Innovations in Cell Line Development to Optimise Biotherapeutic Development

Developing stable and high-producing cell lines to manufacture biologics is complex, multi-stage, and time-consuming, often resulting in development timelines that exceed six months when using classical techniques. This represents a frustrating bottleneck in biotherapeutic development, a market where speed-to-clinic is a priority.

The challenge to overcome these limitations and meet the increasing demand for novel biologics encourages constant innovation in the manufacturing processes. This article presents some of the latest strategies to optimise product concentrations and productivities, and high-throughput automated methods to accelerate screening, while reducing manufacturing costs.

Biologics represent the fastest growing sector of the pharmaceutical industry, and this constant growth has been complemented with significant improvements in upstream process development.[1] Many biopharmaceutical companies are acting upon new insights and experimenting with ways to accelerate traditional cell line development timelines. Three novel advancements that have had a significant impact on these processes are as follows: 1) a novel method to influence the charge profiles of the biologic to improve cell-specific productivity, 2) a technique developed to precisely modify the critical quality attributes of the biologic to optimise effector functions, and 3) an automated, high-throughput approach to search and select the optimum high-yielding proven monoclonal cell line.

Harnessing Citrulline for Improved Cell Culture Productivity

Advances in cell culture systems, focused on improving process efficiency by increasing volumetric productivity, can be applied to facilitate process optimisation. Conventional cell culture strategies generally focus on improving the viable cell density (VCD) during upstream processing to increase volumetric productivity. However, while a high VCD results in higher product titres, such changes often lead to difficulties downstream due to the increased complexity of clarification and the requirement for larger filter surfaces. A higher VCD is also associated with a higher concentration of host-cell-related impurities, which researchers must remove during the downstream processing phase, thereby increasing the overall cost of production.

Recent studies to increase specific productivity show that the use of citrulline is a promising alternative.[2] In contrast to conventional practices, the effectiveness of this technology is based on increasing the cellular volume, rather than increasing the VCD. The addition of citrulline to the culture medium increases the volume of mammalian cells by more than two-fold while simultaneously decreasing the volume of the culture medium by 60%, with no negative effects on total biomass or productivity. Furthermore, citrulline has the potential to modulate the charged profile of a product by lowering the percentage of acidic species in the product's composition.

Citrulline has also been shown to reduce the protein content of host cells by 40% while simultaneously increasing the filterability for clarification by 40% per gram of product. This is due to the increase in cell size and, consequently, the increase in cell-specific productivity. The latter has significant implications for the good manufacturing practice footprint of the cell culture clarification step. With an increase in specific productivity, less cell debris needs to be removed during clarification, resulting in less product loss and, in turn, lower costs.

Enhanced Modulation Strategy for Improved Process Efficiency with

In addition to increasing volumetric productivity, optimising product quality is critical in the production of biotherapeutic proteins. The majority of biologics are composed of recombinant N-glycosylated proteins that are produced in mammalian expression systems, such as Chinese hamster ovary cells. These biologics require human-like post-translational modifications and a cellular system to perform the complex protein folding and glycosylation steps crucial for therapeutic efficacy. Post-translational modifications are dependent on many parameters, including the host cell line and culture conditions, which, coupled with the inherent variability of the cellular factories during bioproduction, can introduce significant issues.

Difficulties occur as recombinant biotherapeutic proteins have the potential to misfold and stress the secretory pathway, resulting in protein degradation or cell death. Multi-chain biotherapeutic proteins may fail to pair correctly, and insufficient or incomplete glycosylation can occur, affecting the biological activity, stability, and immunogenicity of therapeutics. Furthermore, variation in N-glycan species can have an impact on effector functions, including antibody-dependent cell-mediated cytotoxicity and complement-mediated cytotoxicity, both of which are associated with drug efficacy.

Accordingly, engineering approaches to reliably generate complex proteins with the correct post-translational modifications are an area of continuous research and development. Recent findings show that researchers can achieve improved control over the production of biologics by leveraging an advanced process modulation toolbox that draws upon advances in pathway platform knowledge and the requirements for growth and production, integrated with an understanding of the cellular environment.[2] Using the modulatory strategy, it is possible to specifically modify critical quality attributes of novel biologics, including terminal galactosylation, core-fucosylation, and mannosylation to enhance efficacy. In conjunction, it is possible to modulate the charge profile of the product by using

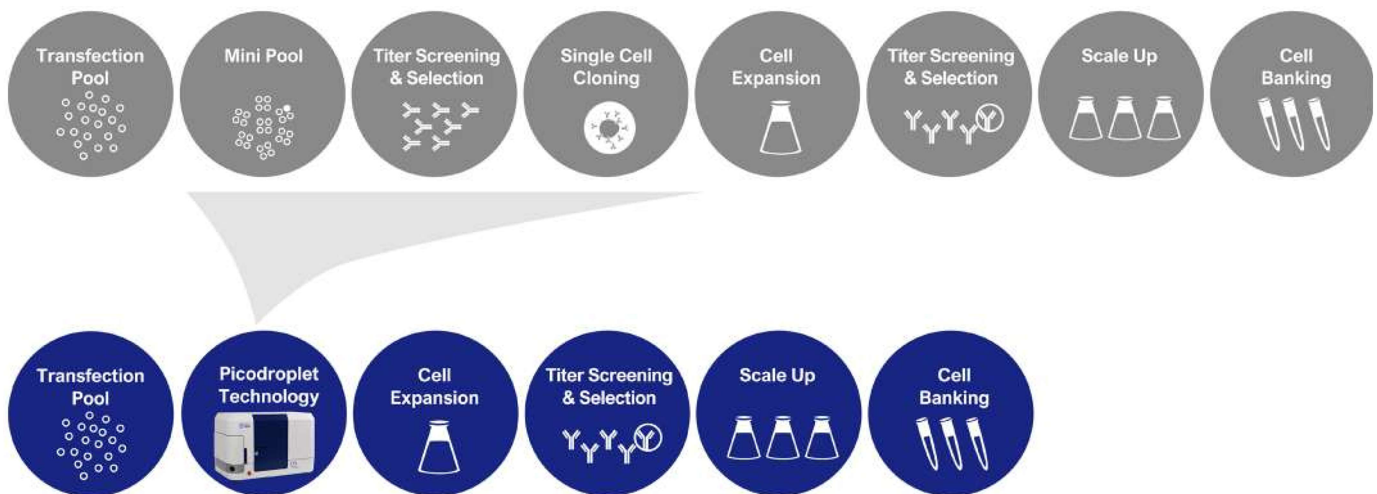

Figure 1. A high-throughput screening workflow to significantly reduce timelines and bring more molecules towards commercialisation.

molecules like citrulline, as described above. Another essential factor to consider is the optimisation of culture media.

Automating Screening and Selection of Production Cell Lines

Several aspects are considered when choosing a production cell line. The optimum cell line will have high cell-specific productivity and titres, demonstrated scalability and stability, as well as well-documented proof of monoclonality to meet regulatory requirements. The subsequent selection process to identify the desired cell line is traditionally a lengthy process. Major bottlenecks include heterogeneity in a clonal cell population, requiring extensive screening for desired traits, and the regulatory requirement for assurance of monoclonality, demanding multiple rounds of single-cell cloning.

Selecting single clones has conventionally been achieved using the classical, resource-intensive method of limiting dilution or semi-automated technologies, such as fluorescent activated cell sorting, colony picking, and single-cell printers. Once isolated, clones are imaged using instruments such as cell-in-well imagers and then evaluated for productivity, before progressing into small-scale bioreactors for batch production. Overall, this is a costly, time-consuming, and resource-intensive process, requiring separate pieces of equipment for each technique, taking up valuable laboratory space and increasing the risk of sample contamination. As a result, the industry's research and development efforts to consolidate steps in the cell line development workflow and alleviate some of these limitations continue to be a major area of focus.

Utilising the combined benefits of picodroplet-based microfluidics and automation, researchers can now bypass the traditionally time-consuming and multi-step selection, isolation, and verification process with a fully automated, high-throughput screening solution. In doing so, optimum cell lines capable of delivering high productivity and antibody quality can be identified through more commercially competitive workflow timelines. Given their potential to reduce critical path timelines, picodroplet single-cell analysis systems have proven to be transformative in the field of cell line development (Figure 1). Studies show that high-throughput microfluidic technologies, such as this, significantly improve the accuracy and efficiency of screening and culture development, enabling large numbers of cell lines

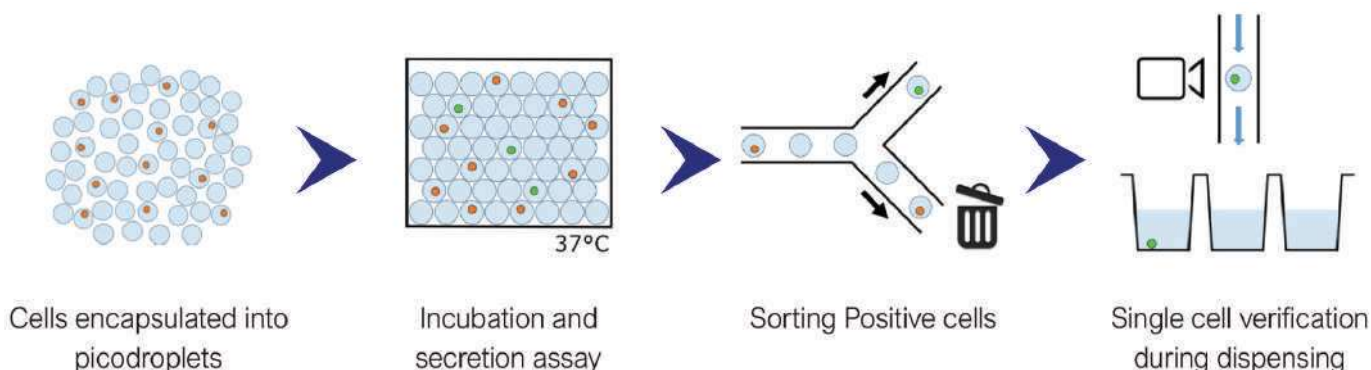

Figure 2. An integrated workflow packaging the screening, sorting, isolation, and verification of high-secreting clones into a fully automated process.

to be assessed early in the development process and providing high assurance and probability of monoclonality.[3,4]

Picodroplet-based microfluidic technologies encapsulate single cells into picolitre-volume aqueous droplets. These miniaturised picodroplets provide a highly controlled microenvironment for studying individual cells, chemical and biochemical reactions, and cell-cell interactions, while preserving cell viability. Picodroplet technologies are increasingly applied to optimise cell line development, offering several advantages over conventional processes, including high single-cell screening throughputs, rapid-yet-gentle cell processing, highly sensitive quantitative assays, and reduced reagent costs.[3,4]

Within a picodroplet, encapsulated cell-secreted molecules quickly accumulate to a concentration that can be measured consistently and quantitatively utilising a Förster resonance energy transfer theory (FRET) assay. In a FRET experiment, two fluorescent probes, the donor and acceptor, will attach to the target secreted molecule. The binding event brings the probes together, resulting in energy transfer from donor to acceptor, seen as a reduction in the donor's fluorescence signal and a rise in the acceptor's fluorescent signal. Picodroplets containing desired cells can then be fluorescently sorted and distributed into individual wells on a microtitre plate for downstream analysis.

Fully integrated instruments, actuated through intuitive single-cell analysis software, also incorporate high-speed imaging into the dispensing step, allowing researchers to recover high value clones with clear visual evidence of monoclonality, without additional screening or instrumentation (Figure 2). During dispensing, as the picodroplet travels through the microfluidic channels, the encapsulated cells are imaged multiple times to provide visual verification of clonality. Additionally, in requiring less equipment, reagents and hands-on time, the integrated instrumentation minimizes operating costs to enable more cost-efficient upstream processing, and enhanced biologics manufacturing capabilities.

Conclusion

Novel developments have had a considerable influence on the cell line development process, helping to increase cell-specific productivity, improve process efficiency and establish high-throughput methods to advance the generation of high-quality clonal cell lines with monoclonality assurance. These innovations in host cell line optimisation, selection, and screening strategies can be combined to improve product consistency, quality, and robustness. In doing so, biologics development processes are streamlined and less labour-intensive, to prioritise speed to clinic.

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