CELL & GENE THERAPY INSIGHTS

SCALABILITY

INTERVIEW

Advancing AAV production scalability: enhancing purity, productivity, and yield

Abi Pinchbeck, Commissioning Editor, *Cell & Gene Therapy Insights*, speaks to **Ashish Saksule**, Principal Scientist, Vector Core Lead, Vertex Pharmaceuticals, about ongoing efforts to improve vector productivity and titer, addressing issues with the separation of empty and full capsids, and increasing scalability in the downstream enrichment of AAV.

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Q What are you working on right now?

AS: I lead the vector core lab at Vertex Cell and Genetic Therapies (VCGT) overseeing end-to-end viral vector production and building in-house capabilities to support research and process development. My primary focus is on developing a scalable and universal platform for AAV, encompassing commonly used serotypes and engineered or modified AAV capsids.

Although modified AAV capsids look similar to the parental serotype of AAV, they can behave very differently during production and purification. There are many challenges in developing processes for new viral vectors, requiring novel innovations and technologies to solve.

Most of my work is focused on the optimization and development of robust purification platforms for viral vectors to efficiently produce higher yield and quality vectors. Recently, I have started transitioning towards a new position in cell therapy, leveraging the potential of stem cells including hematopoietic and pluripotent stem cells. This transition reflects the strategic move to contribute towards accelerating process development in the field of regenerative medicine with the therapeutic application of stem cells.

Q What are the key current challenges and pressure points relating to the scalability of recombinant AAV (rAAV) vector processes?

AS: AAV is a powerful vector technology most used in gene therapy clinical trials. However, achieving scalable packaging and production of rAAV remains challenging across all stages of development. Starting with the preclinical research phase, one key challenge is selecting the right AAV serotype or engineered version of AAV. The selection and development of scalable platforms across these variants of AAV can be challenging. Early developmental efforts into designing a universal platform technology across different AAVs can be beneficial.

Another critical issue is that the processes are not linearly scalable. Scientists often conduct optimization at a smaller volume and consider it to be proportionally linear when scaling up, but due to differences in scale-down and scale-up model devices, processes often cannot be scaled linearly, posing a major challenge during process development. Furthermore, the lack of orthogonal and real-time analytical techniques is a further challenge while developing and scaling up processes. As an industry, we still lack standardized analytical alignment, though there has been significant progress in recent years in providing viral vector-specific characterization tools, in addition to helpful specific guidelines from regulatory authorities.

Another challenge surrounds costs and resources. The scaling up of rAAV vector requires significant resources, including infrastructure, equipment, and skilled personnel. Cost–effectiveness and resource optimization are key considerations in scaling up production to ensure the affordability and accessibility of gene therapy treatments for everyone.

Q How does this compare to experiences with lentiviral processes?

AS: I began working with lentivirus at the beginning of my career, and I find it to be a more challenging vector than others. Lentiviral and AAV processes share some similarities, such as upstream transfection protocols and filtration techniques, but they exhibit notable differences in terms of harvesting, lysis mechanisms, structural characteristics, stability, processing time, and overall process yields.

Lentiviruses can integrate their genetic material into the host cell's DNA, and upon activation, they produce new viral particles that are released through budding and are automatically

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released upon their production without the requirement for an additional lysis method. For AAV, an external chemical or physical lysis method is required, and the clarification will look different based on differences in the lysis method. Chemical or detergent-based lysis introduces additional challenges to the clarification step.

Enveloped viruses like lentivirus exhibit significant challenges around stability during processing, storage, and freeze–thaw cycles compared to non-enveloped viruses like AAV. We have observed significant vector titer losses of >50% of lentivirus if left overnight or through multiple freeze–thaw cycles. In contrast, AAV can be left for a few days and can tolerate multiple freeze–thaw cycles without significant losses in vector titer.

Lentivirus and AAV processes employ similar filtration technology for the purification and concentration of viral particles. However, the specific filtration requirements are different based on their size differences. Lentivirus has a larger size at approximately120 nm, while AAV is one of the smallest parvoviruses at around 20 nm. The sterile filtration of lentivirus poses unique challenges due to its size and the molecular weight cutoff of standard sterile filtration. Thus, there is a need for sterile filtration optimization, larger or multilayer sterile filters, and rigorous testing and quality control to ensure the removal of microbial contaminants while retaining lentiviral activity and titer. Overall, understanding these distinctions is essential for optimizing production processes for both lentivirus and AAV and ensuring the successful application of viral vectors in gene therapy.

Q Where might the required improvements in productivity/yield/titer come from? What promising technological innovations are you seeing within the space?

AS: The improvement of productivity and titer is the million-dollar issue that everyone in the industry is working to answer. These improvements are critical for advancing medical treatment and making viral vector-based therapies affordable and easily available to all patients in need.

First, rather than thinking about increasing productivity, we need to think about increasing the therapeutic index of the drug, thus reducing the dosage level while maintaining efficacy. This can be done in a few ways that utilize vector engineering and design. One method is developing vectors with enhanced selectivity. The greater the selectivity for the intended target or tissue, the less off-target effects and viral toxicity. Incorporating tissue-specific promoters and regulatory elements can be helpful here. Another approach is optimized administration and combination therapies. This includes the development of synthetic vectors, engineered capsids, and hybrid vector systems with optimized properties for therapeutic applications.

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To increase yields and titers, there are many ongoing efforts in upstream processing to optimize bioprocessing parameters such as culture conditions, including transfection optimization and cell line engineering to maximize productivity and yield. Utilizing novel cell culture systems such as perfusion bioreactors for suspension systems or microcarrier culture systems for adherent platforms can help enhance cell growth viability and viral vector productivity.

There is also ongoing innovation that can be incorporated into the purification process itself. Many processes currently used are traditional methods taken from the monoclonal antibody and protein industry and were not developed for use with viral vectors. These lack effectiveness considering viral size and the complexity surrounding the viral membrane and capsids. Advancing purification processes through the development of novel chromatography formats such as membrane absorbers and monolith devices will help to achieve better purification of viral vectors, particularly AAV. Having a universal affinity-based purification method and a scalable ion exchange chromatography system can help streamline the downstream processes to generate high purity and recovery of viruses.

Finally, integrating automation and robotics could enable increases in yield and quality. Having real-time monitoring and automation within workflows can improve process consistency, reducing human error and thus increasing throughput. Automated systems can also enable continuous operation and precise control over the production parameters, thus reducing batch-to-batch variability and ensuring resources are utilized effectively.

Q How are current solutions helping to address the challenge of empty/full capsid separation?

AS: Within AAV processing, the separation of empty and full capsids is a critical topic. We first need to attempt to address this from the upstream point of view. Increasing the efficiency of packaging will reduce the co-production of empty particles and increase the efficiency of producing full genome-containing particles. To further address this issue during purification, we need to introduce more real-time analytics to help identify the differences between the empty, partial, and full AAV particles so that effective purification techniques can be applied. For example, the isoelectric point difference between empty and full particles can be used to enable a baseline separation. However, this difference is very small, so we require novel tools to distinguish these so they can be effectively separated during the chromatography step.

For purification, many processes still utilize traditional methods such as ultracentrifugation. This technique can provide a high degree of separation and has been used for many early

academic or small-scale-based purification processes, however, it is not scalable. We need to develop more scalable processes such as ion exchange chromatography or a similar combination of chromatographic tools to separate empty and full particles utilizing baseline separation.

Q How can we achieve increased scalability in the downstream purification and enrichment of AAVs?

AS: There are multiple approaches to achieving downstream scalability. First, implementing process intensification strategies such as the use of high-capacity chromatography resins, continuous processing systems, and multi-column chromatography setup, can lead to higher throughput and increased scalability. Continuous chromatography systems can enable uninterrupted operation and higher productivity compared to traditional batch processing methods.

Another approach is technology integration. Using a combination of filter and chromatography in a single step or product can be beneficial to reduce the number of unit operations and product loss over multiple steps. One example from a vendor-specific application is the use of clarification with chromatography in a single unit operation. An example of this is Harvest RC chromatographic clarification, which utilizes filtration and ion exchange chromatographic purification in the same step. In our tests, this product has been shown to increase productivity through AAV clarification and reduce the need for an additional chromatographic purification step.

Another option is utilizing high throughput screening and optimization, which can enable the rapid evaluation of various purification conditions and parameters to identify the most efficient and scalable processes. Automated screening platforms can streamline the evaluation processes, thus allowing the rapid identification of critical process parameters. Then, the utilization of design of experiment and quality by design approaches can allow the systematic evaluation and refinement of these process parameters.

Q What are your key priorities, both for yourself and for Vertex Pharma as a whole, over the next 12–24 months?

AS: Currently, I am focusing on expanding my knowledge of scale-up and process optimization for viral vectors, in addition to looking at reducing the cost of goods to ensure these treatments are available at an affordable level to all patients.

Developing and training the next generation of skilled scientists in the field is also one of my key priorities. As I move to new leadership roles, I want to help other scientists develop viral vector-specific skills to help overcome the current shortage of skilled workers.

Finally, I am currently transitioning into the stem cell and regenerative medicine industry, focusing on establishing the cell therapy core lab at Vertex Pharma. These labs will help foster innovation and accelerate process development efforts for cell therapy at Vertex. I am looking

forward to this next chapter of my career so that I can contribute to scientific progress and learn as much as I can.

BIOGRAPHY

ASHISH SAKSULE is an accomplished cell and gene therapy process development expert, specializing in the bioprocessing of viral vectors. With over 10 years of experience, he has worked across various domains, including academic research, clinical stage vaccine development, and process optimization for cell and gene therapies. His expertise spans both upstream and downstream processing for viral vectors, including lentiviruses, AAV, live viruses, and proteins.

Ashish holds a Biotechnology degree from Harvard University and a Chemical Engineering degree from Michigan Technological University. Currently, he serves as a Principal Scientist and Core Lead at Vertex Pharmaceuticals, where he oversees the Vector Core facility and the Cell Therapy Core facility. Prior to this, Ashish contributed to the Rare Disease Gene Therapy program at Takeda and worked at MilliporeSigma on virus and gene therapy process development for AAV and LV. His passion lies in developing cutting-edge bioprocessing tools for cell and gene therapy, with the goal of making these life-changing treatments more accessible and affordable for all patients.

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