BIOPROCESS ONLINE

Article | June 21, 2023

Innovative Platforms For Improved Viral Vector Development And Scale-Up

Source: Oxford Biomedica

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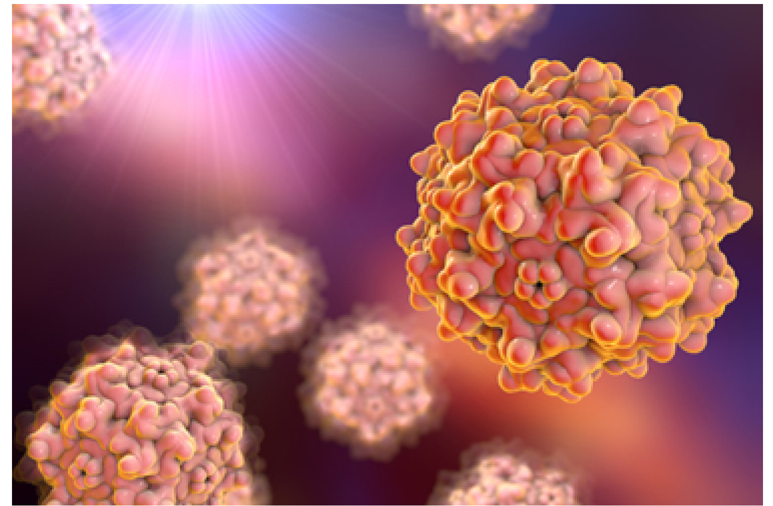

The viral vector manufacturing and development space is facing a host of challenges tied to unoptimized platform technologies, poorly understood biology, and shifting market forces, which can make identifying manufacturing capacity that is well-positioned to translate vectors from the clinic to commercial difficult. Those challenges, alongside others specific to developing adeno-associated vectors (AAVs) or lentiviral based vectors (LVs), necessitates deep expertise in productivity improvements, cell line development, chemistry, manufacturing and controls (CMC) strategy, and platform optimization, among other variables.

There exists a broad demand for expertise in AAV and LV manufacturing across the biotherapeutic sector. Companies that contract with development and manufacturing partners that have a limited understanding of vector CMC, as well as unoptimized platforms for commercial production, could find themselves facing manufacturing delays or clinical holds. Combatting this requires a close look at a development and manufacturing partner's successes in the viral vector space, as well as the innovations they have pursued in vector design, process development, and robust analytics, that have led to improved productivity and high product quality delivered.

The Challenges Limiting Productivity and Scale-Up for AAV and LV

Addressing the challenges of viral vector manufacturing and scale-up starts with establishing deep understanding of the unique biological considerations that serve to define an asset's development strategy. While nearly everyone in the industry faces challenges with transfection protocols, there are a number of factors unique to LV that can result in increased complexity for developers. One of the major factors influencing LV production is the relative "fragility" of lentiviral vectors, which can result in issues with downstream purification. Additionally, due to the distinctive biology of both AAV and LV, the size of the transgene used can have an impact on the vector titer; likewise, translation of the LV genome cargo can significantly reduce vector yields and quality during production. Often, companies will come into a partnership with plasmids obtained from academic sources or other labs that are not truly optimized for a vector, or with a design construct that belies an incomplete understanding of a lentivirus' underlying biology. In these cases, a closer evaluation of the construct often reveals different open reading frames throughout a genome, with truncations in key components, unoptimized promoters, weak Kozak signals, or no codon optimization.

For AAV, similar issues related to poor understanding of their biology can often result in low productivity, poor packaging, and difficult scale-up. Most players in the industry pursue plasmid transient transfection for AAV, a complicated process with varying potential for inefficiencies. Viral vector manufacturing is not standardized across the space; as such, issues surrounding the inconsistent optimization of AAV platform technologies, as well as difficulties in streamlining downstream processing and CMC approaches, can serve to delay and derail these projects. Construct design is equally critical for AAV products – the size and gene sequence of a construct, as well as the promoters selected, can significantly impact the productivity of a vector. Platform optimization for transient transfection is also crucial, as ensuring that plasmids can enter cells efficiently is key to producing target amounts of AAV. While conventional thinking may dictate that more cells in a bioreactor will result in more final product, industry typically utilizes only one to two million cells per milliliter transfection cell density in an AAV transfection process. This is because higher cell densities have resulted in lower productivity in practice, often owing to poor understanding of the necessary environment for productivity at higher cell densities.

State-of-the-Art Platform Technologies for Improved Productivity and Scale-Up

In order to address the complex issues at the heart of viral vector manufacturing, Oxford Biomedica (OXB) and its subsidiary, OXB Solutions, have worked to develop a number of innovations for both LV and AAV products. Focusing on improving quality, speed, and cost, OXB has developed platform approaches for both AAV and LV. Its AAV plug-and-play platform has achieved high productivity and quality, along with accelerated development, scale-up, and technology transfer to GMP manufacturing: its technology has consistently demonstrated bioreactor viral vector genome (vg) titers beyond 1E15 vg/L and final product quality greater than 90% full vector across a range of AAV serotypes and genes of interest. Additionally, OXB Solutions has achieved successful scale-up of its process, from 2L, 50L, 500L and 2,000L, all with consistent vg productivity and product quality. With approximately 1.5E17 vg in drug substance (DS) per 500L batch, OXB Solutions' process can produce significantly more patient doses per batch which helps to significantly reduce the number of batches needed and the overall manufacturing cost for clinical and commercial operations.

OXB's LentiVector® platform has also proven successful in addressing many of the challenges faced when trying to produce and scale high-quality LV vectors. With its Process C, OXB has demonstrated that it can manufacture at the 200L scale and produce significantly more lentiviral vectors from a batch, with additional improvements in both the residual DNA and residual protein content of the final vector product. Process C adopts perfusion coupled with a plug-and-play approach that facilitates the introduction of small molecule enhancers of production, some exemplars of which have already been identified by OXB. The perfusion process employed for LV is designed to achieve high cell densities and allows for the removal of excess DNA and cellular contaminants post-transfection. OXB performs steady perfusion after induction to enable continual harvest of the vectors; this is crucial in LV vector manufacture, as these delicate products tend to become inactive over time at room temperature. By engaging in continual harvest and replenishment, and storing material at 4°C, Process C avoids significant degradation.

By removing many of the residuals that occur in the culture, there is less aggregation of vectors, which in turn aids sterile filtration, increasing recoveries to over 90% for many products. Process C also incorporates small molecule additives to boost titers. These include a small non-coding RNA (called U1) that binds near a vector's packaging signal and results in significant increases in titers, particularly for certain CAR-T therapies. For example, use 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, OXB has demonstrated process yield improvements from two- to ten-fold for a range of therapeutic lentiviral vectors, with process scalability demonstrated in stirred tank bioreactors of up to 200L in GMP.

Early Development Optimization to Support Downstream Success

Part of OXB's LV approach has been to target suppressing transgene translation through the Transgene Repression in vector Production (TRiP System™), which works to transfect a plasmid that encodes the bacterial tryptophan RNA-binding attenuation protein (TRAP) in production cells. A TRAP binding sequence (TBS) is inserted upstream of the transgene start codon in the vector genome, whereafter TRAP proteins bind to the TBS in the vector genome mRNA, resulting in blocked transgene translation. This allows for significant improvements in yield and particle purity, as well as for the development of vectors that carry cytotoxic transgenes or inhibit cell growth.

By engaging in design of experiment (DoE) optimization for the plasmids used in producing a vector, OXB can achieve the desired amounts of both packaging and enveloped proteins for LV production. Researchers at OXB have also begun evaluating the transduction of the cell type selected for a vector – while many in the industry utilize a surrogate cell line such as HEK293 for titer, OXB has begun to pursue optimization of primary T cells for certain applications, such as chimeric antigen receptor products for CAR-T therapies. In doing so, it was found that plasmid optimization targeting envelope composition improves titers for specific cell lines.

Employing DoE optimization, OXB looks at every variable, including plasmid ratios, induction time, and any small molecule enhancers that may boost titers. This process is often performed at small scale, starting with plasmid transfection of HEK293T cells, targeting as many as 100 conditions and finishing with evaluating vector performance on the intended target cell (e.g. primary T cells). For those interested in packaging and producer cell lines for LV, OXB is working toward *in silico* design through optimizing the ratios of various packaging components for different types of vector genomes. Currently, OXB can generate producer cell lines by trialing the packaging cell lines it has in-house, determining which results in the best titers, and pursuing a stable producer cell line from there.

While OXB Solutions uses third-party cell lines for AAV production, it incorporates medium additives in its processes in order to boost productivity. It has also worked to refine plasmid design, focusing on variables such as optimizing pHelper and pRepCap genes to further improve productivity and packaging. In optimizing its plasmid transfection and AAV production, OXB Solutions took a systematic approach to optimizing total amount of plasmid DNA, ratio of plasmids, ratio of plasmid DNA to cells, amount of transfection reagent, ratio of plasmid DNA to transfection reagent, transfection solution mixing time, as well as equilibration time, and was able to identify a set of parameters optimal for its process. Additionally, several key bioreactor operating parameters have been optimized, such as agitation, pH, temperature, dissolved oxygen (DO), and dissolved CO_2 (pCO_2) for cell growth and AAV production.

Next-Generation LV Vectors and Next-Level Process Control

In conjunction with its process improvements, OXB has invested significant resources in pioneering the TetraVecta™ system, fourth-generation lentiviral vectors that improve on previous generations in four key respects. The first variable was to improve the polyadenylation signals in the genome's terminal repeats, halting transcription read-in and out from the gene cassette and enhancing polyadenylation and better increasing protein expression in the target cell. This has resulted in as much as 300% increases in expression levels for select transgenes. Second, OXB has mutated the major splice donor, which, while essential in a lentiviral life cycle, is unnecessary for viral vector manufacture. The result is a vector that will not splice into introns either in the transgene or promoter, increasing full length RNA production. The third feature OXB targeted is a removal of additional lentiviral sequences liberating an extra kilobase of space in the vector and enabling better titers with larger transgenes. Finally, it has enhanced the TRiP System™ of these vectors, preventing transgene expression in the production cells, increasing particles quality and rescuing titer. Another key component of establishing a comprehensive viral vector manufacturing approach is achieving process control. Maintaining efficiency at a paradigm scale is crucial to realizing eventual commercial success; to do so requires a focus on the foundational upstream and early design considerations that impact later development and commercialization. OXB uses validated scale models for its LV processes that it can

adjust according to product specifications, introducing automation starting at the shake flask stage and validating scale up to 200L. OXB Solutions has likewise homed in on equipment engineering and process control for its AAV products, enabling scale of up to 2,000L for these applications.

Conclusion

At OXB, translating processes from concept to commercialization is central to its value proposition for clients in the advanced therapy space; with decades of experience, a wealth of expertise, and a range of capabilities in the AAV and LV markets, OXB can help development and manufacturing partners achieve the necessary speed to market, quality, and scale to bring novel, life-changing therapies to bear. Its work improving productivity in particular could have important cost and time implications for many advanced therapies, ultimately improving the access and economics for patients who need them.

Oxford's next-generation LV and AAV manufacturing platforms incorporate multiple advances in process technology, and its end-to-end development and scale-up approach can help companies produce significantly more patient doses per batch, helping to reduce the overall costs for clinical and commercial applications. Likewise, its fourth-generation lentiviral vectors, which significantly improve titers, increase expression levels, and boost full-length RNA production, represent next-generation advancements for LV vectors. These advancements complement a portfolio that spans packaging and producer cell line development, DoE-driven, cell-specific optimization, titer enhancing and transgene suppressing technologies, and a comprehensive suite of in-house assays and analytics that span characterization, CMC, and custom assay development.

With a long history of success in the gene therapy field, OXB has demonstrated years of stable, dose-dependent gene expression in patients after direct *in vivo* administration, as well as thousands of patients who have safely received *ex vivo* and *in vivo* treatment with products using its lentiviral vectors. Its comprehensive suite of analytical methods for AAV manufacturing have likewise supported multiple successful IND applications to initiate Phase 1 clinical trials in the U.S. Ultimately, it's the continuous improvement efforts, coupled with a longstanding track record of success in the viral vector space that positions OXB to support the innovative, life-changing gene therapy modalities poised to transform healthcare.