Advanced Cell Technologies: Making Protein, Cell, and Gene Therapies a Reality

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Abstract

Researchers and engineers came together in Lisbon at the 27 th Meeting of the European Society for Animal Cell Technology (ESACT 2022), to discuss the latest advances in technologies associated with protein-based biologics production, new modalities and cell, gene and tissue therapies. Main contributions focused on how the capabilities of production platforms can be enhanced, and how to leverage them to generate new products. Some of the advances that were presented are discussed below, including those related with cell line development, metabolic engineering, analytics, CHO and insect cells platforms engineering, vesicle and viral vector production, and gene and cell therapy, along with some concluding remarks on the future of this important field.

Editorial

Advanced Cell Technologies: Making Protein, Cell, and Gene Therapies a Reality

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Researchers and engineers came together in Lisbon at the 27th Meeting of the European Society for Animal Cell Technology (ESACT 2022), to discuss the latest advances in technologies associated with protein-based biologics production, new modalities and cell, gene and tissue therapies. Main contributions focused on how the capabilities of production platforms can be enhanced, and how to leverage them to generate new products. Some of the advances that were presented are discussed below, including those related with cell line development, metabolic engineering, analytics, CHO and insect cells platforms engineering, vesicle and viral vector production, and gene and cell therapy, along with some concluding remarks on the future of this important field.

Improving CHO cell line performance through cell and bioprocess engineering

A continuing goal in mammalian biomanufacturing processes is to evaluate, modify, and ultimately enhance the performance of CHO cells in culture. Indeed, one of the major challenges in CHO cell culture is long term cell line stability. Torres *et al* . took a deeper look into this issue, examining changes in cell culture performance, gene expression, and metabolism over time for two cell lines. Especially interesting was the

upregulation of genes involved in cell proliferation and survival concomitantly with changes in metabolites' uptake and production rates. Acknowledging the impact that culture time has on the cell, researchers are now exploring ways to improve cell line stability, aiming to design more predictable, consistent, and productive expression system. One of such approaches is targeted integration; however, identifying a CHO cell genomic loci capable of supporting high-level protein expression is still a bottleneck. To address this need, Lee *et al* ., implemented the "Thousands of Reporters Integrated in Parallel" (TRIP) high-throughput screening method, identifying several hotspot candidates in the CHO genome exhibiting high transgene mRNA expression. In another study, Marx *et al.* presented a fast and robust method -the nanopore Cas9-targeted sequencing (nCats) pipeline - to characterize cell clones and isolate the most promising ones. This method was able to identify integration sites, the composition of the integrated sequence, and the DNA methylation status in CHO cells in a single sequencing run. Building up on CRISPR/Cas9 technology for mammalian cells, Lee *et al* . developed an all-in one reporter system to quantify gene disruption and site-specific integration (SSI) in CHO cells. Using this system, it was possible to identify specific molecules (inhibitors of DNA repair pathways) that enhance SSI efficiency and thus accelerate cell engineering. Another approach to further improve mammalian cell factories is to ameliorate cell's capacity to handle proteotoxic stress, which can result in cellular apoptosis. In Segatori *et al.* , the challenges and opportunities in synthetic biology for improving these programmable cell factories is detailed. An important contribution to the field of cell line and metabolic engineering is provided by Kontoravdi *et al.* with a review on the current state of CHO genome-scale metabolic models (GEM), their inability to model intracellular metabolism and capture extracellular phenotypes. In addition, Kontoravdi *et al* . presented an improved GEM, iCHO2441, as well as two cell line specific GEMs for CHO-S and CHO-K1 that may serve as foundations for better design and assess next-generation flux analysis techniques. Jimenez del Val *et al* . propose a more compact network model, CHOmpact, that can provide improved interpretations from simulations, including identification of shifts in key metabolic behaviours. This model could also serve as a platform for dynamic models used for process control and optimization. The advancement in process analytical techniques (PAT) and artificial intelligence (AI) has enabled the generation of enormous culture datasets from biomanufacturing processes. AI-based data-driven models permit the correlation of biological and process conditions and cell culture states. This approach was exploited by Lee *et al.*that describe data-driven prediction models for forecasting multi-step ahead profiles of mAbs produced in CHO towards bioprocess digital twins.

A complementary approach to cell line engineering is the development of improved biomanufacturing processes. To address that need, Ben Yahia*et al.* worked on intensified processes by optimizing the feeding strategy and specific power input (P/V) in a high-cell-density (HCD) seed bioreactor operated in fed-batch mode towards improved monoclonal antibodies (mAb) expression in the production bioreactor. Interestingly they report a positive impact of cellular "organized stress" in the seed bioreactor on the production performance. Chotteau *et al* . implemented a Design of Experiment (DoE) approach to design an optimal CHO culture medium capable of supporting the operation of microbioreactors in perfusion at HCD and low specific perfusion rates while maintaining constant specific product quality attributes such as N-glycosylation profile of the produced antibody. Using a similar statistical design methodology, Ladiwala *et al* . fine-tuned specific amino acids levels in reference basal and feed media in order to limit production of inhibitory metabolites, ultimately enhancing peak viable cell densities and product titers. Alternatively, Naik *et al.* looked at adding glycolysis inhibitors to limit the production of lactate as metabolic by-product in CHO cell cultures. They found that specific glucose analogs could lower peak lactate concentrations while also providing an increase in the final titers, although there was some changes in glycosylation patterns indicating an effect on product quality.

Viral vectors, extracellular vesicle, and vaccines

With the rise of new modalities such as viral vectors, extracellular vesicles and vaccines, there is a pressing need to continue improving biomanufacturing capabilities of these biologics. Several presentations at ESACT 2022 were devoted to addressing this topic, some of which being reflected in this special issue as a way of recognizing the enormous efforts of the scientific community to date in this area which are likely to further expand in the future as these modalities become an increasingly important part of the biomanufacturing

landscape.

Scalable and cost-effective bioprocesses for production of viral vectors for gene and cell therapy are currently in high demand. Thus, developing new, innovative technologies for production of these products is an emerging thrust in biomanufacturing. One of the most used vectors in gene therapy is the recombinant Adeno-Associated Virus, or rAAV, produced in a variety of cell types. As an alternative to traditional transient transfection systems using HEK93 cell lines, Escandell*et al* . developed a scalable system for rAAV production using a stable HeLa cell line; it proved to be time-efficient and easy to scale-up while capable of generating high rAAV yields and full to empty capsid ratios. Another increasingly important platform for rAAV production is the insect cells-baculovirus expression vector system (IC-BEVS). To understand the variability in insect populations and its impact on rAAV2 titers, Isidro *et al* . analyzed the Sf9 insect cell transcriptome using single-cell RNA-seq. While transcriptional heterogeneity in Sf9 insect cells prior to infection exists, mainly associated with cell cycle, that is exacerbated upon infection with the differential expression of baculovirus genes and rAAV transgenes. The genes and pathways identified will inform the path forward for cell and process engineering towards improved rAAV2 production. Finally, Yoon*et al.* reviews the advances made in rAAV bioproduction as well as the challenges ahead in making these therapeutics accessible. Lentivirus (LV) are another important viral vector used in gene and cell therapies for which large scale production remains a major challenge. Aiming to solve this issue, Klimpel *et al* . developed suspension-adapted stable packaging cell lines in a scalable and serum-free production process, and compared alternative methods to remove doxycycline in order to initiate LV generation. Oncolytic viruses, another emerging modality, shares the same bioprocess limitations as rAAV and LV. Genzel *et al* . developed an optimized perfusion process for large-scale production of recombinant vesicular stomatitis virus-based fusogenic oncolytic virus (rVSV-OV). Three cell lines (AGE1.CR, BHK-21, and HEK293SF) were evaluated in HCD cultures, with a 15-30-fold increase in volumetric productivities being observed compared to batch process, establishing perfusion as a viable process for large-scale production of rVSV-OV.

In the area of vaccine development, Palomares *et al.* applied phage display technology to produce epitopes (or mimotopes) that when attached to AAV virus-like particles (VLP) can produce an antibody response to recognize and potentially protect against Zika and Dengue viruses. In another study, Lorenzo *et al.* described a process to purify HIV-1 Gag VLPs from contaminating host extracellular vesicles using a series of downstream isolation stages including multiple filtration and chromatography steps.

Another emerging modality for delivery of therapeutics are extracellular vesicles produced by mammalian production hosts, and a number of studies explored methods to further improve their capabilities. In one study, Estes *et al* . identified specific metabolic pathways in HEK293 and CHO-S cell lines capable of improving extracellular vesicle titers using high throughput siRNA screens. Cholesterol biosynthesis was one of those pathways, results showing that the addition of statins to cell culture increased vesicle productivity up to 9-fold. In addition, Belliveau*et al* . investigated the impact of osmotic and ammonia stress on the microRNA (miRs) within extracellular vesicles (EVs) of CHO cells. While normal culture conditions included higher levels of mir-92a and mir-23a in EVs, stress condition resulted in the enrichment of let-7a, let-7b, and let-7c miRs, which regulate core oncogenes and may alter the balance between cell cycle and apoptosis.

Stem cells and cell therapies

Other important emerging modalities reviewed at ESACT 2022 were stem cells and immune cells; there were several presentations examining the development of these cell-based products, some of which are included in this special issue. For example, Vives *et al* . evaluated the impact of 3D printing to preserve critical quality attributes in bone tissue regeneration using natural hydrogel scaffolds. Interestingly, hyaluronic acid, gelatin, and fibrin applied as bioinks enabled optimal recovery of mesenchymal stromal cells while also preserving proliferation and osteogenic capacity by reducing shear stress and providing structural support. In addition, Costa *et al* . described an integrated bioreactor and downstream process enabling the scalability of mesenchymal stromal cells production of extracellular vesicles, which yielded considerably higher yields than those achieved with static cultures while preserving the quality of the EVs and their ability to stimulate angiogenesis. Fed-batch alone or in combination with perfusion were described by Fernandes-Platzummer

et al. as a process to generate bone marrow-derived MsCs and MSC-EV in stirred tank bioreactors. To complement these experimental investigations, Lewis*et al* . proposes an *in silico* approach (using mathematical models) to describe cell-to-cell communications important to immune cells cell functions and ultimately immunotherapies.

An overriding topic at the conference (and the field of biomanufacturing) was the challenges and opportunities of introducing new therapeutic platforms. This special issue starts with a publication by Schaefer *et al* . that discuss the barriers, including technology, business, regulatory and people-driven concerns, that arise when introducing new therapeutic technologies and present enablers for reducing the gaps between advances and innovation in biopharmaceutical manufacturing and final therapy delivery to patients.

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