BIOPROCESS ONLINE

Article | November 29, 2022

Significant Improvements In Total Lentiviral Titer And Quality Seen By Implementing Process C, A Perfusion-Based System

Source: Oxford Biomedica

By Carol Knevelman, Vice President, Head of Process Research & Development, Oxford Biomedica

The advanced therapy space has reached an inflection point – with increasing focus on the novel biotherapeutics that may represent the next frontier of treatment for many rare and intractable diseases, developers are continuing to innovate on the production processes that will enable the safe, efficient, and cost-effective production of these drugs. Viral vectors are one of the most proven and efficient vehicles for gene transfer, facilitating the targeted modification of specific cell types or tissues for the treatment of genetic diseases.

There are several types of viral vectors commonly used in advanced therapy applications today, including lentiviral vectors, which offer a number of advantages as gene transfer tools owing to their ability to transduce both dividing and nondividing cells, efficiently integrate into the host genome, carry large transgenes or multiple genes, and allow for stable, long-term transgene expression. As with many viral vectors utilized in cell and gene therapies, lentiviral manufacture is predominantly achieved following transient transfection of immortalized human cells with plasmid DNA encoding viral components. Whilst this approach enables significant flexibility with vector design and development, production by means of transient transfection presents a number of challenges for manufacturing at larger scales.

Many production processes are initially developed in an academic laboratory setting using an adherent platform and subsequently transferred to industry. While these approaches can be effective in an early phase clinical setting, the difficulty of scaling an adherent process becomes apparent once an application transitions to later stage (e.g. phase III) development – these labor-intensive processes typically require scale out, rather than more efficient scale up, and most developers opt to transition to suspension platforms where volumetric scaling can be achieved using standard engineering principles.

While the shift to suspension is an important step for scaling a drug product, it comes with its own challenges. Because of the environment in which cells are cultured in suspension, the risk of host cell impurities in a product stream increases. Additionally, some impurities can interact directly with the viral vectors being produced and impact viral vector recovery during downstream processing steps. Furthermore, the viral vector transgene produced can potentially interfere with the manufacturing process and downstream processing.

To address these challenges, some players in the space have worked to innovate perfusion techniques aimed at increasing titers while decreasing impurities. In contrast to fed-batch processes, perfusion involves the continuous removal of spent cell culture media, replacing it with fresh media while retaining the production cells. As a result, the production process can be performed at higher operating cell densities than can be achieved in batch mode, enabling increased productivity, as well as improving the purity and increasing the volume/amount of final drug product.

Key to the success of vector production at scale is the formulation and optimization of the transfection complexes required to efficiently deliver plasmid DNA to production cells. Whilst the transient transfection process can be precisely controlled at smaller scales during process development, the additional complexities associated with larger operating volumes and the manufacturing environment have highlighted the criticality of complex stability for ensuring process robustness at scale.

Improving the overall stability of these complexes represents one of the key process improvements necessary to achieving reproducible, scalable transfection. Oxford Biomedica, the premier contract development and manufacturing organization (CDMO) for lentiviral vector production, has worked to perfect the stability of these complexes. This work, coupled with other upstream and downstream process improvements, has resulted in its latest platform process offering, Process C, a transient transfection process utilizing a number of technologies and in particular perfusion that has resulted in process improvements of as much as tenfold without the need for an increase in bioreactor size.

Cell growth is dependent on the nutrient composition within a bioreactor. With perfusion, operators are able to replenish the nutrients inside a bioreactor, allowing for increased cell growth within the same process while removing significant amounts of plasmid DNA as well as host cell and process related impurities. An indication of the improved culture conditions is that at the end of the process there

are more viable cells than in the batch process. This, coupled with additional process improvements, optimized plasmid and construct design, and a decreased cost of goods sold (COGS), make Process C a crucial advancement for lentiviral vector production for the industry.

Optimized, Scalable Transient Transfection with Higher Titers and Fewer Impurities

Within the industry today, the total COGS for lentiviral vector production has been largely limited by the inability to scale beyond a few hundred liters. With its Process C, Oxford Biomedica has demonstrated that it can manufacture at the 200L scale and produce significantly more lentiviral vector from a batch, with additional improvements in both the residual DNA and residual protein content of the final vector product.

Moreover, Oxford Biomedica's vector system and production platforms has been optimized to produce both the ideal safety profile coupled with high titer. The individual application, particularly the amount of vector required to be efficacious, determines the ultimate number of doses per batch; increasing the number of doses produced per batch is crucial to reducing the COGS of manufacturing a drug product. This is particularly critical for the advanced therapeutics space, where individual doses of a drug product can cost hundreds of thousands of dollars to manufacture. Autologous cell therapies, which utilize a patient's own cells to produce drug product, are one of the prime modalities for which these process gains can serve to improve cost.

Process C adopts a plug-and-play approach which facilitates the introduction of small molecule enhancers of production, some exemplars of which have already been identified by Oxford Biomedica. For example, incorporation of U1 (an RNA-based enhancer) during vector production using Process C was shown to result in increased cell-specific productivity for several different therapeutic vectors. U1 has been shown to enhance the quality and quantity of packageable RNA in production cells, thereby enhancing titer. Overall, process yield improvements from two- to tenfold have been demonstrated for a range of therapeutic lentiviral vectors, with process scalability demonstrated in stirred tank bioreactors up to 200L in GMP. Process C also incorporates an improved downstream processing approach to specifically target the removal of unwanted protein-DNA complexes implicated in vector aggregation, which has the added benefit of minimizing process losses across sterile filtration process steps. Process modifications introduced within Process C significantly increase batch productivity and generate lentiviral vectors with improved quality characteristics, including lower levels of residual impurities and an increased proportion of functional vector particles.

Oxford Biomedica, which has performed lentiviral transient transfection in suspension for clients since 2016, is at the forefront of innovation for lentiviral production. Its Process C represents one of the first proven perfusion-based transient transfection processes for lentiviral vector production; its organization includes a team dedicated to identifying opportunities for innovation in the space, and it has plans to continue scaling Process C beyond 200L runs while simultaneously continuing to further optimize the process.

Ultimately, Process C represents a next-generation lentiviral manufacturing platform that incorporates multiple advances in process technology. The platform technologies and comprehensive experience developed at Oxford Biomedica can be leveraged directly to improve timelines and ensure product quality, with teams able to quickly develop a target product profile and then process 5L and 50L scales for research and pre-clinical supply and testing.