Moving Towards Scalable AAV Vector Production with High Volumetric Efficiency

with Prasanna Srinivasan and Graziella Piras

In the world of cell and gene therapy (CGT) development, drug developers have an increasing need for scalable, efficient, and robust processes. Those are especially important in viral-vector production, for which bioreactor titers are still below what is needed to meet market needs. Improving process efficiency requires a thorough understanding of adenoassociated-virus (AAV) bioproduction, which can be achieved by using comprehensive analytics in upstream production.

Prasanna Srinivasan (research scientist at the Massachusetts Institute of Technology's Center for Biomedical Innovation, CBI) and Graziella Piras (senior director of strategic marketing at 908 Devices) presented the REBEL cell-culture media analyzer in a 2024 Ask the Expert webinar. The REBEL cell-culture media analyzer is an at-line device that requires sample volumes of ~10 µL and measures samples in 10 minutes. It can be used in different applications, including process development and feed optimization for bioproduction, such as AAV production by human embryonic kidney (HEK293) cells. The device is designed for at-line use in bioprocess labs and does not require high-level operator expertise. The presenters provided two case studies demonstrating the CBI team's application of the technology.

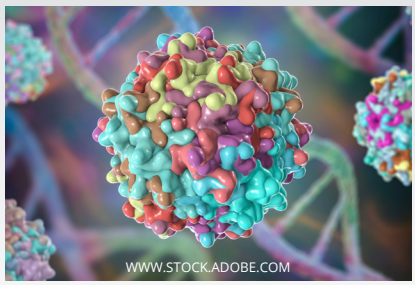
The Presentation

Challenges of AAV Production: Virus-mediated gene therapy requires the delivery of genetic material to patients' target cells via innocuous pathogens, such as AAVs. Such viruses have 24-nm nuclear capsids comprising 60 subunits that shield single-stranded DNA cargo. The genetic elements of the cargo are exchangeable to enable tissue-specific therapeutic gene expression. Tissue targeting is achieved by changing the composition of the capsid proteins using AVVs' ability to infect an array of tissues.

AAV manufacturing generally involves production by transient transfection of host cells or by stable packaging and producer cell lines. The presentation focused on triple-plasmid transfection of mammalian cells. That process mixes three different types of plasmids with cationic transfection reagents to form a polyethylenimine (PEI)–DNA complex that is delivered to cells in suspension cultures. Transfected cells produce both empty and full capsids that must be separated after harvest. AAV batches contain fewer full capsids than empty capsids, which are considered to be product-related impurities. Removal of such impurities is crucial to the efficiency of AAV-vector production and to the safety and efficacy of resulting the gene therapy.

However, separating empty from full capsids burdens downstream processes and increases process-related costs and labor. Downstream purification requires expensive processes such as chromatographic separation and ultracentrifugation. Furthermore, scaling up vector production is a major challenge; critical product attributes must remain the same irrespective of production volume. Impurities left in gene-therapy products can cause undesired immune responses in patients and reduce treatment efficacy. Therefore, an approach is needed that enriches upstream production to decrease impurity levels, which in turn will reduce costs.

Building Mechanistic Models: The CBI team leverages mechanistic models for the development of recombinant



AAV (rAAV) bioproduction. Those models require rich data input, such as information about key nutrient consumption. The REBEL device performs amino acid analysis to build predictive, relevant models for reaching the target quality and quantity of rAAVs. Such data-driven models enable users to identify problems and adjust production accordingly. Sample preparation for the REBEL analyzer is straightforward; samples obtained from a cell culture are filtered, and the filtrate then is loaded onto well plates for analysis. Results from REBEL amino acid analysis, as well as viral-vector titers and empty/full ratios, are fed directly into the mechanistic models, which provide information to improve the process.

Such mechanistic models help scientists understand the timing and pace of viral-DNA replication and inclusion to viral particles during culture growth. Within 24 hours of culturing, the number of viral DNA copies is low. That number begins to increase later, resulting in a time mismatch between viral-DNA replication and capsid production because most capsids are produced within the first 24 hours of culturing. The delayed viral-DNA replication leads to a large population of empty capsids.

Culture-Media Studies: To develop and improve AAV production processes as guided by mechanistic models, CBI

scientists ran and analyzed two culture processes — an untransfected culture and a triple-plasmid transfected culture — both in two different media formulations. Growth rates decreased at 48 hours in both the untransfected and transfected conditions. Viability also dropped at 72 hours from 90% to 80%. There was no appreciable difference in cell growth rates between the two cultures. Next, the team compared spent media amino acid profiles from cultures using the two media. The instrument detected a depletion of glutamine and asparagine in both cultures. With the help of the mechanistic model, those two amino acids were identified as crucial to capsid production.

At ~50 hours, the team observed maximal viral-DNA replication along with decreased levels of glutamine and asparagine in the transfected batch. However, capsid production was occurring at an increased rate, leading to a high proportion of empty capsids. Of note is that capsid titers increased in the transfected cell cultures until the 50-hour mark, when asparagine concentrations dropped suddenly. Information from the MIT–CBI mechanistic model enabled the team to identify bottlenecks in AAV production, and they investigated whether certain proportions of glutamine and asparagine could improve AAV vector production.

At the second step in the experiments, the team tested the effect of glutamine in the process. Asparagine was present in two cell-culture media that were used, and glutamine was present in only one of the tested cell-culture media. In these conditions, the glutamine-deprived, asparagine-containing cell-culture medium resulted in ~2× capsid titer-per-cell increases compared to the conditions in which glutamine was present.

Based on those results, the CBI team proceeded with the next steps of AAV vector production. This scale-up and intensified experiment investigated the use of at-line process analytics for continuous AAV production. The scientists used a 300-mL bioreactor with filters for cell retention and performed triple-plasmid transfection on HEK293 cells. In a process monitored continuously for 11 days, perfusion rates were determined using the mechanistic models. Capsid titer continuously increased over 11 days, as expected. The team drew samples daily to estimate amino-acid concentrations over the culture period. Results showed a positive correlation between amino-acid concentrations and daily capsid production, a negative correlation with glutamine, and a positive correlation for asparagine, as demonstrated in the previous set of experiments. Furthermore, other correlations between amino acids and capsid titers were identified, leading the way for further experiments and development opportunities.

AAV Production's Future: Host-cell performance is the key to producing full AAV capsids. The REBEL device helps users identify the nutrients necessary for maintaining optimal conditions during culture of transfected cells. The CBI team zeroed in on the glutamine–asparagine metabolic pathway. Measurements from the REBEL analyzer enable rapid at-line analytics to inform data-driven models, and thus enabling precise control of AAV production. Currently, the team is running more experiments to understand better how amino-acid metabolism affects high-density transfection.

Questions and Answers

How are models applied to batch and continuous production runs? We build models using data generated from in-house experiments, both in batch and semibatch systems. The bolus is determined based on previous batch and semibatch runs. We are improving models with the REBEL analyzer to predict capsid titers more effectively. We also are generating a viable data set for analysis of continuous production processes.

Do glutamine and asparagine have specific effects on different capsid titers? Although most sequences are conserved in capsid compositions generated by transfected cells, some changes related to certain amino acids can occur. When we compare serotypes based on capsid compositions, asparagine plays a critical role; we have 8–10% asparagine in the composition. The REBEL device enables us to estimate how asparagine and glutamine concentrations vary during production processes.

How are data reported back from the instrument, and what type of software is needed? All analytes are reported in millimolar concentrations. You can export data easily in a comma-separated values (CSV) file. Results also can be exported seamlessly to JMP statistical software for visualization.

Does amino-acid depletion lead to AAV product quality issues such as changes to capsid proteins? Depletion of certain essential amino acids required for AAV production indeed generates impurities. For example, both glutamine and asparagine are required for viral-genome replication, and they are precursors to nucleobase sugars. If we have an amino-acid concentration that is lower than what is required, we produce more empty capsids than filled ones, which results in product impurities being manufactured instead of the actual product.

Based on the reported experiments, how close is the CBI team to reaching the target titer for an AAV production commercial process? We have optimized media supplementation based on glutamine and asparagine pathways and developed a media formulation enabling 3× increases in titers. Currently, we are developing new processes based on that formulation.