CELL & GENE THERAPY INSIGHTS

INTERVIEW

Shear ignorance? Think again: breaking the perception of shear within viral vector manufacturing

Charlotte Barker, *Editor, BioInsights,* speaks to MilliporeSigma's **Ratish Krishnan,** Senior Strategy Consultant, Bioprocessing Strategy Operationalization and **Akshat Gupta,** Associate Director, Global Biopharma Center of Excellence.

Ratish Krishnan & **Akshat Gupta** (pictured from left to right)

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The tangential flow filtration (TFF) unit operation in viral vector manufacturing is a critical step on the path to commercialization. In this episode, Ratish Krishnan and Akshat Gupta discuss best practices – and common misconceptions – when establishing process conditions and utilizing different TFF device formats.

Q How and why is tangential flow filtration (TFF) used in viral vector manufacturing?

RK: TFF is a widely used unit operation in the biopharma industry for downstream processing applications. A typical TFF step employs membranes such as polyethersulfone (PES) or regenerated cellulose of varying molecular weight cut-off limits, to either concentrate a product of interest through volume reduction and/or perform buffer exchange through diafiltration.

Traditional TFF requires multiple passes through a system, using a pump to drive feed material through a filter, and then sending the retentate back to the feed tank for another pass through the entire system. The circuitry of operation is monitored using pressure and temperature gauges, to ensure avoidance of high pressures that can cause damage to the materials of construction of the membranes and the product of interest itself.

It is a very efficient method of separation of the product of interest for a diverse range of modalities, be it monoclonal antibodies, viral vectors such as adeno-associated viruses, lentiviruses, or different types of vaccine platforms that are used today. This step effectively removes undesirable contaminants, like cellular residues, and others, from the product of interest.

Zooming in on a standard viral vector manufacturing process, the TFF step is used a couple of times. Firstly, for volume reduction prior to a capture chromatography step; specifically, the loading step. And secondly, in a final concentration and diafiltration step prior to the final sterile filtration. This ensures the target concentration of the viral vector is reached, and the product of interest is in the desired drug substance matrix or buffer.

Q What are the main types of TFF formats and membranes used in viral vector purification?

RK: There's a lot to unpack in this question. If you're speaking about membranes, referring to the commonly used materials of construction, the answer would be either PES or its modified version or regenerated cellulose.

PES or regenerated cellulose membranes with a molecular weight cut-off of 30 or 100–300 kDa are generally recommended for viral vector production. The rule of thumb is to have a membrane with a molecular weight cut-off of about three-to-five-fold lower than the molecule of interest, which in this case, these cut-offs apply to AAV or lentivirus.

Another point to consider is that TFF filters are available both in single-use and reusable formats, and there are pros and cons associated with each type. When reusable formats are considered, comprehensive cleaning performance qualification and validation studies are required to ensure sanitization, regeneration, and consistent performance for the desired number of cycles that the membrane is intended for.

Single-use formats are more popular among bioprocessing and manufacturing groups, for obvious reasons.

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On the other hand, if you are talking about preferred filter formats then we have largely two options: flat sheet cassettes or capsules and hollow fibers.

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> - Akshat Gupta

AG: As Ratish mentioned, there are two prominent filter formats, which are widely used in the biopharmaceutical industry: hollow fibers and flat sheets.

Hollow fibers have been traditionally used for industrial and biomedical separations and have also been adopted for many biopharmaceutical applications. They are available with modified PES as well as mixed cellulose ester lumens. A hollow fiber filter can be selected based on lumen diameter, length and number.

On the other hand, flat sheet cassettes specifically designed for biopharmaceutical applications are very robust and offer efficient process performance and linear scalability in a compact format. These aspects are very critical for good manufacturing practice (GMP) manufacturing of biopharmaceuticals irrespective of modality.

A lot of research has gone into designing the feed channels of these cassettes and the appropriate feed channel geometry can be selected based on mass transfer, pressure drop, and shear rate requirements for a given application and modality. Cassettes are available with both modified PES and regenerated cellulose membranes.

Another thing to mention is that we recently introduced a new spiral wound format designed to provide high performance and linear scalability, which is an attractive alternative for single-use and closed applications.

Q What are the key factors and best practices when it comes to designing a TFF step?

AG: There are various considerations that need to be kept in mind when designing a TFF step. It starts with identifying the objective.

There are two key applications. The first one is if you are solely targeting the product concentration – this can be done to eliminate some of the processing bottlenecks downstream. The other application would be a typical formulation where the modality needs to be transferred into a specific diafiltration buffer or a formulation buffer and then concentrated to a predefined concentration. This is a step that is carried out at the end of the purification process.

Now, one aspect that we want to consider early on when we are approaching TFF is to keep the GMP considerations in mind, along with the scalability of systems and devices. It is critical to pick a system design and a device format that would be scalable, and the systems need to be characterized for at-scale performance. They should have the right turndown ratios, and you should be able to achieve the desired yields and capacities at the full scale.

The other aspect to keep in mind is that if you are targeting closed processing, both the system as well as the device should be designed for it. It is particularly helpful to have process performance and recovery data available for at-scale systems and at-scale devices.

From a modality standpoint, it is very important to have an idea about the size and the isoelectric point of the target molecule, and also how the key process variables like temperature, shear, and mixing affect the stability of the modality. That information can really come in handy when we are designing a TFF process.

Another aspect is how the impurities clear over the diafiltration. Here we are mainly targeting small molecule impurities, so that would be another consideration to keep in mind.

If we think in terms of process, Ratish introduced the concept that there are two key materials of construction of membranes, PES-based and regenerated cellulose-based, so it would be important to understand how the modality interacts with these materials of construction. Typically, regenerated cellulose has been widely used for applications requiring low protein binding. However, what kind of membranes work well with a given modality needs to be experimentally verified.

Another consideration to keep in mind is the buffer matrix, and how stable the modality is in a given buffer matrix. Sometimes the buffer matrix would be linked to a downstream unit operation, but again the excipients and the buffering system which keep the modality stable should be selected.

Q Thinking about the customers you work with, what are some of the common misconceptions regarding TFF for viral vector purification?

AG: Often when we are starting off with a novel modality, one challenge is that there is very little information available on-hand. That's something that we are seeing with a lot of new viral vector therapies. It's not atypical to make a selection based on certain fundamental observations, and some prevalent perceptions, regarding certain technologies.

One perception that is particularly prevalent in industry when it comes to viral vector TFF is around shear. To assume that envelope viruses and proteins are sensitive to shear is honestly not a bad assumption. But the challenge is there is very limited work that has been done to establish the thresholds for shear susceptibility for these modalities. This is something that needs to be understood, and these levels need to be defined, so we can have a better understanding of whether these perceptions are real or not.

Additionally, there's a generalized perception that hollow fibers introduce less shear stress to the modality, as compared to flat sheets. Shear rates truly depend on feed fluxes and feed channel design. To broadly suggest that hollow fibers would be introducing less shear may not be a correct statement. At the same time, we can also extend that and say that to consider that all flat sheets are the same or similar in terms of shear rates is also an inaccurate assumption.

As we move towards a better understanding of these modalities, their interaction with the physiochemical environment, and their susceptibility to shear, we can progress towards making better processes in the future.

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Q How are your teams working to overcome this perception about flat sheets in viral vector purification?

"...one of our main objectives is to solve our customers' toughest problems in bioprocessing, and we have been generating a large amount of data..."
- Ratish Krishnan

AG: There is a lot of ongoing work, and this is being done at various scales and with a broad spectrum of modalities.

One thing which cannot be ignored is the diversity of viral vector modalities. That diversity would require small-scale or rather ultra-small-scale systems, which can be used to characterize shear, and their interactions with various other process variables.

We are also working with our customers to understand how different materials of construction of membrane and device geometry are contributing towards the performance for processing of these viral vectors.

RK: Those are all great points – there isn't a cookie cutter approach to TFF. You also need to consider the uniqueness of the feed – that shouldn't be overlooked.

Process development scientists have the luxury of leveraging what has worked well in past projects, or relying on information that is publicly available to the bioprocessing community. But as with everything in science, the approach has to be data-driven. No data, no science, as a matter of fact.

Customers have engaged us in both simple and complex design of experiments with their process intermediates. As Akshat mentioned, this may be exploring membrane chemistry, cut-off, trans-membrane pressures, feed flux rate, operating temperature, and load ratio – just to summarize a few – for the intended TFF step.

The performance data with their feed material is then collected and packaged into a comprehensive report by our process development services and our MSAT teams. Our customers usually perform their own analytics after which they engage with us in a holistic understanding of the TFF step. We work together towards either optimization of parameters, scale up into a pilot plant, or a manufacturing facility, as necessary.

Sometimes the scope of our work with customers is to explore and evaluate a new product that is in alpha or beta testing phases. We really appreciate the support we get from our customers, who are instrumental in helping us in bettering an existing product or providing feedback for a new concept.

Oftentimes for the betterment of the scientific community, we author, co-author, or support manuscript preparation of technical articles with customers as well. A perfect example is a recently published article that looks at the scale-down model of a 30 kDa flat sheet cassette in a regenerated cellulose format for the popular serotypes of AAV 2 and 9.

In summary, one of our main objectives is to solve our customers' toughest problems in bioprocessing, and we have been generating a large amount of data – including best practices for TFF operations – to empower them and help them design processes firmly based on data.

We are excited to continue to partner with our customers in their journeys of developing potentially curative solutions for patients using these viral vectors.

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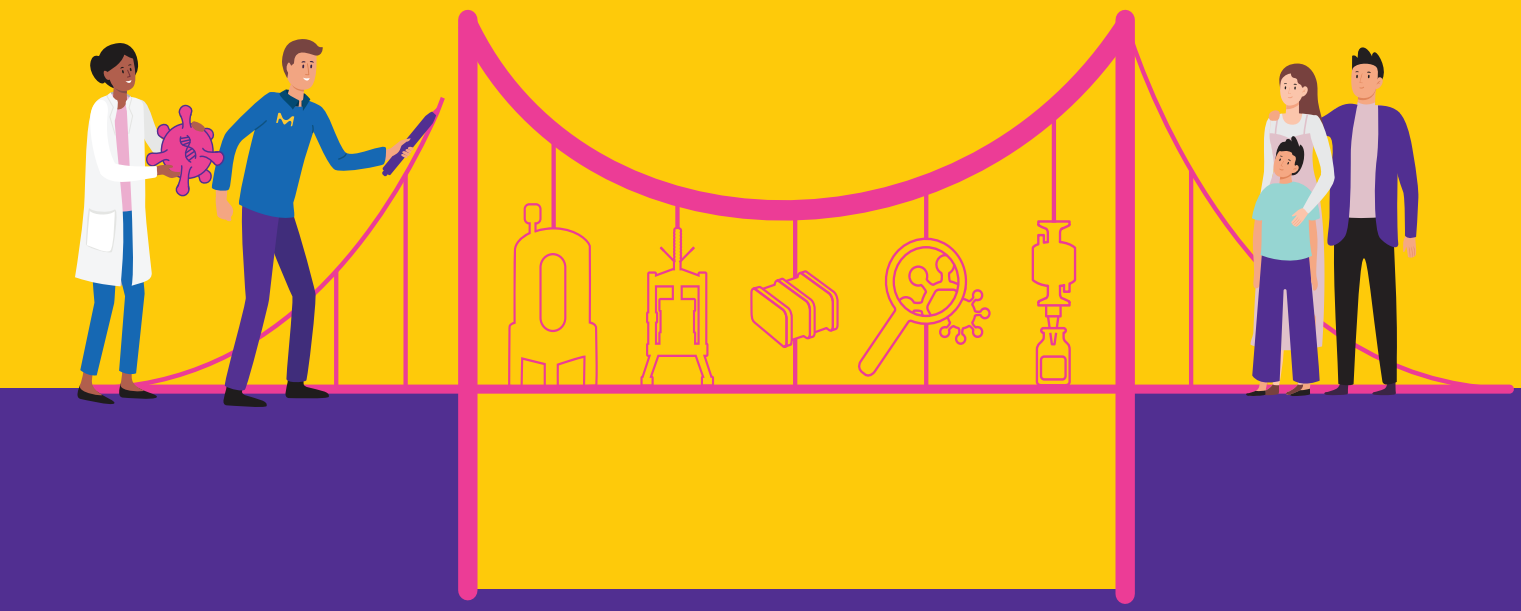

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