant clones. Next, we evaluated a recombinant clone for protein production using the EX-CELL® Advanced™ cell culture media platform and optimized the feed strategy at small scale. Then, we introduced a recombinant clone into 3L Mobius® single-use bioreactors and developed a consistent and robust process. Finally, we scaled that process to 50 L Mobius® single-use bioreactors.

Results and discussion: We observed consistent process performance across the 3L and 50L bioreactor scales in regard to cell growth, titer, and product quality. Bioreactor titers of >2 g/L were achieved, which is comparable to titers observed in shake flasks. Product quality analyses further supported the scalability between bioreactor sizes, specifically the similarity in glycan profiles. This platform has since been utilized for a different recombinant clone and relatively high titers were achieved without any further process development. These combined results demonstrate the value of a commercially available, turn-key platform for production of Biotherapeutic proteins.

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EVALUATION OF CELL CULTURE PROCESS ROBUSTNESS AND CELL LINE STABILITY

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Background and novelty: Robustness, defined as the ability of a process to tolerate changes and variability of materials, process and equipment without negative impact on quality, is a desirable feature of any cell culture manufacturing process.

The objective of this study was to investigate the effect of perfusion rate and glucose concentration in the cell culture medium for the production process of a highly glycosylated protein expressed in a recombinant CHO cell line.

Experimental approach: Cell growth, productivity, downstream processing performance and quality of active pharmaceutical ingredient batches were measured for each experimental condition defined using a factorial 2^2 experimental design. Controlled bioreactor cultures were established in 5 l bioreactors in perfusion mode. Daily samples were taken and after each run a four-step downstream process was carried out for obtaining the pharmaceutical grade recombinant protein.

Results and discussion: Perfusion rate and glucose in cell culture medium did not affect product quality in all the design of space since protein glycosylation, potency, host cell proteins and oligomers contents showed no statistically significant differences. However, maximum cell density, growth rate and productivity were significantly affected by changes in those operative parameters.

These studies prove that the design of experiments applied to cell culture processes has the potential to demonstrate robustness and significantly increase productivity through process optimization, with assurance of product quality consistency.

Finally, since it is essential that the productivity of the expression system remains stable throughout culture expansion for the successful long-term production, the stability of the mentioned highly glycosylated protein producer clone was evaluated after repeated batch culture in shake flasks. Cell growth and viability, productivity of the clone and product quality remained consistent throughout 80 generations.

P-531

SCALE-UP AND SCALE-DOWN OF FED-BATCH CELL CULTURE PROCESS: CASE STUDY

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Background and novelty: Scale-up of a cell culture process is seldom straightforward, more so when scaling up for the first time to bioreactors with higher aspect ratio. The recipe for a successful scale-up includes a thorough understanding of the cell culture process, manufacturability of the process, well characterized bioreactors and efficient utilization of the available scale-up principles. In this case study, we review the scale-up from 5L scale as well as the scale-down approach for a fed-batch process for the manufacturing of a biosimilar mAb.

Experimental approach: For determining the optimal aeration and agitation strategies for the 2000 L bioreactor, several parameters such as tip speed, kLa, power per unit volume and oxygen transfer rate were considered. Based on the process knowledge and the available bioreactor characterization data, a combination of OTR, kLa and P/V were finally used.

Since the next step was process characterization, the need for a reliable scale-down model was paramount. While reviewing historical development data, it was noticed that a lower agitation speed, and consequent higher sparge was enhancing the titer productivity at 5L scale. Contrary to the available literature which indicates that the damage to cells from sparge is substantial compared to agitation, we found that the cells preferred lower agitation at the cost of twice the sparge rates!

Results and discussion:

– The scale-up from bench scale resulted in moderately higher cell density and 35% increase in antibody productivity while not affecting the harvest viability and product quality attributes.

– The antibody titer productivity for batches run with lower agitation was higher by 28% compared to the control batches. The product quality as measured by charge variants and glycan distribution was unaffected due to the changes.

– Scale-down model shall be qualified after completion of additional experiments confirming the impact of lower agitation on productivity.

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A CHO HIGH CELL DENSITY PERFUSION PROCESS WITH IMPROVED GLYCAN PROFILE

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Background and novelty: Perfusion methodology has caught industrial interest due to new technology and the advantages of integrated bioprocess. Together with the possibility of operating at high cell density, enhanced product quality and increased productivity, the combined benefits can offer lower cost of goods. Higher productivity is dependent on high density and extended period of stable continuous harvest. A key aspect is to have consistent quality profile during the intended harvest time to avoid the need to pool the material. In a fed-batch process the specific productivity and the quality profile can vary heavily during the production time. This is not a problem if the product quality in final harvest is reproducible.

Experimental approach: In this study a high cell density perfusion process with alternating tangential flow as cell retention device was developed for a difficult-to-express therapeutic enzyme with multiple glycosylation sites. The approach was to keep as many parameters similar to a fed-batch process adapted for the same target protein.

Results and discussion: The novel perfusion process resulted in stable glycan profile with increased levels of higher order glycoforms during 10 days of steady state at high cell density of 100 million cells/mL. The process yielded increased sialylation and decreased G0F and G1F glycoforms, which can be beneficial for therapeutic proteins. The product specific activity and other key quality attributes were comparable to the results obtained in fed-batch. Interestingly, the cell specific productivity that varied in the fed-batch process did so also in the perfusion setting until the very end of the steady state phase when it was stabilized. Transcriptomic investigation will be performed to further investigate the potential of higher productivity.

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HIV ENVELOPE GLYCOPROTEINS AS IMMUNOGENS – EXPRESSION AND MANUFACTURE

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Background and novelty: HIV-1 envelope glycoproteins (Env) used as vaccine anitgens are among the most promising strategies fighting HIV/AIDS. Given the complexity of the HIV-1 Env homotrimer, manufacturing of these proteins remains challenging. In the framework of the European Aids Vaccine Initiative (EAVI2020) this project aims to define strategies for the efficient and feasable manufacture of different HIV-1 Env variants as prospective vaccine candidates.

Experimental approach: Stable CHO cell clones co-expressing variants of HIV-1 Env and the human furin pro-protein convertase (mediates efficient cleavage of the Env precursor), were generated by BAC vector technology [1]. CHO cell clones were evaluated for their trimer specific productivity by ELISA based on binding to the structural epitope specific anti-HIV mAb PGT145 and for their applicability in larger scale production based on small and mid-scale processes. Affinity purified Env trimers (by mAb PGT145) were characterized based on various PAGE techniques, western blot and SE-HPLC. Additionally, the trimers' glycosylation status as well as native-like appearance by negative-stain EM was determined.

Results and discussion: Characterisation of large scale manufactured and affinity purified Env proteins was performed by BN-PAGE, SDS PAGE and Western blot. SE-HPLC analysis revealed only minor fractions of non-trimeric forms and negative-stain EM imaging as well as glycan analysis confirmed the expected native-like appearance of Env preparations. Hence, our findings strongly support the idea of a fast but still flexible strategy to manufacture HIV-1 immunogens in CHO cells. Ongoing experimental work will put a focus on a better understanding of the intracellular processing (e.g. co-expression of human furin) of HIV-1 Env under conditions of high heterologous protein expression.

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NEW CELL CULTURE MEDIUM COMPONENT TO REDUCE PRODUCT MICROHETEROGENEITY

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Background and novelty: To provide high-quality product to patients, product variants have to be reduced during the bioprocess development. However the control of the microheterogeneity can be detrimental to the process yields. During the development of an IgG production process, it has been demonstrated that a reduction of cysteine is helpful to reduce charge variants. Unfortunately this reduction alone also leads to a titer reduction.

Experimental approach: In this context, alternative molecules performances have been assessed to recover the primary titer in Ambr15 and in 2L bioreactors. A further analysis to gain a deeper understanding by measurement of intracellular metabolites such as glutathione, has been performed.

Results and discussion: We have run four cultures, and based on these experiments, a new cell culture medium component has been identified. This novel component will be revealed in the presentation. The component allows a reduction of charge variants and IgG coloration without significant titer decrease at the tested concentration. The close relationship of this component with oxidative stress can also partially explain its mechanism of action.

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IMPACT OF SINGLE-USE BIOREACTOR AERATION ON CELL CULTURE PERFORMANCE

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Background and novelty: Single-Use Bioreactors (SUBs) have become a widely used tool for the development and manufacture of biopharmaceutical products. Developing manufacturing processes in SUBs early on, can enhance manufacturing opportunities for late stage and commercial production. However, SUBs usually have different design characteristics compared to Stainless-Steel Bioreactor (SSB) which can pose a challenge for process transfer from one to the other system. The oxygen mass transfer coefficient (kLa) can be lower in SUBs. This low kLa can lead to a lack of oxygenation for high cell density processes. To overcome this, suppliers introduced microspargers of various shapes and sizes.

In this study, we assess the differences in cell culture performance between SUBs and SSBs systems and show how the overall performance could be improved by optimizing the SUBs aeration strategy.

Experimental approach: CHO cells producing monoclonal antibodies were cultivated in chemically define media, using identical overall culturing parameters (such as consistent temperature, pH and nutrient). For the production stage, 50L or 200L SUBs were used with different sparger systems (1mm, 0.5mm or 20µm for the SUB) and 80L SSB with a 1mm ring-sparger. In-Process Monitoring was performed daily to assess various performance indicators such as growth and productivity. Dissolved oxygen levels were controlled by using a multi-stage aeration cascade and by agitation speed adjustment during the process.

Results and discussion: We showed that the sparger size can have a negative impact on performance, likely due to both bubble size and gas velocity. This was improved by changing the aeration cascade and/or sparger type. Overall, similar performance between SUBs and SSBs can be achieved, however, depending on sensitivity of the producing cell line, aeration strategy optimization might be required.

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DRY COMPACTION OF SINGLE CHEMICALS AND CELL CULTURE MEDIA

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Background and novelty: Bulk powders like cell culture media (CCM) or single chemicals show physical disadvantages. CCM powders with fine particles show high dust formation and poor flowability. In addition, dissolution is time consuming due to floating of light particles on the water surface. For some single chemicals, appropriate handling is often impeded due to caking of the bulk material. Strong mechanical forces are needed to break up the material. In many cases, these limitations of powders can be overcome by granulated material.

Experimental approach: Bulk powders were dry-granulated by roller compaction. Stability and particle size of granules were analyzed. Flowability, bulk and tapped densities, dissolution kinetics and degree of caking were compared between bulk and granules. Stability studies at ambient and accelerated conditions (temperature and humidity) were performed to assess the ability of granules to reduce the degree of caking.

Results and discussion: Compaction of single chemicals like salts or buffers resulted in granules with high stability and low amounts of small particles. Granules resulted in improved flowability and reduced caking compared to the bulk material. Stability studies at ambient conditions showed that powders formed solid monoblocs whereas granules kept free flowing without critical caking issues up to nine months. Dissolution times between granules and bulk material were similar.

Granules of CCM or feeds showed reduced dissolution times of up to 4.4-fold compared to powder. In addition, dust formation during handling of CCM was tremendously minimized, increasing safety for employees and decreasing contamination risk and cleaning costs. Better handling of granules (Carr Indices 10-16) was achieved due to improved flowability compared to powder (Carr Indices 25-35). Moreover, storage and transport costs can be reduced by granules due to bulk volume reduction up to 40%.

In conclusion, granules may help to improve upstream and downstream processes.

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BIP INDUCER X: AN ER STRESS INHIBITOR FOR ENHANCING MAB PRODUCTION

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Background and novelty: Endoplasmic reticulum (ER), a central organelle of the secretory pathways, is responsible for regulating protein translocation, protein synthesis, and early post-translational modifications, including folding and glycosylation. Chemical additives such as dimethyl sulfoxide (DMSO) and sodium butyrate (NaBu) have been widely used for enhancing monoclonal antibody (mAb) production in recombinant Chinese hamster ovary (rCHO) cell cultures. It increases specific productivity of target proteins but simultaneously causes ER stress and apoptotic cell death in a dose-dependent manner. To cope with ER stress and cell death induced by ER stress, it is necessary to identify new media additive that can reduce ER stress.

Experimental approach: To find an effective ER stress inhibitor for rCHO cell cultures, three different ER stress inhibitors, including Bip inducer X (BIX), were evaluated as chemical supplements for rCHO cells producing mAb. Of these, BIX showed the best effect on culture performance, and was added to rCHO cell cultures under ER stress induced by DMSO addition. The ER stress level and UPR levels were measured to understand the effect of BIX on mAb production and quality. Finally, BIX was evaluated as a chemical additive in fed-batch cultures under ER stress induced by DMSO addition.

Results and discussion: ER stress was induced in rCHO cell culture, especially with the addition of q_{mAb}–enhancing chemicals such as DMSO and NaBu, which negatively affected cell growth and mAb production in rCHO cell cultures. Among the three ER stress inhibitors tested in this study, BIX showed the best mAb production performance in rCHO cell cultures. Co-addition of BIX and DMSO significantly enhanced mAb production while increasing the galactosylated form of the mAb. Thus, BIX is an effective ER stress inhibitor for use in rCHO cell cultures for improved mAb production.

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IMPACT OF ACETYLATED AND NON-ACETYLATED FUCOSE ANALOGUES

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Background and novelty: The biological activity of therapeutic antibodies is highly influenced by their glycosylation profile. A valuable method for increasing the cytotoxic efficacy of antibodies, is the reduction of core fucosylation, as this enhances the elimination of target cells through antibody-dependent cell-mediated cytotoxicity. Development of fucose analogues is currently the most promising strategy to reduce core fucosylation without cell line engineering. Since peracetylated sugars display enhanced cell permeability over the highly polar free hydroxy sugars, this work sought to compare the efficacy of peracetylated sugars to their unprotected forms.

Experimental approach: Two potent fucose analogues, 2-deoxy-2-fluorofucose and 5-alkynylfucose, and their acetylated forms were compared for their effects on fucosylation. Incorporation of the fucose analogues was confirmed through mass spectrometry data.

Results and discussion: 5-alkynylfucose proved to be more potent than 2-deoxy-2-fluorofucose at reducing core fucosylation but was associated with a significant higher incorporation of the alkynylated fucose analogue. Acetylation of the sugar yielded only slightly lower fucosylation levels suggesting that acetylation has a minor impact on cellular entry. The general low impact of acetylation may be explained by differences in the mechanism of cellular transport when comparing fucose and other carbohydrates. In the literature, acetylation of ManNAc and a glucosamine derivative was reported to enhance the passive diffusion. In contrast, fucose as well as the analogues may be transported in cells via a transporter. The sodium/myo-inositol transporter is expressed in CHO cells and was reported to transport L-Fucose due to the high structural similarity to myo-inositol. The hypothesis that fucose derivatives can be recognized by the transporter is further supported by the fact that fucose analogues are also recognized by other key enzymes in the fucosylation pathway.

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CHO BASED ANTIBODY PRODUCTION IN 200L CUBICAL STIRRED SU BIOREACTOR

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Background and novelty: Mammalian suspension cell based antibody production is often accomplished in large scale stirred bioreactors due to their acceptable mass transfer (mixing and aeration) capabilities and their ease of scaling up. Nowadays, single-use bioreactors dominate high-value antibody processes, due to their advantages in terms of fast time-to-market, easy and safe product change, and cost reduction. The Allegro STR from PALL Biotech elegantly combines these two features through its design, with its cubical shape mimicking baffles. Excellent mixing performance is seen through a bottom mounted direct driven agitation technology, that is coupled with a large 45° elephant ear bottom-mounted impeller, held either 0.4 or 0.5 impeller aspect ratio. Furthermore, due to the single stirrer no limitations in liquid level operation capability occur when applying feeding strategies.

Experimental approach: CHO suspension cells (CHO S-38) producing IgG were grown in the Allegro STR 200L and in a cylindrical shaped single-use stirred bioreactor. The stirrer speed was adjusted to the same volumetric power input for each bioreactor and the aeration strategy was kept identical based on constant vvm sparging. A fed-batch strategy was applied. In-process control included cell count and viability, metabolite profile and analysis of IgG quantity and quality.

Results and discussion: The results in both bioreactors are comparable. The average peak cell density was $2.15 \cdot 10^7$ cells·mL^{-1}. The specific growth rate during the exponential growth phase was 0.028 h^{-1}. As expected, the mAb titer increased to an average level of 2.18 g·L^{-1} at the culture harvest. To summarize, a very close variant profile to the reference antibody was detected and only small amounts of aggregates or low molecular weight species were found. This allows to conclude that the shear stress acting on the cells under the selected process parameters does not impair the IgG quality in the Allegro STR 200.

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DEBOTTLENECKING YOUR TIMELINE USING AN INTENSIFIED PERFUSION PROCESS

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Background and novelty: Integrated continuous biomanufacturing (ICB) process has gained tremendous interest and momentum as the front runner in next generation manufacturing platform in our industry. Perfusion process is one key aspect of an ICB process, which often time associate with some concerns including long duration and large volume of media usage. These challenges can be overcome with a highly productive short-term perfusion process, which will provide the added benefits in the overall development timeline by accelerating late stage development and beyond.

Experimental approach: In the past year, we have focused on development of a highly intensified short-term dynamic perfusion process which has similar culture duration as any typical fed-batch processes. The intensified process utilized concentrated media at low volume to overcome the large media usage challenge inherited from historical perfusion processes. Additionally, this work was demonstrated at bench-scale as well as pilot scale, and repeated in multiple cell lines to ensure that the platform is widely applicable.

Results and discussion: Our team has demonstrated the capability to overcome both of the pain points mentioned above to showcase that a viable perfusion process can be short term and utilize low volume of media. By utilizing concentrated media, our process requires only 0.5 vvd (volume of media per bioreactor volume per day). Average volumetric productivity level up to 5 g/L/d was accomplished in a 14-day perfusion process. The downstream is integrated with the upstream, and can accept 8-fold titer variability. The mass output of our process can produce > 10-fold higher than a fed-batch process in the same duration. The benefits of such a high productive and flexible process is not only driven by cost per gram of drug substance, but also can be realized in the overall development timeline by having a commercial-ready process during early phase development.

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FED-BATCH PROCESS OPTIMIZATION FOR ANTIBODY PRODUCTION AT 2000 L SCALE

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Background and novelty: One of the current main challenges faced during monoclonal antibody development using CHO cells is to get a high productivity on a Good Manufacturing Practices (GMP) scalable process without any changes on the quality attributes of the molecule. In this project, the aim was to re-develop a process which was previously successfully transferred from 3L [Mobius] single-use development bioreactors to 2kL [Mobius] single-use GMP production in order to increase the titer and better control the pCO_2. The additional constraints were 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 60 different feeding and supplementation strategies using spin tubes to identify conditions with high titer and productivity. In parallel, the parameters of the 3L single-use bioreactors were optimized to reduce the pCO_2 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 after a 200L pilot batch.

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 feeding 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 can be considered as an efficient approach to foster high productivity with a consistent product quality on a GMP scalable process. The single-use technology additionally insured the feasibility of this project within tight timelines which are crucial in today's drug development.

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DEVELOPMENT OF GIBCO™ QP-CHO™ MEDIUM THROUGH MULTI-OMIC ANALYSIS

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Background and novelty: Traditional CHO cell medium development relies heavily on the stoichiometric analysis of metabolites in spent medium with emphasis on amino acid, glucose and select water soluble vitamin consumption. Analysis of these basic metabolites in an empirical design of experiment (DOE) has consistently resulted in incremental increases in titer generally through increased viable cell density (VCD) and viability of the culture. It has been long hypothesized that an upper limit to titer and product quality would be reached using traditional medium development techniques. Through the use of multi-omics analysis, we are developing a hypothesis based method to design media through analysis of critical pathways focused on specific productivity.

Experimental approach: Metabolomics and proteomic analyses were conducted on two medium formulations with disparate growth and production characteristics. Medium formulation 1 (M1) demonstrates moderate peak VCD with a high specific productivity (qP) over a 14 day growth performance assay utilizing a recombinant IgG producing CHO-S cell line and DG44 cell line. Medium formulation 2 (M2) demonstrates a high peak VCD with moderate qP under the same conditions and cell lines. A comparative analysis of metabolite abundance and enzyme regulation identified that M1 had greater flux in the sorbitol pathway verses glycolysis, the TCA cycle was upregulated to a greater degree than M2.

Results and discussion: A Design of Experiment (DoE) study based on metabolomics and proteomic data was developed to increase the specific productivity of M1 without decreasing the VCD to M2 levels resulting in a superior volumetric titer. Simultaneously, we utilized traditional empirical approaches to increase the qP of M2 in a parallel set of experiments. We describe here the path to develop a new catalog medium through both approaches.

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CELL CULTURE PROCESS PARAMETERS FOR MODULATING MAB AFUCOSYLATION

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Background and novelty: The extent of afucosylation, which refers to the absence of core fucose on Fc glycans, can correlate positively with the antibody-dependent cellular cytotoxicity (ADCC) activity of a monoclonal antibody (mAb). Therefore, it is important to maintain consistent afucosylation during cell culture process scale-up in bioreactors for a mAb with ADCC activity. However, there is currently a lack of understanding about the impact of pCO_2—a parameter that can vary with bioreactor scale—on afucosylation.

Experimental approach: Using a small-scale (3-L) bioreactor model that can modulate pCO_2 levels through modified configurations and gassing strategies, we identified three cell culture process parameters that influence afucosylation of a mAb produced by a recombinant CHO cell line: pCO_2, media hold duration (at 37°C), and manganese.

Results and discussion: These three independent parameters demonstrated a synergistic effect on mAb afucosylation; increase in pCO_2, media hold duration, and manganese consistently increased afucosylation. Our investigations into the underlying mechanisms through proteomic analysis indicated that the synergistic interactions downregulated pathways related to GDP-L-fucose synthesis and fucosylation, and upregulated manganese transport into the CHO cells. These new findings highlight the importance of considering potential differences in culture environment and operations across bioreactor scales, and understanding the impact of their interactions on product quality.

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ATPS PHASE SEPARATION FOR INTEGRATED CLARIFICATION AND PURIFICATION

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Background and novelty: Therapeutic monoclonal antibodies (mAb) are used for the treatment of numerous serious diseases, which led to an increasing demand over the last decades. Intensified upstream processes led to high challenges for the subsequent clarification and capture operations in the downstream process. A selective mAb extraction via an aqueous two-phase system (ATPS) directly from the cultivation broth might be a promising approach, integrating clarification and purification. The major challenge of the subsequent phase separation was the similarity of the two ATPS phases, which both consist of approximately 80 % water.

Experimental approach: An efficient purification of the mAb was accomplished by the ATPS composition. Phase separation was realized by a newly developed membrane based phase separator, where a selectivity between both phases was achieved by membrane modification.

Results and discussion: A mAb producing industrially relevant CHO cell line was used as model. Yields up to 98 % in the light phase with simultaneous reduction of process related impurities were achieved after aqueous two-phase extraction (ATPE) itself. Phase separation performance was characterized for different ATPS regarding flux and purity of the target phase. Contact angles of the phases on the modified membrane enabled a good predictability of the separation performance. ATPE directly from the cultivation broth in combination with the new membrane based phase separation led to a recovery of 83 % mAb with simultaneous reduction of DNA, HCP and an entire cell removal.

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IMPACT OF CELL CULTURE MEDIA ADDITIVES ON IGG GLYCOSYLATION

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Background and novelty: Glycosylation is a key critical quality attribute for monoclonal antibodies and other recombinant proteins because of its impact on effector mechanisms and half-life. In this study, a variety of compounds were evaluated for their ability to modulate glycosylation profiles of recombinant monoclonal antibodies produced in CHO cells.

Experimental approach: Compounds were supplemented into the cell culture feed of fed-batch experiments performed with a CHO K1 and a CHO DG44 cell line expressing a recombinant IgG1. Experiments were performed in spin tubes or the ambr®15 controlled bioreactor system, and the impact of the compounds at various concentrations was determined by monitoring the glycosylation profile of the IgG and cell culture parameters such as viable cell density, viability and titer.

Results and discussion: Results indicate that the highest increase in high mannose species (+85.8 %) was achieved through 15 µM kifunensine supplementation. Fucosylation was reduced by 76.1 % through addition of 800 µM 2-F-peracetyl fucose. An increase of 40.9 % in galactosylated species was achieved through addition of 120 mM galactose in combination with 48 µM manganese and 24 µM uridine. Furthermore, 6.9 % increased sialylation was detected through addition of 30 µM dexamethasone. The studied cell culture media additives are efficient modulators of glycosylation and are thus a valuable tool to produce recombinant glycoproteins.

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SCALE-DOWN PERFUSION METHODOLOGIES FOR RAPID BIOPROCESS DEVELOPMENT

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Background and novelty: Scale-down devices enable cost effective, high-throughput screening for the development and optimisation of bioprocess. While a wide range of scale-down devices exist for fed-batch processes, there are few comparable devices for high cell density perfusion cultures. This work aims to develop a 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, to operate for, extended periods compared to fed-batch cultures.

Experimental approach: Cultivation approaches have been developed using a GS-CHO cell line in both 24 well microwell plates (MWP) and in a custom-designed micro-bioreactor (MBR), with working volumes of 1.2 and 250mL, respectively. Quasi-perfusion in MWP was achieved using either sedimentation or centrifugation for supernatant removal followed by media exchange. Perfusion in the MBR was achieved via connection to a tangential flow filter (TFF). Perfusion in both systems commenced on day 3 at a rate between 0.5 and 2 vessel volumes per day (VVD).

Results and discussion: Quasi-perfusion culture methods in MWP achieved cell densities over 4× and volumetric productivities up to 2× greater than fed-batch. Media screening and analysis of a range of perfusion rates show these methodologies are robust and sensitive to changes in feeding strategy. Perfusion culture in the MBR achieved cell densities >40×10^6 cells/mL for >14 days while maintaining viabilities >90% for a range of feeding strategies. The results demonstrate that the characteristics of perfusion culture can be effectively mimicked in MWP systems, with the MBR successfully achieving well-controlled small-scale perfusion. The combined use of MWP systems for high-throughput screening in early-phase development, supported by a small volume MBR system for the study of selected conditions represents a powerful tool for the development of perfusion processes.

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IMPACT OF MEDIUM FILTRATION ON CELL GROWTH AND CHARGE VARIANT PROFILE

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Background and novelty: It was observed that sterile filtration of basal medium had a large impact on the cell growth performance and the charge variant profile of a monoclonal antibody produced using a mammalian cell culture process. The identified root cause related to the type of medium filter used for sterile filtration of the basal medium. To develop a further mechanistic understanding, an additional investigation was initiated to elucidate the potential filter-medium interactions, including leachables/extractables contamination and adsorption of medium component(s) to the filter.

Experimental approach: The multidisciplinary investigation comprised of 1) a filter pre-treatment study to elucidate the impacts of leachables/extractables and/or adsorption, and 2) spiking studies to confirm and understand the impact of adsorption. The cell growth performance and charge variant profile was studied in shake flasks and 3-L bioreactors. Moreover, analysis of (spent) media (LC-MS, NMR, ICP-MS) and the filter membranes (SEM-EDX, FTIR, ICP-OES) was performed to identify potential adsorbed components and/or leachables/extractables.

Results and discussion: The studies suggest that adsorption of one or more medium component(s) likely occurred, which impacted cell growth performance and charge variant profile severely, whereas leachables/extractables did not have an impact. This was confirmed by spiking studies with complete basal medium which demonstrated restoration of cell growth. This presentation shows the results on the various analytical and cell culture experiments to uncover the medium component(s) adsorbed to the filter.

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PROCESS OPTIMIZATION FOR FAB PRODUCTION IN EXPICHO-S™ CELLS

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Background and novelty: The increasing need to accelerate drug development programs led to the establishment of Chinese hamster ovary (CHO) based transient expression methods, which circumvent the production host changes between early developmental phases and the clinical phases. For monoclonal antibodies and antibodies fragments (Fab), the maintenance of the production hosts is especially important, once post-translational modifications are highly dependent on the host cell used for expression.

With this study we aim to optimize Fab production in ExpiCHO-S™ cells by integrating upstream and downstream processing engineering.

Experimental approach: The ExpiCHO-S Expression System™ was used for Fab production by transient transfection. Upstream optimization experiments were performed to evaluate the impact of different parameters on Fab production. Productivity was assessed after ion exchange chromatography based purification and product quality was evaluated by SEC-HPLC.

After establishing the best upstream procedure, we have tested Capto L, KappaSelect and CaptureSelect affinity chromatography resins for Fab purification. Finally, a 4-Fold scale-up of the optimized protocol for Fab production was performed.

Results and discussion: Two key parameters for the Fab expression optimization were identified and their synergetic effect resulted in 12-Fold increase in the final productivity. Purification strategies based on affinity resins were effective on Fab capture, nevertheless product aggregation was observed, resulting in drastic reduction of final productivity. The optimized production process was reproducible and scalable up to 4-Fold, without impact in final productivity and product quality.

In summary, our results highlight the potential of CHO based transient processes for the supply of pre-clinical products during drug development programs.

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OPTIMIZATION OF FAB PRODUCTION IN CHO-S CELLS: THE IMPACT OF FEEDINGS

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Background and novelty: Chinese hamster ovary (CHO) cells are the main hosts used for the clinical grade production of monoclonal antibodies. However, the low transfection yields of CHO cells prevented their use during early drug development. Recently, new transfection reagents and transfection kits have been commercialised to boost CHO based Transient Gene Expression. In this study, we performed a stepwise optimization of parameters for transient production of an antibody fragment (Fab) in FreeStyle™ CHO-S cells.

Experimental approach: The FreeStyle™ CHO-S cells were used for transient gene expression. Fab production optimization was performed to evaluate the impact of parameters such as: transfection reagent (PEI or FectoPRO®), temperature, DNA concentration, DNA/transfection reagent ratio, cell concentration, transfection mix medium and nutrient feeding. Cell concentration and viability was monitored daily and Fab productivity was assessed by SDSPAGE and Western-Blot analysis.

Results and discussion: For the PEI based transfections, Fab production was only detected when culture feedings were included. The subsequent tests performed with the selected key parameters resulted in enhanced productivity.

In the initial FectoPRO® based transfections, Fab production was detected however cell viabilities were severely impaired, resulting in early culture harvest. Further optimizations indicated that using higher cell densities at transfection had a positive effect on Fab production.

In summary, our stepwise optimization strategy identified optimal protocols for Fab production using either traditional or modern transfection reagents.

Our results demonstrate the potential of CHO based transient processes in the early phases of drug development programs.

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PROCESS INTENSIFICATION STRATEGIES FOR AN INDUCIBLE CHO CELL LINE

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Background and novelty: There is a growing interest toward the use of inducible mammalian expression systems, which open up new challenges and opportunities for cell culture process development. In simple batch mode, culture performance is limited by the inability to achieve productive high cell density induction, due to nutrient limitations and/or by-product inhibitions. Unlike for cell lines with constitutive expression, very little work has been devoted specifically to the development of culture strategies when using inducible mammalian expression systems.

Experimental approach: In this study, we have conducted process development at small scale and in bioreactors for a cumate-inducible CHO-GS cell line expressing rituximab. We have evaluated and compared the use of fed-batch, sequential medium replacements and continuous perfusion strategies applied during the pre-induction period (growth phase) to enhance process performance in terms of product yield and quality.

Results and discussion: In small-scale shake flask cultures, both fed-batch and medium replacement strategies were shown to significantly increase the integral of viable cell concentration and antibody titer compared to batch cultures. Further enhancement of antibody yield was achieved by combining bolus concentrated feed additions with sequential medium exchange; however, product galactosylation was reduced compared to fed-batch, as a result of the prolonged culture duration. In bioreactor, combining continuous perfusion of the basal medium with bolus daily feeding during the pre-induction period and harvesting earlier during the production phase is shown to provide a good trade-off between antibody titer and product galactosylation. Overall, our results demonstrate the importance of selecting a suitable operating mode and harvest time when carrying out high cell density induction in order to balance between culture productivity and product quality.

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RAPID FED-BATCH DEVELOPMENT USING MODELING AND HIGH THROUGHPUT TOOLS

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Background and novelty: Recently, an Upstream Technology Team within Janssen BioTherapeutics Development (BioTD) was tasked with increasing the productivity of a legacy fed-batch process platform with new media and feeds to meet pipeline demand. Using multiple tools and technologies, the team was able to create new proprietary basal and feed media in less than two years that achieved a titer increase of 2-7-fold while achieving product quality targets. An implementation strategy was deployed for the new fed-batch platform to ensure smooth and consistent performance upon adoption by existing process development teams and manufacturing sites.

Experimental approach: The Upstream Technology Team used a combination of screening studies, omics technology (e.g., metabolomics) and metabolic modeling to design reformulated media powders. Quantitative metabolomics data was used in a two-fold approach; first, the data was used as an input into a genome-based metabolic model that was used for amino acid optimization of the feed media. Second, metabolomic data was used to rebalance media components not included in the model optimization. In addition to model-based media optimization, Ambr® bioreactor systems were used to screen and test multiple media and feed iterations in a short timeframe.

Results and discussion: The new proprietary basal media resulted in about a 50% improvement in process titer. Reformulated and concentrated feeds contributed an additional 50% improvement in titer, resulting in a total titer increase greater than 2-fold. These results were consistent across multiple cell lines tested with the new fed-batch platform. The new platform has been implemented successfully at multiple stages of clinical development and manufacturing through the strategic use of a liaison program to communicate challenges and guide teams as they became more familiar with the updated platform.

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TEMPERATURE IMPACT ON MABS CHARGE IN FOUR INDUSTRIAL PROCESSES

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Background and novelty: The charge profile is a major quality attribute of monoclonal antibodies (mAbs) that is monitored from the early steps of cell line and process development to GMP manufacturing. Variations of acidic and basic forms proportions may be the result of many post-translational events. Although the criticality of these variations in antibody activity and safety is still debated and investigated, a consistent charge profile minimizes the risk of drug efficacy variability and is key in the process consistency demonstration. High expectations are thus expressed towards early reference charge profile similarity and consistency across the development steps. Here, we present the charge profile fine-tuning in four cases of mAb production process developments in two different CHO cell types in fed-batch mode for clinical GMP production.

Experimental approach: The upstream processes were developed in 3L single-use [Mobius] bioreactors and scaled-up to 200L and 2000L single-use [Mobius] bioreactors. The charge profiles were characterized by capillary isoelectric focusing using the [iCE3] system. For one case, the effects of chosen culture parameters on the charge profile were evaluated using a DoE approach at 3L scale.

Results and discussion: The mAbs' charge profiles were adjusted to the target during the upstream process development and were maintained across the downstream steps. The profiles were successfully maintained from development to GMP manufacturing scale. In three cases, the culture temperature was a key parameter influencing the charge profile (2 to 7-fold increase of the basic forms at low temperature) whereas it had no significant impact in the last case. The predominance of the temperature effect over the other parameters was confirmed by the DoE model. These case studies show that, despite general tendencies emerging, the impact of temperature on charge remains to be evaluated for each clone individually.

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HI-INTENSITY LOW-VOLUME PERFUSION – OPTIMIZATION, BENCHMARK, SCALE-UP

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Background and novelty: After somewhat disappointing results with more classical steady-state approachs to cell culture using perfusion[1], we developed a cell culture process making use of the advantages of cell-retention/perfusion, but that delivers a significantly higher volumetric productivity using much lower volumes of perfusion medium.

Experimental approach: Through experimentation with a dozen different CHO cell lines producing protein therapeutics we designed a highly-intensified perfusion cell culture process integrated with a continuous downstream process designed for a single use system. Unlike more classical continuous cell culture perfusion processes, this process does not seek to achieve a steady-state condition (no cell bleed). The process is of comparably short duration (12-18 days), yet because of very high cell densities, similar or higher volumetric productivities are achieved compared to more sustainable and highly medium intensive perfusion processes. Like any non-steady-state process, significant changes in process conditions over the course of the cell culture result in small instantaneous product quality (PQ) changes, while PQ of the pooled, purified drug substance has a similar overall profile to that produced in a fed-batch culture.

Results and discussion: We will describe the various control and optimization parameters of the process and explain the "cell-controlled" perfusion control which allows continuous cell feedback precisely delivering the required volume of perfusion media to maintain continuous near exponential growth during the expansion phase. We will show representative data from our benchmarking experiments with eleven antibody producing glutamine synthetase-CHO cell lines in a standardized high-intensity low-volume perfusion process.

Finally, we will show representative data from some of the cell culture results of the process when scaled up to the pilot stage (100-L working volume) for a limited number of cell lines.

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A VERSATILE TOOLBOX FOR RAPID DEVELOPMENT OF INTENSIFIED CHO PROCESSES

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Background and novelty: Providing patients with affordable biopharmaceuticals requires next-generation processes with improved economics. Intensified processes enable plants with lower cost of goods, reduced footprints and increased flexibility, rendering compact bioreactors suitable for commercial production. CHO clones from traditional cell line development differ in responsiveness to process intensification, so efficient approaches for developing tailored processes are needed.

We have implemented a toolbox for rapid development of such USP processes. It comprises parallelized scale-down models for clone selection and media screening as well as different types of intensified formats. We demonstrate the application of this platform to different CHO clones.

Experimental approach: Preliminary clone assessment was performed in TubeSpin® reactors for different CHO clones originally developed for fed batch. An ambr15-based perfusion mimic served to optimize media compositions and to evaluate clones under controlled conditions. Upscale was performed in rocking motion-based reactors for (N-1) perfusion and full perfusion. A high-inoculation fed batch process was developed in the ambr250 and transferred to the 5L scale.

Results and discussion: Most fed batch clones showed significant productivity improvements in the intensified process formats. For some, limited improvements or even reduced productivity were seen, underscoring why scale-down models are key for identifying intensification-ready clones already in cell line development. Scale-up to larger volumes was straightforward and productivity increases of up to 3-4-fold were achieved in highinoculation fed batch and >5-fold in perfusion, respectively, without optimization. This illustrates how the new toolbox can support unleashing unutilized performance reserves of CHO production clones.

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SYSTEMATIC EVALUATION OF HIGH-THROUGHPUT SCALE-DOWN MODELS

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Background and novelty: Many biotherapeutics are produced in mammalian cell culture systems with large-scale bioreactors. Scale-down model development for systematic studies of cell culture performance is a critical part of process characterization and validation of the manufacturing process. Establishment of reliable high-throughput scale-down models could greatly accelerate biologics development and characterization timelines.

Experimental approach: A systematic analysis was done to evaluate high-throughput scale-down models (ambr®250) for 500 L or 2,000 L single-use bioreactors (SUB) using engineering approaches. A main scaling criterion, gas throughput (vvm), was studied for scale-down model development after analyzing bioreactor hydrodynamic environment and gas transfer characteristics (i.e., oxygen and carbon dioxide volumetric mass transfer coefficients, from both surface and sparging). Two different processes with distinguished peak cell densities (12 – 14 vs. 20 – 25 × 10^6 cells/mL) for a monoclonal antibody (MAb) were evaluated at various agitation speeds and air sparge rates. As a confirmation, two other MAbs were evaluated using the same approach.

Results and discussion: We demonstrated that scaling-down cell culture processes using similar vvm as the criterion in ambr®250 was feasible in reproducing the large-scale gas transfer characteristics for a wide range of cell densities. The same approach was applied to two other MAb fed-batch cultures with comparable results across scales. Practical aspects of ambr®250 operations, especially the impact of the background air sparge rate and antifoam addition on dissolved oxygen (DO) control and gas transfer, will also be discussed. Understanding and managing the gaps between small- and large-scale bioreactors is expected to streamline scale-up effort, and equally importantly scale-down activities for process characterization.

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OPTIMISATION OF FEED AND TEMPERATURE IMPROVED EPO-FC PRODUCTION

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Background and novelty: CHO cells have been the main host to express recombinant (r-) therapeutic proteins. Improvements in CHO culture processes have enabled the increase of final titres (up to 10 g/L) and productivity (20-30 pcd) for some r-proteins, although they present low titres and productivities for some proteins (known as difficult-to-express (DTE) proteins). Strategies focused on optimising of feeds or environmental parameters have proven to increase r-protein production, but these have mainly focused on easy-to-express proteins. Therefore, further understanding of these strategies on systems producing DTE proteins is needed to enable the development of more productive and efficient processes.

Experimental approach: A stable CHO cell line producing EPO-Fc was generated and cultured in batch (B), fed-batch (F) and perfusion (P) systems. F and P cultures were initially operated in B mode for 48 h and then simulataneously fed and shifted from 37 to 32°C. Customised feeds for F and P cultures were designed using metabolomic data of preliminary B cultures. The culture performance was analysed by considering growth, r-protein production and key metabolites, and calculating physiological parameters.

Results and discussion: Customised feeds prolonged the culture period and increased the peak of viable cell density (by 150%) and the r-protein production while specific productivity (q_p) remained the same. Decrease in temperature improved viability, r-protein production (by 80%) and q_p (by 50%) in all cultures, whereas cell growth rate (μ) decreased. This strategy improved the culture performance of this cell line in both fed-batch and perfusion. Lactate and ammonia concentrations did not reach detrimental concentrations for controlling both growth and productivity. We observed a strong negative correlation between the q_p and the μ in all culture conditions, suggesting that strategies for improving DTE protein production should consider a compromise between these parameters.

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SERICIN, CULTURE SUPPLEMENT IMPROVING CELL CULTURE

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Background and novelty: In mammalian cell culture mammal-derived factors are supplemented to the culture media. But mammal-derived factors should be replaced by non-mammal factors because of zoonotic disease. We previously reported that addition of sericin peptides improved the proliferation and survival of various cell lines.

We aimed to elucidate the mechanism how sericin promotes the proliferation and the survival of mammalian cells. For the purpose, we focused the sugar consumption and EGF signal pathways.

Experimental approach: Murine hybridoma cells were cultured in RPMI medium supplemented with ITES. Using glucose-free medium, glucose concentration was decreased and the effect of sericin on the glucose-limited cells was studied.

Human keratinocyte was cultured in Keratinocyte-SFM medium supplemented with EGF and BPE. One day later, the medium was replaced to that without both EGF and BPE in order to reset the cells from growth factors. 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: Glucose limitation inhibited the proliferation of the cells. But addition of sericin improved the proliferation of the cells in the low glucose medium and decreased the glucose consumption rate.

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.

Our results imply that sericin activates TCA-cycles and decreases sugar consumption and that sericin promotes the cell proliferation through MAPK, JAK/STAT and PI3K signaling.

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OPTIMIZING QUALITY AND PRODUCTIVITY OF AN FC-FGF21 FUSION PROTEIN IN CELL CULTURE VIA A CONTINUOUS PERFUSION PROCESS

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Background and novelty: Fed-Batch (FB) cultivations for the production of an Fc-FGF21 fusion protein revealed a gradual increase in degradation rate over the whole process, leading to purity values as low as 39.9% at the end of the production process. Protease cleavage at residues Pro171 and Ser172, known for other FGF21 fusion proteins (Hecht et al., 2012), was considered as one possible source. Therefore a 30-day perfusion process with continuous product harvest was developed in small-scale in order to mitigate the proteolytic effect.

Experimental approach: Suspension cells producing a recombinant Fc-FGF21 fusion protein were cultivated in a stirred bioreactor at a 1.5 L working volume coupled with an alternating tangential flow (ATF) system for cell retention. A continuous medium, glucose and glutamine supplementation was assured to maintain high cell densities in bioreactor. Biomass control was achieved using estimations of an on-line capacitance probe and an automatic cell bleeding. Productivity and product quality attributes were evaluated at different cell densities and cell-specific perfusion rates.

Results and discussion: The results obtained showed mitigated product degradation with purity values above 92% and consistent glycosylation patterns over 30 days of perfusion. In addition, perfusion allowed for 7-fold higher volumetric productivities compared to the standard FB process. This study demonstrated that the yields of processes with sensitive products and/or cells with low specific productivities can be significantly improved by shifting to continuous perfusion production platforms. Additionally, a recent assessment indicated that a scale-up to a 40 L stirred bioreactor can provide similar quality attributes and productivities than the ones obtained at 1.5 L scale, ensuring a robust continuous perfusion process.

Acknowledgements & Funding: Hecht, R. et al. Rationale-Based Engineering of a Potent Long-Acting FGF21 Analog for the Treatment of Type 2 Diabetes (2012) PloS one 7, e49345

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Hecht, R. *et al.* Rationale-Based Engineering of a Potent Long-Acting FGF21 Analog for the Treatment of Type 2 Diabetes (2012) PloS one 7, e49345

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REDUCTION OF UNIT OPERATIONS IN ANIMAL CELL CULTURE PROCESSE

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Background and novelty: The majority of unit operations in biopharmaceutical industry is executed by the combination of several unit operations in batch mode. Process intensification offers the possibility to reduce the required scale of operation and the number of unit operations.

Experimental approach:
Process analytical technologies: If a new unit operation is introduced to replace multiple batch unit operations, the new unit operation is usually more complex than the existing batch steps. Therefore, a higher level of control is required. We evaluated different PAT technologies for their potential to control the new intensified unit operations.

Continuous Bioprocessing & Harvest Technology: Beside analytical technologies, new equipment is needed to replace the existing batch unit operations. We compared devices for continuous cell culture and harvest steps with regard to their potential to increase the efficiency of the overall process.

Results and discussion: For specific CHO processes, we selected the best suitable PAT methods for process control, the best equipment for continuous process steps and the best process strategy. Via the implementation of the intensified unit operations, we could achieve a dramatic reduction in process scale and operational costs.

Acknowledgements & Funding: We would thank the Isabelle Schoenauer, Christian Schantz and Roman Hirschauer from Roche Pharma Research for the support during the equipment evaluation.

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THE RELEVANCE OF CELL SIZE IN A CHO CELL FED- BATCH PROCESS

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Background and novelty: Cell size is an important characteristic that together with the viable cell concentration determines the active biomass concentration. In this study the growth phase (NI phase) of a CHO cell fed-batch culture is followed by a phase where cell division stops, but cell growth continues in the form of an almost threefold increase in cell size (SI phase). The aim of this work is to obtain more insight in this cell size increase with respect to metabolism and gene expression.

Experimental approach: Three 10 liter fed batch cultures were done using a CHO-K1 clone producing an IgG1. ActiCHO-P was used as a basal medium and ActiCHO feed A&B were added daily as a bolus from day 3 on. Samples were taken in the NI and SI phase for cell cycle, metabolic flux and transcriptome analysis.

Results and discussion: Cell cycle analysis showed that the cell size increase is related to an arrest of cells in the G1 phase in combination with continued biomass formation. In the SI phase accumulation of fatty acids occurs. Furthermore, the mAb specific productivity per cell increases linearly with the cell volume. Since specific nutrient uptake rates remain constant, the yield of product on nutrients increases in the SI phase. Metabolic flux balancing showed that also the yield of product on oxygen consumed is increased in the SI phase, which means lower gas-flow rates can be used to reach the same volumetric productivity. Transcriptome analysis showed up-regulation of cyclin-dependent kinase inhibitors and down-regulation of various cyclin-dependent kinases and cyclins, which is in agreement with the cell cycle arrest. The expression of genes in the upstream and downstream pathways of mTOR show a strongly synchronized pattern to promote the mTOR activity and stimulate protein translation and lipid synthesis, which agrees with the continued biomass increase.

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PERFUSION MEDIA DEVELOPMENT FOR SCALABLE PROCESSES

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Background and novelty: Cell culture perfusion processes are considered optimal for a truly integrated continuous biomanufacturing pipeline. The nutrient-rich but balanced media should be designed to support very low cell-specific perfusion rates (CSPR) that minimize media consumption but maximize viable cell days and productivities. Optimized processes at low CSPR drastically reduce equipment costs, lab space, and product dilution. Finally, operating at very low CSPR enables running mammalian cell bioprocesses as true chemostat cultures in the future. We demonstrate a general workflow to develop high-performing perfusion media using small-scale models and transferred the process to 50 L scale at CSPR of 20 pL/c/d.

Experimental approach: Recombinant CHO cells were evaluated at small scale in shaking tubes. Cells were grown in HyClone™ ActiPro or CDM4NS0 basal media, and optimal spike concentrations of HyClone Cell Boost™ supplements were determined using a DoE-supported workflow. The identified high-performing perfusion medium was applied to ReadyToProcess WAVE™ 25 and XDR-50 bioreactor runs. Different strategies were tested to find the critical minimum CSPR and maximum supported viable cell density (VCD). The obtained product profile was compared between scales, as determined by glycan-, charge-, and size-variant distribution.

Results and discussion: Scale-down models were leveraged to define high-performing media and applied to bioreactor runs at constant volumetric perfusion rate, VCD, or CSPR. CSPR values as low as 10 pL/c/d at 2×10^8 c/mL were achieved. These results make high-density perfusion processes suitable for inoculum preparation (N-1) or high cell density cryopreservation. The developed perfusion processes supported steady-state production at constant 5×10^7 c/mL by applying a continuous cell bleed and were scaled to 50 L.

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A ROAD MAP TO LICENSURE FOR MULTICOLUMN CAPTURE IN A MAB PROCESS

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Background and novelty: Application of multicolumn chromatography for affinity capture in process scale manufacturing of monoclonal antibodies has significant impact on cost savings from reduced sorbent and buffer requirements compared to conventional batch operation. A case study is presented investigating the Cadence® BioSMB system to support incorporation of multicolumn chromatography for capture into an existing commercial antibody drug platform. Here we take an approach that relies on modeling breakthrough curve data to derive process conditions that demonstrate multicolumn chromatography can achieve capture efficiency and product quality consistent with current process specifications. By leveraging mechanical stability of the sorbent, we demonstrate multicolumn chromatography can operate within time constraints for capture with only 7% of the sorbent volume currently required for processing. A risk based approach is then explored to identify aspects relevant to column performance, bioburden management and process integration. In this approach, data from both single column and multicolumn experiments help us to better characterize the space for operation and provide justification to satisfy requirements for regulatory submission.

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INTENSIFIED SEED EXPANSION & SIMPLIFIED CLARIFICATION OF FED-BATCH

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Background and novelty: A conventional production fed-batch process involves a multi-stage seed expansion and elaborate clarification steps to isolate the protein of interest from other process impurities. The current clarification technology utilizes centrifugation and depth filtration trains that are complex and limited in their ability to operate in a sterile or low bioburden manner. With an increase in demand for production of protein therapeutics, it is essential to simplify the traditional fed-batch processes to result in better yield, shorter time and smaller footprint.

Experimental approach: Repligen developed a simplified & scalable single-step clarification method: High Productivity Harvest (HPH) using the XCell™ ATF system. HPH helps maximize the yield of a fed-batch bioreactor in a sterile manner while eliminating centrifugation, depth, and sterile filtration steps. The current project explores the benefits of combining the HPH method with a perfusion enabled (n-1) seed train bioreactor.

Results and discussion: The case study focuses on performing (n-1) bioreactor in a perfusion mode that enabled to inoculate production fed-batch bioreactor at 10e6 cells/mL, 20x higher compared to traditional seeding density. Increased seed density facilitates reducing the fed-batch culture duration by half. Implementing the HPH clarification process using XCell ATF system resulted in an enriched environment with a 150% boost in the overall yield in half the fed-batch culture time. The HPH process is performed as a single step in a sterile manner with a 0.2µm clarified product as the output. The product is readily available for either batch or continuous chromatography without additional processing.

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DEVELOPMENT OF AN AUTOMATED PERFUSION BIOREACTOR 'AMBR® 250 PERFUSION'

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Background and novelty: In recent years a strong trend towards intensified and continuous biopharmaceutical processing has gathered momentum, enabled by key cell culture technologies such as ATF and TFF. However, small-scale application has been limited to traditional benchtop bioreactor formats that are manually intensive, relatively low throughput and costly to operate. Automated high throughput, single-use, mini bioreactor systems with new capabilities to support perfusion culture, including new ambr 15 capability and the novel 'ambr 250 perfusion', can facilitate and significantly accelerate an industry wide transition to intensified and continuous perfusion cell culture processes.

Experimental approach: Working in collaboration with biopharm industry development partners, the design of the 'ambr 250 high throughput' bioreactor system has been modified to include hardware, software and single use components required to operate up to 24 parallel bioreactors with ATF or TFF cell retention modes. Iterative prototype testing with development partners has resulted in a novel ambr 250 system design, shown to be capable of operating for extended culture durations and supporting high cell densities.

Results and discussion: Technical description and cell culture results presented for 'ambr 250 perfusion' outline system capability for intensified cell culture applications. Case studies will be presented on the utility of new ambr 15 system features for perfusion mimic applications, together with a range of industry case studies and novel performance data for the new 'ambr 250 perfusion' system. As previously established with ambr 250 for fed-batch processes, 'ambr 250 perfusion' has the potential to provide the biopharm industry with a step change in perfusion process development capacity, enabling implementation of DoE based approaches for perfusion process optimization and characterization.

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MEDIA SUPPLEMENT INDUCED SIGNALING IN CHO CELLS VIA TRIPLE SILAC-MS

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Background and novelty: Process intensification of CHO cell culture often involves supplementation with (small) molecules affecting cellular productivity or proliferation. However, screening for positive candidates remains time and cost intensive. Identification of underlying cellular mechanisms e.g. by signaling studies using quantitative phosphoproteomics [1,2] would allow targeted application. In a triple SILAC-MS approach of mAB producing CHO cell lines we aimed to identify cellular targets and to elucidate effects of cell growth enhancing agents on a molecular level.

Experimental approach: CHO cells were cultivated in CDM (Xell AG). For SILAC-MS experiments media with either lLys/lArg, mLys/mArg or hLys/hArg were utilized and glutamine was supplemented if indicated. Insulin-like growth factor (IGF) was added at day 3 and a few minutes later aliquots of the culture were harvested and pooled. Bottom-up (phosho)proteomic analysis of signaling events was done by nLC-ESI Orbitrap MS with subsequent data analysis based on MaxQuant and Fusion software [3].

Results and discussion: IGF supplementation induced rapid changes in signaling cascades directly linked to cellular metabolic processes (AKT, ERK etc.) and subsequent changes in metabolic behaviour were observed. Separately, IGF and glutamine supplementation resulted in an increased max. VCD. Surprisingly, the combination of IGF and glutamine yielded in reduced max. VCD. To further elucidate this, the benefits of directly comparing two agents within one triple SILAC MS approach were used and resulted in reliable MS data and deep insights into CHO cellular signaling. For visualization of complex data including dynamic protein phosphorylation patterns, we established and successfully applied a semi-automatic expression data mapping on cellular pathways.

Acknowledgements & Funding: We would like to thank the AIBN (Brisbane) for providing the CHO lines and Larissa Leßmann for excellent support with western blotting.

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A SMALL-SCALE MAB PLATFORM WITH A CONTINUOUS AND INTEGRATED DESIGN

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Background and novelty: Most processes for monoclonal antibody (mAb) production are based on inefficient and expensive batch operations: a batch or fed-batch bioreactor followed by several chromatography steps, and virus inactivation and filtration steps in batch mode in between. Integration of the upstream and downstream processes, and conversion to continuous manufacturing would allow to increase productivity, decrease equipment size, and reduce the manufacturing costs. An integrated and continuous bioprocess already at the earliest stages of process development allows for an easier and quicker scalability and a more efficient production of small-scale drug candidates. The process presented here is a proof-of-concept of an end-to-end monoclonal antibody production platform at small scale.

Experimental approach: In this work, a mAb platform at small scale was implemented in a compact design with minimal equipment required: a 200 mL ATF perfusion bioreactor, and a single lab-scale chromatography system (ÄKTA pure) for all downstream steps. The downstream process, consisting of three chromatography steps, including a periodic twin-column capture with protein A resin, followed by a virus inactivation step, a cation exchange step in bind-and-elute mode, and an anion exchange step in flow-through mode was compactly implemented in a single chromatography system, with a purification time of less than 4 hours.

Results and discussion: MAbs were produced for 17 days in a high cell density perfusion culture of Chinese Hamster Ovary (CHO) cells and the downstream process was run in a total of 14 days. A consistent glycosylation pattern of the purified product was ensured by the steady-state operation of the process. Regarding the removal of impurities, a 5-log reduction in the host cell protein (HCP) levels was achieved. The recovery yield was up to 60 %, and a maximum overall productivity of 0.8 g/L/day was obtained.

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MEDIA & FEED SUPPLEMENTS ENABLE FINE-TUNING OF HIGH MANNOSE GLYCANS

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Background and novelty: The biosimilar development resulted in substantial interest in the modulation of the glycan profile of recombinant therapeutic proteins as the increased number of publications[1, 2, 3]. The structural features shape their inherent biological activity, and thus, the Fc RIIIa receptor binding affinity is highly dependent on the level of fucosylation of the antibody. In this study, we developed a novel approach to fine-tune the degree of mannosylation, leveraging media and feed supplementation of various compounds in combination with feeding strategies.

Experimental approach: The media and feed supplements including alkaloids and antiprotozoal agents were tested in fed-batch culture experiments, using industrial relevant Chinese hamster ovary cell lines expressing recombinant antibodies. The experiments consisted in a joined variation of bolus feed concentrations and feed time points. Viable cell density, viability and product titer were monitored, and purified supernatants underwent N-glycan analysis by 2AB-UPLC.

Results and discussion: The combination of low supplement levels in the media and the addition at various culture timepoints resulted in high mannose (HM) levels below 20%. Moderate concentrations of kifunensine and maduramicin exhibited a gradual increase within that range. As a result, the approach enables HM fine-tuning for product quality adjustments. Our data show cell culture medium and bolus feed supplementation allow to induce small, precise and consistent variations of the HM level of antibodies.

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CHO SEED CULTURE INTENSIFICATION AND ITS CELLULAR EFFECTS ON N-STAGE

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Background and novelty: Reducing the plant size and footprint of today's manufacturing processes is one key aspect of sustainable and economic production of biopharmaceuticals. One approach during mammalian cell cultivation covers the intensification of the seed train by applying perfusion as N-1. Currently, latest research mainly assesses the impact of perfusion on cell cultivation by examining product quality and capacity, but ignores cellular responses initiated by process intensification strategies. In this study, both features are considered in fed-batch (FB) processes based on an intensified N-1 seed train using perfusion.

Experimental approach: An industrial relevant CHO cell line expressing a monoclonal IgG was used for an intensified and established perfusion process in a 2-D rocking motion bioreactor intended to generate inoculum cells for subsequent FB cultivations. These inocula were obtained with increasing viable cell concentrations (VCC) on days 3, 5, and 7 of the perfusion seed culture. Subsequently, regular 12-days FB processes conducted in single-use, small scale automated bioreactors (15 mL) were inoculated with these cells. Cellular impacts were evaluated using flow cytometry, while critical quality attributes were investigated by microfluidic electrophoresis.

Results and discussion: The presented data will contain results of FB processes inoculated from intensified seed trains. VCCs of >100E6 c/mL were obtained within 7 days in the RM based N-1 perfusion process allowing to reduce the required inoculation volume about 30 times compared with a VCC of a standard seed train of 3.5E6 c/mL. As cellular responses, both time-dependent cell-cycle distributions and apoptosis were analyzed for the N-stage FB processes and compared with data from N-stage FB processes obtained from regular seed trains. Moreover, applying perfusion as a method to generate high cell concentrations allowed to compare the IgG-product profiles between both process strategies.

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SEED TRAIN INTENSIFICATION IN ROCKED 2D-BIOREACTORS AFFECTING CQAS

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Background and novelty: Historically low seed ratios of 1:5 [1] require intensive seed-trains to inoculate production processes. Current progresses in seed train handling, media formulation and cell lines allow to shorten the seed trains significantly. Furthermore available process analytical technologies (PAT) can easily be implemented for better process control. Omitting steps from the seed train will result in reduction of footprint and streamlined production facilities.

Experimental approach: In earlier works an industrial relevant fed-batch process using a CHO-DG44 cell line was transferred to perfusion mode using small scale multi-bioreactor systems [2]. This platform process was used to serve as a high cell density seed trains [3]. The seed culture was expanded by maintaining a given cell specific perfusion rate of 50 $pL \cdot (cell \cdot d)^{-1}$ resulting in high growth rates and viable cell densities. Seed cultures were run in rocked bioreactors (Biostat® RM) in different scales, ranging from 1 – 25 L working volume. Online sensor were used to monitor the seed culture. The cells were used for inoculation of subsequent fed-batch processes in stirred tank reactors after reaching 100E $c \cdot mL^{-1}$ at day 6.

Results and discussion: The presented data will comprise scaling of high cell density seed cultures in rocked bioreactors from 1 – 25 L working volume using perfusion mode. Viable cell densities of 100 – 170 E6 $c \cdot mL^{-1}$ were achieved while maintaining viabilities well above 95 %. Subsequently inoculated fed-batch cultures in shake flasks and stirred tank reactors showed that these seed cultures can successfully be used for inoculation, even by extending the seed ratio down to 1:200 and more. Critical quality attributes like glycan profiles in fed-batch cultures originating from normal and intensified seed trains are compared. Furthermore the effect on overall process costs will be discussed using available cost models.

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HIGH-PERFORMANCE PERFUSION PROCESSES USING PREDICTIVE DIGITAL TWINS

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Background and novelty: Continuous cell culture processing is being increasingly used in the production of biologics to achieve consistent product quality and intensify the process. The key challenges in developing a continuous cell culture process are longer development times and higher labor and material costs. While high-throughput small-scale perfusion bioreactors alleviate the labor and material costs, the development times are still very long. Here, we present the Insilico Digital Twin that not only helps optimize the continuous process with a minimum number of experiments but also in a fraction of the time required traditionally.

Experimental approach: The Insilico Digital Twin mimics the dynamics of a cell culture process by combining a model of the perfusion process and a model of the genome-scale metabolic network of CHO cells. This novel approach uses recurrent neural network to determine the dynamics between the extracellular metabolite concentrations and the intracellular flux distributions which results in breakthrough predictive power. Therefore, it can be used for predicting process performance and product quality in a multitude of experimental scenarios.

Results and discussion: In this presentation, we show a case study wherein the Insilico Digital Twin predicts the media design required to achieve high titers at optimal cell-specific perfusion rates. In conclusion, the Insilico Digital Twin not only optimizes a continuous process based on a limited number of experiments, but also significantly reduces the development times.

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CONSIDERATIONS FOR ACCELERATING READINESS TO PROCESS QUALIFICATION

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Background and novelty: In order to be ready for biologics license application, process design and process qualification need to be completed. These represent very significant workpackages and require quite a lot of time. Due to the acceleration of projects, it may not be possible to ensure clinical exposure to material produced with the commercial process and there may be very little opportunity for engineering runs and non for GMP production prior to process performance qualification (PPQ). Here, we present an innovative approach to PPQ readiness by leveraging accumulated small scale studies, engineering considerations, GMP clinical batches, commercial technical runs and statistical approaches.

Experimental approach: A risk assessment was carried out to identify potentially critical process parameters (pCPPs) from the upstream mammalian fedbatch process (ICH Q8R2). The impact of those pCPPs was assessed (i) by performing process characterization studies (PCS) at 2L scale when a suitable small scale model exists; (ii) by using engineering concepts and data at both clinical and commercial scales for parameters that are facility fit dependent.

Results and discussion: To accelerate readiness to PPQ at commercial scale, PCS started before the identification of final critical quality attributes (CQA) and the establishment of the commercial scale process. PCS were therefore performed with a small scale model of the pilot clinical manufacturing process. All preliminary pCQA were analysed. In total 16700 data points were generated at 2L scale during PCS using appropriate sample and data management system, automation and High Throughput technologies. Statistical approaches, targeted process ranges at clinical manufacturing scale and engineering concepts like mixing time and shear rate simulation were used to establish the operating ranges of PPQ at commercial scale.

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ALTERNATE SUGARS AS ENERGY SOURCE IN CELL CULTURE PROCESS

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Background and novelty: Mammalian cells require carbohydrate source for growth in cell cultures. When glucose level is in excess, large amounts of lactate is produced through anaerobic glycolysis. Lactate accumulation in cell culture medium (CCM) will cause low viable cell density (VCD) and decreased cell viability, and thus has detrimental effects on production yield.

Studies have shown that in addition to glucose, other carbohydrate sources, such as fructose, galactose and mannose that can be utilized by most cell types have been reported. However, there are no conclusive studies about how these carbohydrate sources will affect production yield and product quality when use as single or in combination with other carbohydrate sources. The purpose of this project is to find if alternate carbohydrate source (sources) for CCM are feasible without compromising growth, titer and protein quality.

Experimental approach: Six sugars including monosaccharide (glucose, fructose and galactose) and disaccharide (sucrose, maltose and lactose) were tested on CHO cells under both batch and fed batch modes. The level of each sugar in each group was decided according to design of experiment (DOE). Protein quality was assessed by intact mass and charge variants analysis.

Results and discussion: Here we demonstrated that fructose, maltose, galactose can significantly increase cell VCD and titer when added in CCM. In one of the conditions, two-fold increase in VCD and titer was achieved at fed batch mode. And the higher VCD possibly results from lower level of cytotoxic lactate accumulation. Alternate sugars addition didn't significantly affect protein quality, except that galactosylation was increased by the addition of galactose. The study shows that alternate sugars can be used as means to fine-tune monoclonal antibody glycosylation and charge variants profile as needed. Adding maltose, fructose and galactose in CCM can serve as levers to obtain higher VCD and titer in manufacturing process.

P-573

DEVELOPMENT OF A CD MEDIUM FOR RECOMBINANT CHO-GS CLONES

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Background and novelty: Several CHO cell lines (CHO-K1, CHO-DG44 and CHO-GS) are currently being used in the industry for the development of biologics. Each cell line exhibits different nutritional requirements; resulting in variations of their growth profile and quality of protein being produced. Medium development and optimization is an important strategy for bioprocess optimization during the production of biotherapeutics. The use of the GS-knockout CHO host cell line allows for the rapid selection of high producing CHO clones, with little selection pressure (MSX) and low production of toxic reagents such as lactate or ammonia. However, because this cell platform is relatively new compared to CHO-K1 and CHO-DG44 cells, the characterization of the GS-based selection system has not been fully substantiated yet leading to a gap for commercial media specifically designed for CHO-GS.

Experimental approach: This study focuses on the development of a new chemically defined (CD) and animal component free (ACF) medium for CHO-GS clones. Multiple parameters such as buffering capacity, amino acid profile, vitamins and culture conditions were considered. The performance of the new media was evaluated in terms of cell proliferation, culture viability, recombinant protein production, and the glycan profile of the proteins. Each fed-batch experiment was performed in shake flasks and bioreactors to prove their scalability. The newly developed CD medium had better pH control and nutrient utilization profile when compared to competitor media.

Results and discussion: Future work involves understanding the interaction of amino acids, vitamins, trace elements and other culture conditions, to obtain an optimized media for CHO GS clones that would perform well across all CHO platforms by improving cell proliferation, viability and recombinant protein production.

P-574

LEVERAGING MEDIA AND SUPPLEMENTS FOR DESIRED PROTEIN GLYCOSYLATION

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Background and novelty: Obtaining desirable and consistent mAb protein quality attributes such as glycosylation is critical in today's bioproduction industry, especially for biosimilar molecules. The cell line, cell culture media, and process all contribute to the glycosylation profile. While modifications to the cell line and process can require extensive time and effort, optimization and supplementation of cell culture media offers a rapid option for achieving the desired glycosylation profile.

Experimental approach: Cell culture was performed in 24-well shaking deep-well plates (Enzyscreen) and in 125mL shake flasks for a CHO-K1 line and a CHO–DHFR line. Cell growth was determined using a Vi-Cell® XR (Beckman Coulter). mAb production was determined using an Octet® Qke system (Pall). For N-Glycan analysis, mAb purification was performed using Protein A cartridges on the Agilent AssayMAP Bravo® Platform. N-glycans were 2-AB labeled and analyzed using fluorescence detection on Waters UPLC system. Data analysis was done in Minitab © 2019 Minitab Inc.

Results and discussion: A library of 42 distinct in-house CD media was screened for both production and N-glycan profiles of a mAb produced by CHO cell line. Optimal selection of the media required consideration of both overall production and the desired glycosylation profile. A partial least squares model was fitted to screening data and media components in these formulations could be identified, which were correlated to both production and N-glycan profile. Additionally, media supplements were developed, which can increase mAb protein galactosylation without impacting cell growth and production. In batch or fed-batch cultures, galactosylation profiles could be easily modified. These results demonstrate that supplementation of cell culture media enables modification of the glycosylation profile without the need to reengineer the cell line or change the production process.

P-575

DEVELOPMENT OF A CHEMICALLY DEFINED FEED FOR CHO GS CELLS

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Background and novelty: With current advancement in biopharmaceuticals and emerging demands of biosimilars for existing monoclonal antibodies (Mabs) and biologics, the need for optimized chemically defined (CD) and animal component free (ACF) feed supplements and media is as critical as the other stages of biologics development, e.g. cell line development, process optimization and downstream processing. A majority of current Mabs are produced using different Chinese Hamster Ovary host cells (CHO), and these different lineages exhibit different nutrient requirements and they differ from each other in growth profile, metabolism and quality of proteins generated. CHO GS system is increasingly being preferred in the industry as an efficient expression system owing to its shorter development time, high productivity and clonal stability. Kerry has developed a ACF CD feed, which works synergistically with CD media for CHO GS cells, improving viable cell density, cell viability and recombinant protein production.

Experimental approach: Kerry's new CD feed has been optimized for CHO GS system to achieve enhanced protein production focusing on metabolic pathways e.g. amino acids and other nutrient utilization. The performance of this CD feed is demonstrated in several fed-batch experiments in shake-flasks and the scalability evaluated in bioreactors. Efficiency of the CD feed has been compared to commercial product by analyzing cell density & viability, nutrient consumption, metabolite production, recombinant protein production and glycosylation heterogeneity of Mab.

Results and discussion: This Kerry CD feed resulted in equal or superior cell growth performance and IgG productivity compared to competitors' commercial CD feeds. Additionally, here we illustrate the glycosylation profile of Mab protein is comparable to that of when using commercial feed products. Future work will involve testing the versatility of this new feed in different lineages of CHO cells.

P-576

DEVELOPMENT OF SERUM-FREE MEDIUM FOR RECOMBINANT PROTEIN IN CHO CELLS

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Background and novelty: Chinese Hamster Ovary (CHO) cells are widely used in biopharmaceutical industries to generate recombinant biotherapeutic proteins. Cell culture media/ feeds/supplements are some of the important tools for generating high yield and high quality recombinant proteins. Over the years, culturing CHO cells in biopharmaceutical industry has switched from using serum-containing media to serum-free media (SFM media) or chemically defined media (CD media). Though CD media are touted as a solution to the variability seen with the use of serum-free media containing hydrolysates, this is far from reality. Media supplements such as plant-based hydrolysates are designed to provide a boost to productivity, are quick and easy to use, and are compatible with most media formulations. Kerry's Sheff CHO supplements are carefully optimized hydrolysate blends making them superior to competitor products in terms of lot-to-lot consistency. This study focuses on the development of a new SFM media with AmpliCHO CD medium as the basal medium fortified with Sheff-CHO supplements which is totally animal component-free (ACF).

Experimental approach: The new SFM was evaluated using CHO-GS and CHO DG44 clones. Fed batch cultures were performed with and without a feed in shake flasks and bioreactors. The cell growth and metabolic status were measured with Bioprofile Flex, the quantity of productivity was measured with HPLC and the quality (glycosylation of the IgG) was measured with FLR-UPLC.

Results and discussion: The new SFM performed well in terms of cell proliferation and IgG production. In addition, the quality of the product was also found to be consistent with other media tested. This SFM works as a good platform media for users pragmatic about the use of plant hydrolysates in their bioprocess optimization.

P-577

TEMPERATURE DOWNSHIFT AFFECTS SIALYLATION GENE EXPRESSION IN CHO CELLS

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Background and novelty: An effective strategy for process intensification is a temperature downshift (TDS) from 37 °C to 28-34 °C during the exponential growth phase. This has been reported to induce changes on transcriptomic level 24 – 48 h after the TDS[1]. While most regulated genes belonged to energy metabolism, also genes relevant for product quality, like *Neu2*, coding for a cytosolic neuraminidase, were upregulated. This might negatively impact product quality and counteract the positive effects of TDS on process productivity. We were interested in long-term effects of TDS on transcription of genes being relevant for protein sialylation and investigated not only sialidases but also sialyltransferases.

Experimental approach: CHO-K1 cells were cultivated in 2L-bioreactors in fed-batch mode with and without TDS from 37 °C to 33 °C after 3 days. Samples for Illumina sequencing were taken right before the TDS, 24 h after and 2-7 days later. Sequencing was carried out in paired-end mode on two lanes of an Illumina HiSeq1500. Mapping to the genome assembly from Rupp et al.[2] was performed with *HISAT2* and differential gene expression analysis with *DESeq2*.

Results and discussion: Similar to results from Bedoya-López et al.[1] we found *Neu2* to be 2.37-fold upregulated 24 h after the TDS compared to control. Interestingly, 3 days after the shift, the expression dropped to the initial value before the TDS and 8 days after, the expression was even slightly downregulated by 1.16-fold. In contrast *ST3GAL4,* the highest expressed sialyltransferase, was upregulated 1.35-fold 24 h after TDS and expression increase remained constant for the next 7 days. In control culture *ST3GAL4* expression was 1.22-fold downregulated after 7 days of cultivation. These results indicate, that temperature downshift in CHO fed-batch culture influences glycosylation relevant genes in a time-dependent manner and might have positive effects not only on process productivity but also on product quality.

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P-578

STRATEGIES TO IMPROVE SCALE UP AND SCALE DOWN OF UPSTREAM PROCESSES

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Background and novelty: Boehringer Ingelheim (BI) is a leading biopharmaceutical manufacturer with more than 35 years of experience and many successful transferred biopharmaceutical products to market. Our novel BI-HEX® CHO K1 expression platform enables fast-track development of high-quality, high-titer processes including scale up to clinical and commercial scales for producing biopharmaceuticals from CHO cells. For the development of cell culture processes, equipment knowledge is essential, especially since a process is typically scaled up and transferred numerous times in large pharmaceutical companies. The need for a thorough equipment understanding has been also recognized by the FDA in their PAT-publication. To gain such an understanding at full production scale, Boehringer Ingelheim has put tremendous effort in fully characterizing our multiple bioreactors at different scale across multiple sites.

Experimental approach: Computational fluid dynamics (CFD) modeling is utilized which allows the evaluation of integral parameters including energy input, mixing time, shear forces and mass transfer coefficient (kLa). In addition, we have constructed a 15000L acrylic bioreactor model which provides opportunities to validate simulation results with experimental data.

Results and discussion: This presentation will give insight on our novel BI-HEX® CHO K1 expression platform and present our scale up strategy supported by CFD data to optimize the process transfer to our commercial facilities for the manufacturing of biopharmaceutical products.

P-579

LIQUID ENGINEERING: THE OPTIMUM MEDIA ENVIRONMENT FOR BIOPROCESSING

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Background and novelty: One of the key components of bioprocessing is the media environment in which producer cells are grown. Each production run varies and must be tailored to the producer cell and product. Media optimisation remains a fundamental aspect of this process development. The addition of small molecule enhancers (SME) to media, for example, to regulate cell cycle, protein production and the critical quality attributes (CQAs) of products are being tested with increasing frequency. Addition of SMEs to media offers a cost-efficient way to subtly modulate specific aspects of bioprocessing at critical stages during growth and productivity of cells. Suitable culturing platforms are required to streamline this process and offer a range of SMEs for the development of optimium media environments for lead clones. Valita®DESIGN offers one such approach for the development of bespoke media recipes in-house.

Experimental approach: A number of SMEs were selected for screening using the Valita®DESIGN platform, a custom developed culturing methodology which enabled analysis of growth and productivity in a mAb producing CHO cell line. The resulting top performing SMEs were selected for further evaluation based on improving growth and titer. Top SMEs were analysed in a number of applications, including; Day 0 addition, delayed addition (Day 3) and as individual, or as combinatory, additives.

Results and discussion: A number of SMEs were found to improve the growth and titer without negatively impacting cell health, IVCD or qP. We discovered a novel SME, Valita®MOL V3, which improved cell growth and titer and did not negatively impact IVCD, qP or the N-glycosylation of the mAb product. Valita®DESIGN offers companies a SME screening platform to ultimately generate a more tailored, bespoke media environment, targeting specific cell pathways and responses.

P-580

NOVEL DIPEPTIDES FOR CELL CULTURE MEDIA AND THEIR CELLULAR RESPONSE

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Background and novelty: High performance media for efficient biopharma processes need to be closely adjusted to cells needs. Thereby, nutrients must not only be available in sufficient amounts but also stable and easy to use in media preparation, storage and cultivation. This is of special relevance for slightly soluble/instable amino acids (tyrosine, cystine, glutamine). While glutamine containing peptides are applied since decades, tyrosine (beside alanyl-tyrosine and glycyl-tyrosine) or cystine containing dipeptides are rarely available, although there is demand. Moreover, there may be more suitable carriers than in presently established tyrosine dipeptides.

We show a comparative study wherein effects of replacing more challenging amino acids by dipeptides on cell growth, productivity and cellular response on transcriptomic level of different CHO cell lines are evaluated.

Experimental approach: To investigate the potential of dipeptides in cell culture, selected amino acids were replaced by different dipeptides in medium or feed. CHO K1, DG44 and GS cells were used in batch and fed-batch shake flask cultivations to test growth and productivity. To test for relative expression levels of di- and tripeptidases, qRT-PCR analyses were performed.

Results and discussion: Starting from an initial set of more than 35 alternative compounds, 7 different dipeptides were further tested regarding their impact on cell growth and productivity. We found that replacing amino acids by dipeptides in batch and fed-batch result in different growth performances, titers and productivities. Upcoming experiments focus on the production phase to increase the impact of different compounds. Additionally, out of 942 *in silico* predicted relevant peptidases 14 were identified as di- or tripeptidases. Further experiments should reveal new insights into expression profiles of CHO cells after dipeptide treatment.

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SCALE-UP MODEL OF A MAB PROCESS FROM MICOBIOREACTOR TO XDR-1000

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Background and novelty: In process development it is desirable to test process parameters in small scale and later scale up the process into larger production scale. This work demonstrates scalability from micro reactors up to large production-sized reactors. A theoretical scale-up study was performed on a mAb process based on physical characterizations done on the Xcellerex XDR platform. The theoretical study was the basis for the scalability study in XDR systems from 10 to 1000 L. Agitation and gassing settings suitable for the cell line and cultivation process were calculated and tested. Gas settings included sparger selection, gas management, and appropriate oxygen-to-air ratio for efficient CO_2 removal.

Experimental approach: An in-house CHO cell line in ActiPro medium was cultivated in a fed-batch process in scales from 15 mL to 1000 L in the following bioreactors: ambr 15, XDR-10, XDR-50, XDR-200, and XDR-1000. From day 3 the reactor was fed with Cell Boost 7a and Cell Boost 7b once a day. Glucose addition started at day 5 when glucose levels decreased below 2 g/L. Each bioreactor was sampled daily; viable cell density (VCD), viability, product titer, pH, gases, nutrients, and metabolites were analyzed. Product quality (charge variants, glycosylation pattern, and aggregate to main fragment ratio) was analyzed from samples taken at day 7 and at harvest (day 13).

Results and discussion: All investigated bioreactor systems displayed similar trends over time and acceptable variability between cultures for VCD and viability profile, product titer, lactate, and product quality. The work demonstrates process scalability from ambr 15 to XDR systems up to 1000 L scale. It also establishes general agitation and gassing settings suitable for the CHO-based mAb production process.

P-582

DEVELOPMENT OF CHEMICALLY DEFINED MEDIUM FOR VERO CELLS

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Background and novelty: Cell culture media, including currently available animal component free media, contain undefined polypeptides. Knowledge of the exact media composition can accelerate optimization of cell and virus culture processes. For instance, the development of feeds for the virus production phase or fed-batch or perfusion processes. A first step is the development of a chemically defined medium. In collaboration with Biological Industries, the development of an animal component free and chemically defined medium for the growth of adherent Vero cells, and subsequent virus production, was pursued.

Experimental approach: Vero cells were cultured in several media formulations and cell growth was compared with VP-SFM (Life Technologies), a commercially available animal component free (ACF) culture medium. After each set of experiments the formulations were adjusted to optimize cell growth rates and maximum cell concentration in batch. Initial experiments were performed in static T-flasks while the final optimization rounds were carried out in spinner flasks and bioreactors, using Cytodex 1 microcarriers as growth support for the Vero cells.

Results and discussion: The developed medium, commercially available as NutriVero™ Flex 10 (Biological Industries), is chemically defined and does not contain plant or animal components. It supports Vero cell growth in static and bioreactor cultures and is able to support propagation of different type of viruses. In addition, yields for Vero cell growth and infectious virus particles are comparable to a less defined commercially available ACF medium.

P-583

MEDIA DEVELOPMENT FOR SEED TRAIN INTENSIFICATION AND CRYOPRESERVATION

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Background and novelty: Media composition plays a critical role for biopharmaceutical production as well as seed train expansion. Typical seed train operations start by thawing of a single vial followed by several expansion steps. Reaching sufficient absolute cell numbers for production bioreactor inoculation is time-consuming, which leads to a lack of flexibility.

Combining media that are especially designed for their purpose in the production campaign step as well as the application of cryopreserved process intermediates could minimize these challenges: High cell density cryopreservation (HCDC) is a method of freezing cells in bags instead of vials, at higher cell densities. This leads to the advantage that expansion and production can be decoupled: both steps can be separated in space and time. Room classification may decrease due to fully closed processing and reproducibility increased due to a reduction of manual handling steps.

Experimental approach: For designing a seed train expansion medium, a scale down model which represents a typical production campaign was established. This expansion medium was combined with EX-CELL® Advanced HD Perfusion as medium designed for production to ensure constantly high bioreactor productivity.

Additionally, a single-use bag assembly was developed that supports closed filling and inoculation. These were used to simulate a production campaign in lab scale, using a frozen intermediate for inoculation of the perfused N-1 bioreactor, followed by a steady-state perfusion bioreactor step.

Results and discussion: We could show that with this combination of technology, flexibility increases in manufacturing without a loss of cellular productivity. Furthermore, our results indicate that some combinations of media in seed train expansion, including a perfused N-1 bioreactor step, and final production bioreactor, can increase cellular productivity in the final perfusion bioreactor.

P-584

CONTROLLING FAB TERMINAL SIALYLATION OF ANTIBODIES

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Background and novelty: Safety and efficacy of antibody drugs has been linked to carbohydrate structures found on the antibody, termed N-linked glycans. Glycans are mainly found within the Fc-region of an antibody but 20% of IgG antibodies also contain Fab glycans[1]. Glycans are composed of sugars whose presence affects the quality of a drug. Sialic acid is one such sugar; its role is to "cap" the glycan chain, protecting internal sugars[2]. The presence of sialic acid is linked to an increase in half-life along with a reduced inflammatory response[3]. It has been established that bioprocess conditions directly impact glycan composition.

Experimental approach: The effect of pH, temperature, and copper were investigated in regards to their role in affecting Fab sialylation. A mAb was produced in 3 CHO clones, each with varying levels of innate sialylation. Process engineering strategies were conducted on an ambr 15® system. A downward pH shift of 6.8 from 7.1 on days 0, 4, 6 and a day 4 shift to 7.3 were explored. Temperature was shifted from 37°C to 32 °C on day 4. Copper was added at varying concentrations on day 0. Elucidating the mechanistic nature of these strategies and clonal differences, has been performed through a combination of *in vitro* techniques. Glycan analysis was undertaken using a novel method for triple-quadrupole MS and verified with 2AB and LabChipGXII.

Results and discussion: A pH shift was found to have a differential effect, depending on the clone. Cell growth was negatively affected in all three clones, while antibody productivity (qmAb) increased. Effect on sialylation was positive in two of the clones at reduced culture pH. The extent of the effect was correlated to how early the pH shift occurred. A temperature shift was found to negatively affect both sialylation and qmAb in all clones. Copper feeding was found to increase qmAb and overall sialylation in all clones. In parallel, we have studied the mechanism by which the pH influenced enzymatic activity *in vitro.*

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P-585

TROUBLE SHOOTING CASE STUDY: REDUCTION OF COPPER DURING SCALE-UP

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Background and novelty: The design of potent cell culture media is a challenging endeavor. In addition to performance, robustness of stability during storage and preparation are key for a successful medium formulation. Here, we present a case study illustrating observed challenges during scale-up of a fed-batch cell culture process from 2L development scale to manufacturing scale in 12,000L.

Experimental approach: After observation of significant differences in process performance between scales, a structured trouble shooting approach was performed, indicating quality issues for the nutrient feed medium prepared in large scale. In a second step, raw materials and preparation and filtration procedures were investigated applying a broad spectrum of statistical and analytical tools. Ultimately, the identified root cause was verified in lab and manufacturing scale experiments by analytics and cell culture experiments.

Results and discussion: In-depth data analysis did indicate that the quality of nutrient feed medium prepared in manufacturing scale was impacted by preparation procedures. It was clearly demonstrated that copper was reduced in manufacturing scale media preparations. The causal correlation of limited copper availability with reduced process performance was proven in lab scale and manufacturing scale. In a first step, lab scale cultivations with purposely-reduced copper availability could mimic process performance in manufacturing scale. In a second step, a process was designed to reintroduce copper robustly into manufacturing scale. The at-scale verification did demonstrate the restoration of anticipated process performance. In addition, the mechanism of Copper removal during media preparation was investigated. Here, increased hold times of nutrient feed medium caused changes in media quality and ultimately loss of Copper during filtration of the cell culture medium.

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ACHIEVING OPERATIONAL EFFICIENCY IN BIOPROCESS DEVELOPMENT

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Background and novelty: Fragmented development activities combined with the increasing application of high throughput technologies make it challenging for biopharmaceutical companies to keep up with the rapid pace of the highly competitive biotherapeutics market. The increase in the amount of data to be processed and interpreted has also become a major bottleneck. However, the development of safe, effective and life-saving therapeutics relies on data-driven decisions. Therefore, new automation solutions, which unify data stored in silos, and streamlines processes and applications are needed to accelerate the development of manufacturing processes.

Experimental approach: We have co-developed an innovative platform in close collaboration with leading biopharmaceutical companies that supports the entire bioprocess development workflow. It is an E2E platform (Genedata Bioprocess®) that centrally integrates data from post-discovery all the way through transfer to manufacturing. It is tailored to large-molecule therapeutics and provides full traceability of all cell lines, batches and other materials, together with analytics data. It directly integrates with existing IT infrastructure and other scientific data management systems and laboratory instruments.

Results and discussion: We show concrete use cases illustrating the ability to search and track all development candidate molecules, expression constructs, cell line lineage, upstream and downstream batches, samples, and analytical test results within one integrated system. All types of novel biotherapeutics – protein and cell therapeutics – can be managed. We show how the system automatically captures output data (online, at-line & offline) from various equipments. Finally, we demonstrate that a systematic assessment of cell lines, raw materials, processes and molecules can be performed at any process development stage leading to a significant increase in the operational efficiency and throughput.

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MEDIA REFORMULATION; CHALLENGES FOR PROCESS DEVELOPMENT AND SCALE UP.

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Background and novelty: Removing Animal Sourced Material (ASM) from basal medium in cell culture processes can present challenges on scale up and technical transfer. This work describes how a next generation NS0 process for the production of a therapeutic Monoclonal Antibody (MAB) was transferred to a manufacturing facility where formulation changes and operational practices revealed some unexpected process performance effects.

Experimental approach: The basal medium, feeding formulation and feeding strategy changed significantly as part of the process development. During scale up to pilot and manufacturing scales it was identified that this change affected medium stability, cell culture process performance and protection from mechanical stress. It was determined that the mode of addition of media and cholesterol components to the bioreactor was important for cell culture performance and to protect from mechanical stress, particularly during transfer of cells to bioreactors. The newly developed feeding regime necessitated particular attention during the technical transfer to manufacturing scale owing to the impact of pH extremes on culture conditions. Additionally, media hold conditions routinely used in manufacturing operations were found to be unsuitable for this formulation and required modification to ensure the ability of the medium to support cell growth and overall process robustness.

Results and discussion: An NS0 process was developed for the production of a MAB by removing an ASM, increasing the titre output and generating product of comparable quality to the existing process. Challenges encountered on scale up were related to the reformulation of the media and operational practices at manufacturing scale which specifically impacted cell growth and viability, medium stability and protection of cells from mechanical stress. These issues were resolved using bench and shake flask models to ensure process robustness ahead of Process Validation.

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EARLY STAGE PROCESS DEVELOPMENT AND SCALE UP USING HTP BIOREACTORS

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Background and novelty: From the 1980s to the 2000s the product yield of mammalian cell culture processes increased 100-fold by improvements in media composition and process control and led to final product titers of around 1 g/L. By further improvement in cell line development and intensified process strategies it was possible to increase product yields. Nowadays, final product titers of 3 g/L are common in the pharmaceutical industry. This yield is necessary to satisfy the API demand for clinical trials.

Experimental approach: To withstand the competitive environment, Bayer has implemented a platform process, which covers the above mentioned elements for all process scales. Interdisciplinary and interdepartmental collaboration have proven to be a crucial factor to successfully meet this challenge.

Results and discussion: This contribution shows the successful scale up of a current pipeline process from research to GMP manufacturing. This also includes the mayor improvements that have been made during early stage development. High troughput experiments have been conducted using an Ambr15 device. Optimization in feed composition and initial cell concentration led to a significant increase of final product concentration. The optimized process was systematically scaled up to a 1000 L single use production facility yielding similar titers compared to small scale processes.

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A CFD BASED KLA MODEL IN MICROTITER PLATES FOR CELL CULTURE SCALE-UP

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Background and novelty: Microtiter plates are a common tool for clone selection in biopharmaceutical development. A way of visualizing and evaluating these systems and key processes parameters is the application of Computational Fluid Dynamics (CFD). CFD is a powerful tool for the modelling of hydrodynamics and mass transfer parameters. In this work, CFD was used to determine the specific surface area, the volumetric power input and the oxygen mass transfer coefficient kLa for two different microtiter plates with different scales (100 µl – 5 ml).

Experimental approach: For this purpose, a new method of predicting the kLa is presented and calibrated with literature data. Scaling effects in shaken microtiter plates are evaluated by comparing two culture volume scales under various operating conditions. To test validity of these models, three different BI proprietary CHO production cell lines with different growth characteristics were cultivated using the respective microtiter plates under different conditions until limitations in growth and viability were observable. The cell culture data then was compared to different parameters obtained by CFD.

Results and discussion: The calculated kLa values match the cell culture performance in the 96-deepwell plates by predicting lowered oxygen transfer with increasing culture volume and decreasing orbital velocity. The same cells behave differently in the 6-deepwell scale. Here, the overall larger shear stress might cause physical stress for the cells. The kLa model predicts overall higher shear rates for this system, supporting the experimental findings.

P-590

HIGH DENSITY PERFUSION OF HUMAN/ANIMAL CELLS IN SMALL-SCALE BIOREACTOR

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Background and novelty: Process intensification in mammalian cell culture-based recombinant protein production has been achieved by high cell density perfusion exceeding 10^8cells/mL in the recent years. As the majority of therapeutic proteins are produced in Chinese Hamster Ovary (CHO) cells, intensified perfusion processes have been mainly developed for this type of host cell line. However, the use of CHO cells can result in non-human posttranslational modifications of the protein of interest, which may be disadvantageous compared with human cell lines. In this study, a high cell density perfusion process of Human Embryonic Kidney (HEK293) cells producing recombinant human Erythropoietin (rhEPO) was developed.

Experimental approach: A small-scale perfusion system from commercial bench-top screening bioreactors was developed for <250 mL working volume. Steady states for medium (20 x 10^6cells/mL) and high cell densities (80 x 10^6cells/mL), normal process temperature (37 °C) and mild hypothermia (33 °C) as well as different cell specific perfusion rates (CSPR) from 10 to 60 pL/cell/day were applied to study the performance of the culture.

Results and discussion: The volumetric productivity of rhEPO linearly increased with the cell density, remaining unchanged with mild hypothermia and decreased by 37 % when an extremely low CSPR of 10 pL/cell/day was applied. The shift from high to low CSPR strongly reduced the amino acid uptake rates by 45-70 % as their availability became limited. In spite of the low perfusion rate for a very high cell density and consequently reduced metabolism, the culture remained highly stable with respect to maintenance of the cell density and viability. The results from our study show that human cell lines, such as HEK293 can be used for intensified perfusion processes.

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P-591

BAC VS PLASMID – EXPRESSION OF THE "DIFFICULT-TO-EXPRESS" PROTEIN CD19

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Background and novelty: CD19 (Cluster of Differentiation 19) is a transmembrane protein, expressed exclusively on normal and malignant B-cells, which makes it an attractive target for immunotherapy. However, the extracellular domain of CD19 (CD19-ECD) is classified as difficult-to-express protein, characterized by very low product titers and formation of disulphide-bonded oligomeric aggregates attributed to incorrect protein folding. In this study, we designed two different CD19 fusion constructs and investigated their recombinant expression using bacterial artificial chromosomes (BAC) versus conventional plasmid vectors in CHO-K1 cells.

Experimental approach: For proof of concept, stable recombinant CHO-K1 cell lines were generated by application of the same expression construct in plasmid and BAC vectors. Growth behavior and cell line specific productivities were monitored under semi-continuous perfusion conditions. Additionally, various chemical chaperons were tested regarding their influence on product generation. The purified CD19 fusion constructs were characterized relating to aggregate formation and correct protein folding.

Results and discussion: Dependent on the protein construct we were able to observe significant differences between expression yield and maximum cell densities of plasmid and BAC transfected cell lines. In both cases, the addition of valproic acid, known to reduce cell proliferation has proven to be highly beneficial in terms of product titers. Moreover, after size exclusion chromatography, a stable monomeric CD19 fusion protein fraction was obtained, which could apply especially in diagnostics for monitoring of chimeric antigen receptor (CAR) -T cell patients as well as analytics for research and development.

P-592

MITIGATE ADVENTITIOUS AGENT CONTAMINATION RISKS IN CELL CULTURE MEDIA

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al safety is a major concern for biopharmaceuticals. Contamination cases have been reported in the industry. Animal-derived materials are often suspected to be the source of contamination. Risk mitigation could be carried out by inactivating or eliminating viruses of cell culture media. The three common methods are HTST (High Temperature Short Time), UV irradiation or nanofiltration.

Experimental approach: The impact of HTST and UVc technologies was assessed in small scale cell culture systems for different media used in cell culture processes. Different conditions were evaluated: Irradiation time and Flow for UV-C, Temperature and Exposure time for HTST. For each condition, process performance and product quality profiles were measured in comparison with a non-treated media. In parallel, media were characterized to quantify the impact of inactivation methods on critical components (trace elements, vitamins or amino acids).

Results and discussion: For HTST treatment, some slight changes in media and feed compositions can have different effects depending on the process considered. The HTST treatment can slightly modify some of the media and feed components which may in some cases lead to a negative impact on the process performance. HTST can also trigger some precipitations and thus result in clogging of media filters. Thus, for some processes HTST treatment can be directly implemented, while for others it could require further development.

For UV-c treatment, the dose of irradiation that doesn't impact process performance was defined. For higher doses, vitamins were highly degraded due to their high absorbance at wavelength close to the chosen irradiation. Additional experiments have shown that vitamin-supplemented medium is not the solution to offset the strong impact of irradiation. Subsequently, it is necessary to determine if best conditions selected allowed to assure viral inactivation thanks to viral clearance tests.

P-593

A SOFTWARE IMPLEMENTING QUALITY BY CONTROL TO BIOPROCESSES

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Background and novelty: In biopharmaceutical processes quality is still mainly ensured by the use of fixed process conditions and extensive product testing. The process analytical technology (PAT) initiative aims for deeper process knowledge through monitoring of critical process parameters and understanding of their influence on critical quality attributes. Accordingly, the implementation of PAT into production processes allows the transition from the quality by testing to a more flexible quality by design approach.

Experimental approach: In this project we are establishing a software, which allows PAT integration to bioprocesses and enables advanced process monitoring and control. An extensive data set, on which base mathematical models were developed, was generated. Therefore, a design of experiments set-up was applied to Chinese hamster ovary cell culture processes at several scales, reaching from 300 mL up to 100 L. Intensified process monitoring was achieved through a broad analytical platform and the application of multiple advanced sensor systems.

Results and discussion: The developed software is capable of full data-integration, -processing and –management. It allows straightforward application of mathematical models to historical data, as well as running processes. Hence, the software can be used in real-time for model based and predictive control and is thereby paving the way to state of the art control strategies like feed on demand.

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P-594

USE OF A 24-WELL MICROBIOREACTOR AS PROCESS DEVELOPMENT TOOL

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Background and novelty: In recent years, microbioreactors have become an integral part of research and development in the biopharmaceutical industry. This work demonstrates the use of Applikon's® micro-matrix as process development tool. it explores the effect of several bolus and continuous feeding strategies on a GS-CHO cell line in the microscale before translating a selected protocol to the benchtop scale. To our knowledge, this is the first time continuous feeding was documented in a microbioreactor system.

Experimental approach: the dual indicator system for mixing time was employed to determine the mixing time and the static gassing out method was used to determine volumetric mass transfer coefficient (kla) values. The liquid feeding module of the micro-matrix was used to test various feeding strategies for the cultivation of a GS-CHO cell line. characterisation data of both a benchtop bioreactor (5 L) and the micro- matrix were used to perform a scale translation between the systems via either matched mixing time or a combination of mixing time and kla. the success of the scale translation was determined by comparing the growth and production kinetics.

Results and discussion: The resulting mixing times ranged between 0.9–41.8s and are thereby in agreement with previous microwell studies. the kla was mainly affected by the shaking speed and the working volume, whereas variations of the gas flow rate had no significant effect. The kla values ranged between between 2.4–240.8 h-1. The tested feeding strategies yielded no significant differences ($p<0.05$) in both viable cell concentration and final titre. Therefore, the simplest strategy was selected for scale-up. While the scale translations yielded similar production kinetics between the systems, maximum viable cell densities tended to be higher in the micro-matrix. This work demonstrates how the micro-matrix can be used as a process development tool and provides groundwork for further scale translation and optimisation studies.

P-595

INCREASING HD-BIOP3 SEEDING EFFICIENCIES USING VIPS

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Background and novelty: Single cell cloning (SCC) and associated cloning efficiency (colony outgrowth) is currently regarded as an important and discrete step in stable cell line development and cell engineering. The advent of new gene editing technologies, new cell lines, supplements and automated technologies for single cell cloning are rapidly increasing the efficiency of cell line development.

We have previously demonstrated the capabilities and advantages of the VIPS platform over manual limiting dilution for several cell lines. The VIPS results in higher seeding efficiencies over manual limiting dilution whilst giving confidence in clonality thereby reducing the number of plates required for screening.

Experimental approach: In this poster we use the Horizon Discovery HD BIOP3 GS Knockout CHO KI cell line in combination with the VIPS seeding and optimised outgrowth conditions to produce a significantly higher number of colonies per plate, thereby reducing the number of plates needing to be screened.

The experiment was designed to assess the outgrowth of the HD-BIOP3 by manual limiting dilution and VIPS under a variety of conditions.

Results and discussion: VIPS seeding of the HD-BIOP3 cell line with a cloning supplement for outgrowth resulted in approximately twofold increase in clonally derived colonies per plate when compared to manual limiting dilution. VIPS also resulted in reduction in the number of colonies derived from multiple cells. Using the VIPS also allowed the non-clonally derived colonies to excluded from the analysis early on.

Overall, our data show that the combination of the VIPS for high seeding efficiency and the improved outgrowth conditions using the cloning supplement with the HD-BIOP3 cell line is a powerful platform for cell line development.

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HIGH-THROUGHPUT DOWNSTREAM PLATFORM FOR BIOSIMILAR MAB DEVELOPMENT

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Background and novelty: Biosimilar development of monoclonal antibodies is showing a trend increase in recent years since it provides delivery of affordable drugs to the patients. The downstream process is one of the main challenges in biosimilar development due to the critical quality attributes (CQA). Especially aggregates, host cell protein, residual Protein A and residual DNA should be within the limits not to cause any immunogenic reaction in the clinics. A high-throughput platform was developed for development of a high quality biosimilar IgG1 molecule with high tendency to aggregate.

Experimental approach: In order to understand how our molecule behaves with different resins, nine different protein A resins were selected and a high-throughput screening method was developed using pipetcolumns. DoE approach was used and all the quality attributes including aggregate, host cell protein, residual protein A and residual DNA were measured.

Results and discussion: Firstly, the dynamic binding capacities, a range of loading capacities and elution parameters were determined for the protein A pipetcolumns. Using this knowledge and DoE approach in 96 well plate, protein A chromatography was optimized providing the lowest impurity levels and highest yield. For intermediate and polishing purification steps, a fully flow through mode was compared to bind&elute mode in terms of impurity removal using the developed high-throughput platform. Coupling the high-throughput platform with the flow-through mode for intermediate and polishing steps provided us to develop a high quality biosimilar IgG1 molecule with lower aggregate level (≤ 4%).

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P-597

ADVANCING N-1 PERFUSION PROCESS DEVELOPMENT

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Background and novelty: Process intensification via N-1 perfusion and subsequent ultra High Seeeding Density (uHSD) processes has been shown before to dramatically increase space-time yields when compared to regular fed-batch processes. However, process development for N-1 perfusion processes is often hampered by the lack of small-scale high throughput systems resulting in final processes with further optimisation potential. Within this contribution we show that through usage of a novel multiperfusion system, development of a N-1 perfusion process could be dramatically enhanced. Moreover, different feeding strategies in subsequent uHSD processes further increased space-time yields in comparison to regular fed-batch processes.

Experimental approach: The usage of a small-scale (2L) single-use multiperfusion system allowed us to set up a Design of Experiment (DoE) in order to investigate the effect of the cell specific perfusion rate and classical process parameter (e.g. pH) on cell growth and cell viability. Furthermore, uHSD processes were inoculated from N-1perfusion stages and different feeding strategies were applied resulting in variable process performances.

Results and discussion: The DoE approach allowed the identification of single and interaction effects of process parameters on cell growth and cell viability. E.g. interaction effects of cell specific perfusion rate (CSPR) and pH indicated that overflow metabolism at increased pH values could be compensated by increased perfusion rates. With the established DoE model a numerical optimisation was performed resulting in optimal process parameter conditions. Through this approach total media consumption, a major factor for perfusion process development, could be reduced dramatically. Subsequent uHSD processes were optimized through usage of different feeding strategies leading to titer increases of 37% and 87% when compared to regular fed-batch processes.

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CELL GROWTH RATE DETERMINES THE QUALITY OF A MONOCLONAL ANTIBODY

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Background and novelty: The quality of a monoclonal antibody (MAb) is key for its use as a pharmaceutical. In addition to its primary structure, quality is determined by a series of posttranslational modifications that can be chemical or enzymatic. Therefore, both cellular and environmental factors determine antibody quality. Even when several previous research has investigated the effect of culture conditions on the quality of a MAb, few have used continuous well controlled cultures where the effect of a single variable is determined.

Experimental approach: To investigate the effect of growth rate on the quality of a MAb, perfusion cultures operated at the same constant cell concentration but at two different cell growth rates, 20 and 50 % of the maximum growth rate, were performed and compared to fed batch and batch cultures. Perfusion cultures were maintained at steady state for three weeks, and samples were taken to determine the N-glycosylation profile and isoform distribution. A Student's t test was used to determine statistical significance at ($p \leq 0.05$).

Results and discussion: The concentration and specific productivity of the MAb were higher at low growth rate. At high growth rate, a glucolytic lactate producing metabolism was observed, while at low growth rate, a glutaminolytic metabolism resulted in the production of ammonia. Low growth rate decreased basic forms and increased neutral forms. No significant change on acidic forms, which originate extracellularly, were observed. High growth rate decreased the abundance of mannosylated glycoforms from 15 to 5 %, and increased fucosylated glycans. The results show that it is possible to fine tune the characteristics and productivity of the MAB by manipulating the cell growth rate. It was also observed that intracellular events had a more important impact than extracellular events on the quality of a MAb.

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ADDRESSING CHO CLONAL DIVERSITY USING MULTI-OMICS ANALYSIS

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Background and novelty: The development of high-performing feeds for recombinant Chinese Hamster Ovary (CHO) cell lines creates a series of challenges for nutritional needs based on clonal diversity. CHO cell feed development has relied on stoichiometric analysis of metabolites in spent medium to assess the trending and ultimately the depletion of metabolites. The use of design of experiment (DOE) with these metabolites has reliably led to, albeit limited, increases in titer through viable cell density and improved culture viability. Further, the incorporation of novel medium components using a DOE approach is labor intensive, time consuming, and often results in components that are at best, clonally specific or are at worst, ineffective at increasing titer. A plateau has been reached using these traditional cell culture medium development techniques.

Experimental approach: Through the use of multi-omics analysis, we are developing a hypothesis based method to design feed media through analysis of critical pathways focused on specific productivity. Previously, metabolomic and proteomic analyses were conducted on medium formulations with contrasting growth and production characteristics with a CHO-S cell line, and incorporated into a DOE study designed to increase specific productivity. The medium components identified from these studies were incorporated into feed media designed to increase titer and specific productivity.

Results and discussion: The leading feed formulation was tested with Qp-CHO™ Medium with an IgG producing CHO-S cell line resulting in a 287% increase in titer compared to a benchmark fed-batch process. When tested with an IgG producing CHO K1 cell line, there was a 40% decrease in titer compared to the benchmark process. This disparity between CHO cell lines highlights the need to explore a multi-omics approach to identify the differences between clones that will aid in the development of feed media that address the metabolic needs of multiple clones.

P-600

CONTROL OF LIPIDS IN MAMMALIAN CELL BIOPROCESS BY SYNTHETIC BIOLOGY

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Background and novelty: Due to market demand for monoclonal antibodies, a dramatic improvement in fermentation has made primary recovery and purification the bottle neck of the bio-pharmaceutical process. Despite the advantage for increasing yields and shrinking development process there is an increasing pressure in downstream platforms. Process related impurities such as host cell proteins, nucleic acids, lipids and product impurities reduce the ability to purify the product. Lipid impurities change the matrix making the purification process less reliable after repeated cycles of feed applications. To improve the purification of monoclonal antibodies produced by mammalian cell culture, lipid removal by lipase addition was evaluated.

Rational design of industrial host cells to improve bioprocess is a holistic alternative to maximize product manufacturability. An auto-lipolytic chassis using HEK-293 and CHOK1SV derived IgG4 producer cell lines were engineered to raise the levels of robustness achieved previously in primary recovery by exogenous lipase addition.

Experimental approach: A workflow was developed that combined ultra-scale down centrifuge and filtration with design of experiments to screen, solids remaining, lipid concentration and depth filter capacity according to the viability of the cell culture, enzyme addition and the equivalent flow rate to centrifuge.

In addition, inducible lipase was integrated by random non-homologous end-joining into the host cell genome in mammalian cell lines

Results and discussion: Results showed a statistically significant impact of lipase treatment on clarification, when CHO cell broth with low viability and high concentration of lipids was exposed to high shear forces. Depth filter capacity improved (L/m2) throughput can be obtained by addition of 1.035 mg/mL of *Candida Rugosa* lipase.

Moreover, induced lipase expression was not cytotoxic and auto-lipolytic HEK-293 cells reduced by 60% the amount of lipids in the supernatant.

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DEVELOPMENT Of A >10 G/L High-Titer Fed-Batch Cho Platform Process

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Background and novelty: Roche's Global Cell Culture Platform using chemically-defined media has been successfully used in process development for numerous molecules. Several minor version changes have been implemented since its inception mainly to further optimize product quality requirements. However, high lactate levels have been observed in several projects using CHO-K1host cell lines, resulting in sub-optimal culture performance when not addressed by process modifications (e.g., off-platform pH changes, etc.). Understanding the triggers for undesirable lactate metabolism and identifying levers to control lactate metabolism are keys in improving process robustness and enabling further advances in platform process optimization towards higher titers required for high-demand products.

Experimental approach: Using a lactogenic model cell line, we examined numerous potential lactate levers including starting osmolality of production media and other factors that can mitigate the buildup of in-process osmolality (e.g., media components, media powder concentration, feed strategies, and process parameters). The results from these studies were then used to further optimize our existing platform media and process to develop a high titer fed-batch process.

Results and discussion: Bioreactor studies identified starting osmolality as a key lever to minimize lactate production for some cell lines. Additional studies with numerous mAb producing cell lines demonstrated that the newly developed process was applicable across our platform host cell lines, with final titers ranging from 10-15 g/L. The optimized fed-batch process, which still fits within our current manufacturing network, will significantly reduce cost of goods and runs required to support clinical and commercial production of our biopharmaceutical proteins.

P-602

ARE YOU FEEDING MORE CELLS THAN YOU THINK?

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Background and novelty: Perfusion-based process intensification has gained lot of attention in recent years as a biomanufacturing platform because of its high cell densities that result in increased volumetric productivities. However, the process to work with these high cell densities has its own challenges driven by the high oxygen and nutrient demand. The cell culture medium developed in this work had previously been developed to supply the needed nutrients[1]. In this study we show how changes in the process, with adjustment of specific non-nutritional cell culture media components, can enable the increase of cell density by 2.5-fold, reaching concentrations above 250x10^{6}vc/mL at 2vvd (CSPR<10pL/cell/d) – while maintaining or increasing viability by minimizing the cell damage caused due to the generation of shear stress and foam primarily caused by the spargers.

Experimental approach: We modified bioreactor process parameters such as agitation speed, gas flow rates and the ratio of macro/micro bubbles to maximize kLa while decreasing shear. Concurrently, we evaluated the effect of non-nutritional media components on cell growth and viability. We then combined this knowledge in cell culture perfusion bioreactors.

Results and discussion: We determined the inhibitory effect on cell growth and viability generated by a specific component that is required in the culture. At the same time, we identified a method to reverse this loss on growth and viability. The application of this optimized process in a dynamic perfusion (no bleed) at 2vvd resulted in a cell density of 250x10^{6}vc/mL with viabilities of 97% and a volumetric productivity of 2g/L/d (2.5-fold increased from previous processes).We applied the same process to steady state perfusion maintaining target cell densities ranging from 50 to 200x10^{6}vc/mL at 2vvd (CSPR from 40 to 10pL/cell/d) or lower.

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P-603

SIMULATING LARGE-SCALE BIOREACTOR HETEROGENEITIES IN MINI-BIOREACTORS

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Background and novelty: When scaling up from bench-top to production-scale, bioreactor mixing times increase making heterogeneities within large-scale vessels more likely. Most studies in literature simulate these heterogeneities using bench-scale stirred tank reactor (STR) compartmental models with microbial fermentations. However, similar work on animal cell culture is sparse. One way to assess the effect of these heterogeneities on the overall performance of a particular culture requires a number of modelling approaches including computational fluid dynamics (CFD) and population balance (PB) models [1,2].This work aims to establish a combined experimental and modelling approach to simulate overall cell growth and antibody formation kinetics in the heterogeneous environment found in production-scale bioreactors, using a novel approach with two miniature bioreactor systems.

Experimental approach: Initial experimental work has focused on establishing a methodology on the Micro24 and Ambr15 systems, which enables the precise fluctuation of process conditions, specifically pH and dissolved oxygen (DOT). Performance metrics such as growth kinetics, productivity, and metabolite concentrations, are used to assess the effects of the fluctuating conditions.

Results and discussion: The work presented compares results from the systems, which were both successfully able to fluctuate pH and DOT with varied duration and frequency. Fluctuating DO has been shown to have a cell line-dependent effect on culture performance. A CFD model is being developed to better inform future experiments. Data from more cell lines could then be fed into a population balance model to predict their performance at large-scale. This work has the potential to improve cell line screening strategies and help to identify cell lines that are affected by heterogeneities.

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P-604

INSECT CELL PROTEIN PRODUCTION: NOVEL HIGH VOLUME SHAKER FLASK TECHNOLOGY

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Background and novelty: The production of recombinant proteins in the baculovirus expression vector system (BEVS) in R&D laboratories utilises either shake flasks or cell culture bags for insect cell culture scale-up. Standard insect cell culture in shake flasks utilises wide bottom Fernbach flasks with a limited cell culture volume, typically 30% maximum, in order to ensure adequate culture aeration. To scale up past the 1L volume, multiples of such cultures may be used or, more commonly, growth in a cellbag-based system, such as the Wave BioReactor, where volumes of 5L or 10L are typically conducted. Shifting to a cellbag system requires investment in rocker platforms, air pumps, media pumps and tube welders to facilitate the transfer of sterile media. However, we have determined that recombinant protein expression levels in BEVS are not restricted to these traditional volumes and, contrary to expectations, insect cell cultures demonstrate standard growth curves and protein production levels when the culture volume represents as much as 90% of the flask volume. This allows for the elimination of cellbag technology for scale-up protein production.

Experimental approach: Insect cell growth curves were carried out at various volumes in various sizes of Fernbach flasks with a variety of insect cell types. In addition, protein production levels using various cell culture volumes were carried out and compared for a variety of proteins.

Results and discussion: The data demonstrate that insect cell culture volumes up to 90% of the capacity of Fernbach flasks show typical cell growth curves, and that the level of protein production typically mirrors that obtained using traditional culture volumes. This has allowed Pfizer's La Jolla Labs to eliminate cellbag technology completely from its protein production platform, and to deploy simple shake flask technology for protein production requirements.

P-605

A MULTI-SCALE APPROACH TO CHO MEDIA BENCHMARKING

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Chemically-defined cell culture media have become a golden standard of modern CHO production processes facilitating regulatory approval. A well-chosen combination of production clone, process conditions, and cell culture media can ensure optimum process performance with regard to product titer and product quality. This often necessitates finding suitable clone/media combinations before embarking on process development unless a platform process is already in place.

We have applied a multi-scale approach for media benchmarking making use of the ambr15 mini-bioreactor platform for media screening of various clone/media combinations. The results obtained in micro-bioreactors were validated in 5L stirred-tank reactors, suggesting scalability of the processes to production scale. Media and clones were compared to a leading Chemically-Defined (CD) commercially available medium in regards to growth and productivity performance as well as N-glycosylation profiles. The results suggest higher performance of Sartorius CHO-CD medium in terms of cell growth, viability, titer, and glycoprofile compared to the reference medium.

The results were compared against those obtained by traditional shake flask screening and highlight the benefit of conducting media benchmarking studies and near-process conditions in controlled environments.

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AN END-TO-END CELL CULTURE PROCESS DEVELOPMENT PLATFORM AND ITS APPLICATION TO BIOSIMILAR PRODUCT DEVELOPMENT

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To overcome the challenges related to cost of development, timelines, scalability, productivity and product quality, a proprietary cell culture process development platform was developed. This platform contains specifically selected host cell lines, proprietary expression vectors, and highly productive cell culture media and feeds (chemically defined), as well as an optimized upstream and downstream process. We applied this platform successfully for several projects and resulted in very productive cell culture processes. The performance of this platform met and, in most cases, exceeded the results obtained from commercial platforms. Without any optimization high cell densities (30 MM/ml), high viabilities (>90%), and high titers 4-6 g/L could be obtained in a 14-day fed-batch culture. In this work we will present the details of this platform and the data obtained in one of the biosimilar development programs in our pipeline. Cell culture performance and analytical characterization data (peptide mapping, MS analysis, glycosylation, charge variants, subunit analysis, etc) will be discussed.

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VALIDATION OF CHEMICALLY DEFINED MEDIA FOR VACCINE AND GENE THERAPY PRODUCTION

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Key Words: BHK-21 cells; Sf9 cells; rabies virus; AAVs; media benchmarking

BHK-21 and Sf-9 cell lines has been utilized for a number of years as successful platforms for vaccine manufacturing. However, in many cases the limiting factor in regulatory approval of these systems for clinical applications is the presence of serum, as the world health organization has set limits to the amount of bovine serum in vaccines, or the use of a non-chemically defined medium due lot-to-lot variability in raw materials. Thus, media selection play an important role in the optimal cell growth and cellular production to maximize virus titer yield. In this study, a variety of Sf9 and BHK-21 media were tested to assess cell growth, viral titer production as well as analysis of cellular metabolism.

In the first study, a novel prototype Chemically-Defined (CD) Sartorius (SSB) medium was benchmarked against a leading serum-free commercially available medium termed reference medium. Sf9 cells were successfully adapted to CD SSB-Insect medium used for rAAVs production, with constant cell growth rate being achieved 11 days after thawing. Cells were inoculated at 1.106 cell/mL in both media and the growth kinetics were assessed. rAAV were produced in Stirred Tank Bioreactors STB (n=3) using the IC-BEVS and CD SSB-Insect medium, and production yields benchmarked against reference medium (n=3). The infection kinetics showed no significant differences being observed between the two culture media. On the one hand, in terms of cell metabolism, glucose consumption rate in CD SSB-Insect medium was at least 2-fold higher than in reference medium. On the other hand, lactate production rate was significantly lower in CD SSB-Insect medium than in reference medium. Ammonia was consumed in CD medium while in the reference medium it was produced. No significant differences were observed in glutamine consumption rate for both culture media. Production of rAAV using insect Sf-9 cells adapted to CD SSB-Insect medium and IC-BEVS was successfully demonstrated at bioreactor scale, with rAAV titers (in both per cell and per volume basis) in CD SSB-Insect medium higher than in the reference medium. These results demonstrate the feasibility of utilizing the CD SSB-Insect medium for successful growth of insect Sf-9 cell culture and high-titer production of rAAV with improved metabolic profile.

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AN EASY-TO-USE MEDIA SUPPLEMENT FOR INCREASED BIOMOLECULE GALACTOSYLATION

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Background and Novelty: Glycosylation, especially galactosylation, is a critical quality aspect that can significantly alter antibody (Ab) binding, function, and therapeutic effect. Herein, Irvine Scientific developed an effective, easy-to-use media supplement designed to increase galactosylation during the production of biomolecules. This 'Optimized Supplement' significantly increased Ab galactosylation without negatively impacting cell growth and titer. In comparison studies, the Optimized Supplement outperformed a similar supplier's supplement and resulted in better Ab galactosylation and function in in vitro cytotoxicity assays.

Experimental Approach: FUJIFILM Irvine Scientific's approach of Rational Culture Media Design® was applied to the development of the Optimized Supplement. Design of experiment (DoE) studies were done in fed-batch cultures of Chinese hamster ovary cells expressing an IgG1 Ab against CD20. Cell growth, Ab titer, and glycan analysis were assessed by a Beckman Coulter Vi-CellTM XR, a Pall FortéBio Octet® QKe, a PerkinElmer LabChip® GXIITM, respectively. For the functional complement-dependent cytotoxicity (CDC) assay, Abs were incubated with lymphoblast cells and normal human complement serum. Cell cytotoxicity was assessed with a Promega CytoTox-GloTM kit.

Results and Discussion: With a bolus day 7 addition at 0.4-1.8% (v/v), the Optimized Supplement increased Ab galactosylation from 20% in the control to a plateau of 50%. The other supplier's supplement reached a maximal galactosylation of 45% at 2% (v/v). Both the Optimized Supplement and the other supplier's supplement had little to no impact on cell growth and titer. In in vitro CDC assays, the Abs from the Optimized Supplement showed an increased killing efficacy and decreased EC50 values compared to the control Abs and Abs from the supplier's supplement. Overall, the Optimized Supplement effectively and efficiently increased Ab galactosylation and function.

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