Not All Viruses Are Created Equal

Workflows for adeno-associated virus purification and analysis.

After a year like the one we've just survived—in fact, given the year we're still muddling through—one could be forgiven for casting a jaundiced eye at viruses (thanks, SARS-CoV-2).

But it's worth noting not all viruses are created equal—and not all of them are our enemies. Some viruses are instrumental to biotherapeutics delivery, with adeno-associated virus (AAV) vectors falling into this category.

AAVs, as they're called, are getting an especially healthy dose of attention right now, representing one of the most promising systems for human gene therapy currently available. But the wide variety of AAVs on offer, and the processes for their purification and analysis, present some challenges.

To get a better understanding of how to overcome these issues, we sat down for a master class on AAV purification and analysis with Bill Evans and Heidi Vitrac, Ph.D., applications scientists at Tosoh Bioscience LLC.

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BIOPHARM INTERNATIONAL: What are AAVs and what is their significance in biopharmaceutical research?

HEIDI VITRAC: AAVs were originally discovered as a component of adenovirus preparation, hence the name. Simply put, an AAV is a protein shell surrounding and protecting a small, single strand of DNA. This shell, called a capsid, has an icosahedral structure that's 20 nm in diameter. AAVs are non-enveloped viruses belonging to the parvovirus family and depend on coinfection with other viruses—mainly adenoviruses—to replicate, which makes wild-type AAVs nonpathogenic.

That nonpathogenic character has sparked enormous interest in the development of AAVs as gene-therapy vectors, such that AAV is currently one of the most actively investigated gene therapies available. Researchers have used this property to engineer AAVs that lack this viral DNA, which basically makes them a protein-based nanoparticle that can traverse the membrane of a target cell and ultimately deliver its DNA cargo into the cell's nucleus. Then that DNA is translated to produce the therapy protein of interest within those cells.

There are at least 12 different human AAV serotypes that differ in their tropism, or the type of cells they can infect, and that makes them very useful in preferentially transducing specific cell types.

BIOPHARM: There are many ways to purify AAVs. What method do you generally prefer for the most cost-efficient workflow?

BILL EVANS: To determine the best methods for setting up an efficient workflow purification preparation, you want to look at your virus preparation's impurity profile, and at your product's chemical properties in relation to that impurity profile.

AAVs are generally grown in live host cells, such as human embryonic kidney 293 cells. Viruses can be either secreted into the media or lytic. Fortunately for AAVs, most serotypes are secreted into the cell supernatant, which significantly reduces impurities.

The overall strategy for AAV purification involves clarifying the cell supernatant by filtration to remove particulates such as host cell proteins, host cell DNA, empty capsids, etc. Purification then progresses to one or more chromatography steps followed by the final formulation for delivery, which is a drug product.

AAVs have a high physical and chemical resistance, which provides a large working range of conductivity and pH. The major technique used is anion-exchange chromatography conducted in the bind-and-elute mode at neutral to basic pH. Because an isoelectric point difference exists between full and empty capsids, this method has the potential to separate the two.

Several serotypes with an affinity for divalent metal cations have been reported, which are used in mixed-mode hydroxyapatite chromatography. Affinity chromatography would be serotype-specific. Some serotypes have shown heparin affinity, which could be leveraged using some of our heparin substitutes, such as TOYOPEARL® Sulfate-650F.

For some serotypes of AAVs, cation exchange and hydrophobic interaction chromatography are shown to be a good method of choice for the bind-and-elute purification.

BIOPHARM: What purity and recovery can be expected using these AAV purification methods?

EVANS: The most well-documented is probably anion-exchange chromatography, where recoveries greater than 90% have been reported. The primary impurity here is the empty capsid, with concentrations as low as 4–8% being observed. For other impurities, such as host-cell proteins and DNA, fairly low levels are achievable.

BIOPHARM: How do you select the appropriate resin for a particular application?

EVANS: Resin selection will largely depend on the serotype and impurity profile of the specific type of AAV suitable for scaling to large-scale purification.

You can use nontoxic mobile phases that don't pose a hazard when handled in large quantities. Along those lines, it's best to avoid organic solvents, which present both a handling hazard and a disposal issue.

Generally, when selecting a resin, make sure you have something that helps meet your purity goals, works well enough, is efficient and has high binding capacity—all of which yield purer and more stable products in fewer cycles.

BIOPHARM: After purification, the challenge is analyzing and characterizing the purified AAV. Can you share your expertise on getting over this hurdle?

VITRAC: Various product-related impurities can remain even after purification, including host-cell DNA, helper-virus DNA, vector aggregate and even empty capsids. These impurities aren't active but may present safety concerns. You therefore need reliable methods for their measurement and characterization.

Size-exclusion chromatography (SEC) has the advantage of being able to separate monomeric AAV capsids from any higher-order aggregates and other impurities, including polydispersed viruses. SEC is widely used as a polishing purification step for viral vectors and has been developed as a characterization method for both virus-like particles (VLPs) and influenza particles.

SEC can be enhanced by combining it with a MALS detector to determine the absolute molecular weight and molecular size of AAVs. This is very important because AAV yields aren't usually very high, and AAVs don't generate high UV signals. So, you need a very good detector to determine molecular weight and DNA content. With this technique, full vs. empty capsids of AAVs can also be accurately determined.

BIOPHARM: What validation steps or techniques should one think of before undertaking AAV purification?

EVANS: You want to focus on robust methods that work well enough to meet your purification goals. You really need to define the acceptable impurity profile for your final product and then work toward that.

In terms of validation steps, one of the more important ones is cycling—evaluating the performance of the chromatography media for repeated use and cleaning. In a robust process, the run-to-run variation in product quality and impurity profile should be very low.

We'd look for a step that can tolerate a large number of cycles. You need to establish the AAV identity, determine the concentration, analyze the ratio of empty to full capsids, determine host-cell protein and DNA content and then characterize any product-specific impurities and identify specific ways to remove them. A viral-clearance validation would usually be necessary.

BIOPHARM : What do you see for the future of AAV production and purification?

EVANS: AAVs are a still an emerging class of therapeutics that show great promise for gene therapies. Being fairly new, their related methodologies are still being established. I look toward the development of purification platforms like those we have for monoclonal antibodies. Tosoh will always be developing new and improved resins for these purposes.

VITRAC: I agree with Bill, and think new and improved resins will come down the pipeline, leading to the creation of improved and integrated workflow processes. And in that spirit, I think continuous chromatography may play a big role in the scale-up of AAV purification workflows. This will really help address the need to increase the supply and yield of AAV production, while also decreasing the cost of production for these innovative medicines.

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