Moving Closer to Affordable Advanced Therapies:
A Review of Current Trends in Downstream Processing

Viral vectors are a crucial tool within the advanced therapy sector, providing an elegant solution for gene delivery in areas such as gene therapy and vaccine development. These viral particles, often uniquely engineered for a specific application, can effectively deliver genes into target cells, sometimes even enabling long term expression of the therapeutic gene of interest. However, producing clinical grade viral vectors comes with significant challenges and cost, partly due to comparatively low productivity of viral vector expression systems, in tandem with complex and onerous downstream processes.

In this article, we review viral vectors as a tool for advancing novel medicinal products and investigate the key steps and main challenges of viral vector purification. Finally, we will explore some of the emerging trends that hold the potential to transform access to advanced therapies.

Viral Vectors: The Essentials of Advanced Therapies

In the decades since their discovery, many viral vectors have been developed for gene delivery *in vitro*, however, only a small subset of these have been established as safe for therapeutic use, including:

- **Adeno-associated Viruses (AAVs):** AAVs are non-pathogenic and can integrate into the host genome, offering long-term gene expression.
- **Lentiviral Vectors (LVs):** Derived from HIV, LVs can infect dividing and non-dividing cells, allowing stable gene integration and expression.
- **Adenoviral Vectors (Ads):** Derived from Adenoviruses, these vectors can carry large DNA inserts and are effective for transient expression of genes.
- **Retroviral Vectors (RVs):** These vectors integrate into the host genome, but primarily infect dividing cells.

There is significant variation between these vectors in terms of efficiency, stability of gene expression and versatility. The most appropriate vector, payload and dose is largely dictated by the target therapeutic application. For gene therapy applications, integrating non-pathogenic viruses will be favourable when delivering therapeutic genes to correct genetic disorders, such as cystic fibrosis or haemophilia.

On the other hand, when looking at cancer treatment, vectors with specific tropism and activation mechanisms will be preferred in order to induce apoptosis in malignant cells. Alternatively, vectors with high integration efficiency and long-term stable expression will be used to genetically modify cells ex-vivo, before re-administrating them to the patient to elicit an immune response.

However, use of viral vectors as a therapeutic agent, or as a raw material in the production of advanced therapies, also carries risk to the patient. This is mainly due to immunogenicity, where the viral vector itself triggers an immune response in the patient. Whilst in some cases an immune response may simply reduce the effectiveness of a therapy, in more serious cases it can lead to adverse responses and other safety concerns, including the risk of insertional mutagenesis or oncogenic potential.

Regulatory agencies, therefore, closely monitor the use of viral vectors in therapeutics, requiring extensive preclinical and clinical data to ensure safety and efficacy.

The Key Steps of Downstream Processing

Downstream processing for viral vectors typically begins with the bioreactor, where techniques used for harvesting vary depending on several factors, including the expression system and vector of choice. Where viral vectors are predominantly secreted by their expression system (*i.e.* the virus is mostly contained in the supernatant) it is preferable that the cells are not harvested and lysed, as this will increase the purification challenge further downstream. Conversely, where the vector is not secreted, a lysis step is usually required to extract the vectors from the expression system. Lysis may be carried out chemically, by addition of surfactants and detergents, but can also be done physically, using pressure or temperature – although the latter often proves more difficult to scale. Harvesting conditions must be selected carefully to achieve high product recovery, while maintaining particle stability and purity.

Upon harvesting of the vector, the feed stream must be clarified to remove cell debris and other larger contaminants. At small scale, this can be achieved using centrifugation or microfiltration, although clinical and commercial settings often utilise filtration with depth filters as the preferred method due to its high recovery and linear scalability. This technique uses inert filters with decreasing pore size, able to trap larger debris whilst allowing viral particles to pass through. Here, it is crucial to select a filter composition that ensures high product recovery, although certain compositions may also offer increased removal of process-related impurities such as host cell protein (HCP) and host cell DNA (hcDNA).

Following filtration, the final major step that remains is purification, often requiring several stages to achieve the final product. There are a wide array of purification methods currently in use, each with its own distinct benefits and drawbacks. Chromatography is highly effective for both capture and polishing of viral vectors, however, it is typically an onerous process step, due to raw material cost, and may require extensive development depending on the type of chromatography.

Affinity Chromatography

Affinity chromatography selectively binds viral vectors

to an immobilised ligand within the column, allowing for targeted purification based on the surface properties of the virus. This is widely accepted as the 'gold standard' method for product capture, offering high recovery but generally relying on the availability of an antibody against the target product. Furthermore, traditional antibody-based affinity chromatography requires harsh elution conditions to effectively separate virus from ligand, which can have a detrimental impact on virus infectivity. This process may also result in leaching of the animal-derived ligand into the final product, a process-related impurity that is highly scrutinised by regulators. Clearance of these impurities is a high priority during downstream processes, and must be demonstrated through rigorous quality control testing.

Added to this, affinity chromatography reagents currently on the market are expensive and offer much lower reusability compared to antibody-based reagents for other modalities. This method is also agnostic to product related impurities and will effectively co-purify non-target and non-intact viral vectors.

Ion Exchange Chromatography

Ion exchange chromatography separates viral vectors based on charge, which allows for additional purification and concentration. As the presence of a genetic payload modifies the overall charge of the viral particle, this method is widely utilised in large-scale production for separation of target from non-target viral vectors. While they are typically highly reusable and cost-effective, this method also often relies on harsh conditions to recover the virus from the column, which will have a detrimental impact on vector infectivity.

Alternative chromatography methods are available to purify the product based on other properties, (*e.g.,* Size Exclusion Chromatography to remove smaller contaminants while concentrating the viral vector) but may be more challenging to develop or scale up.

To achieve the desired product profile, chromatography is usually used in tandem with filtration-based methods. Filtration is interesting operationally, as it is relatively simple to develop, scale and execute in a manufacturing environment relative to chromatography, and can often be less onerous, dependent on the choice of filter.

Dead-end Filtration

The most common, and perhaps simplest, type of filtration is dead-end filtration, where the complete feed flow is forced through the membrane, and filtered matter is accumulated on the surface of the membrane. Dead end filtration is most widely used to reduce bioburden or ensure sterility by passing the feed through filters with pore sizes between 0.22–0.45µm. Dead-end filters may also be functionalised using identical ligands to chromatography, particularly ion exchange ligands, which are smaller in size and easier to use. By functionalising a filter, filtrates can be retained based on other properties than size alone. This proves particularly useful when considering hcDNA, which can be easily removed from the feed material using a filter functionalised with ion exchange ligands.

Cross-flow Filtration

Cross-flow filtration, also known as tangential flow filtration (TFF), gets its name because the majority of the feed flow travels tangentially across the membrane surfaces rather than through them. The main advantage here is that filtrate accumulation on the membrane surface is substantially reduced, increasing filter lifetime and capacity.

TFF can be deployed in ultrafiltration or diafiltration mode. In ultrafiltration, the viral vector is concentrated by using a membrane that will only allow particles below a certain size to filter through. This is predominantly used to bring the viral vector concentration up to the target dose concentration, but may also serve as an intermediate step to reduce sample volume and the overall downstream processing time. In diafiltration mode, often performed alongside ultrafiltration, the product is exchanged into a different buffer, removing salts and other small molecules.

Diafiltration is one of the final steps of a traditional downstream process, as it is the preferred method to exchange the product into its final formulation buffer. Certain stabilisers (*e.g.* sugars or surfactants) may decrease the diafiltration efficiency, and result in aggregation of product and contaminants, and are only added after completion of ultrafiltration and diafiltration.

Filtration is typically the last purification step applied to any viral vector product, as the material must be processed through a 0.22 µm sterilising filter to ensure that remaining contaminants such as bacteria are removed prior to long term storage. Once the virus is in its final storage condition, Quality Control on the final product may be performed, to ensure the vector meets purity standards.

By combining these different purification techniques, manufacturers can create a final viral vector product with a suitable product purity profile, however, there must be balance between purity, recovery and cost-effectiveness. Although increasing the number of purification steps may result in higher purity, each step comes with additional costs (**e.g.,** raw materials, time, overhead), and usually reduces the total recovery of the viral vector product. Currently, recovery as low as 20% of the harvested amount is deemed acceptable, which raises questions as to the commercial viability of these products.

When low recoveries are looked at alongside particle infectivity, which is also reduced through standard purification techniques, the need for simpler, more cost-effective and gentler viral vector purification processes is evident.

New Technologies Revolutionising Downstream Processing

Many new technologies are emerging that promise to address some of the current challenges in viral vector purification. Synthetic affinity reagents, offering specificity and selective binding to viral vectors, have been demonstrated to offer gentle, high recovery purification. These ligands can be designed rationally, using molecular modelling tools that allow for targeting of specific surface epitopes, giving greater confidence that infectivity and tropism will not be affected through the bind and elute mechanism. This targeted approach also allows scientists to tailor for engineered viral vectors, which present a greater challenge due to their inherent uniqueness.

Synthetic affinity ligands are synthesised from simple monomers, meaning they are chemically-defined, free of animal-derived components, and far more cost-effective than their antibody counterparts. The polymeric nature of these ligands also makes them highly resistant to extreme conditions (pH, temperature, pressure, shear), improving cleanability and reusability compared with traditional reagents.

Advanced solid supports for ligand immobilisation also offer an avenue for gentler, more cost-effective purification. Magnetic particles functionalised with affinity ligands can specifically and selectively bind viral particles, and then magnetically isolated and concentrated from the solution. When paired with gentle elution conditions, functionalised nanoparticles could be an excellent replacement for current column-based chromatography, alleviating the risk to viral integrity and infectivity posed by shear and pressure. The use of nanoparticles is also advantageous operationally, as material can be processed faster, and equilibrium conditions can be leveraged for higher binding capacity and recovery. Gold, silver and silica-based nanoparticles have also been used for small-scale purification, although they are typically not commercially viable at scale.

Alternative means of separating viruses from their specific ligands are also being investigated. Electrochemistry-based methods are a good example, where short electric impulses applied to the solid support can replace traditional methods of separation (pH, salt concentration). This offers several distinct advantages, including being gentler on the viral particle, and decreasing total buffer consumption, ultimately reducing downstream process costs.

With increasing reagent availability, high throughput testing methods are becoming increasingly important, particularly when looking at the increased throughput of upstream and analytical workflows. High-throughput testing methods in upstream process development is nothing new, and is essential for selecting optimal growth and transfection conditions to obtain the highest possible yield and purity. However, high-throughput methods for purification condition screening are less commonplace, meaning that downstream process development can only begin after the upstream conditions have been optimised and scaled. High-throughput downstream testing, for example microfiltration plates or miniature chromatography columns, are allowing us to bridge the throughput gap between upstream and analytics, and are increasingly deployed in industry for screening of large numbers of reagents and conditions. However, these methods typically require automated liquid handling and some machine learning to be performed at an appreciable pace, and the development of these automated screens may be resource intensive.

The Prospective Challenges in Downstream Processing

Despite a surge in the development of new and innovative technologies, some challenges remain unaddressed. While our understanding of the potentially deleterious effects of non-target viral vectors has increased greatly, our ability to produce and separate target vectors still has a long way to go. To date, much of the focus in this area has centred around increasing the yield of target vectors. Although this will undoubtedly be crucial to making advanced therapies widely available, development of more selective reagents

to effectively separate the target vector may hold the key to commercial viability.

This is particularly pertinent when considered alongside recent regulatory changes on the requirement for adventitious virus reduction/inactivation where possible. This requires two orthogonal methods, and is traditionally met by performing one inactivation step, consisting of a hold at low pH followed by a virus removal filtration using low nominal pore size filters, normally performed prior to the final formulation. Non-enveloped viruses such as AAVs may be amenable to such treatments given their higher pH tolerance and small size, however, enveloped viruses and those with lower pH tolerance are generally not amenable to either method. Although in these cases analytical methods are available for detection of adventitious virus contamination, their cell-based nature makes testing slow, and inadequate for in-process control. Consequently, there is significant demand for novel solutions to increase patient safety, de-risk manufacturing, and increase compliance.

Conclusions

Effective collaboration between regulatory bodies, technology developers, and therapeutics manufacturers will be essential in defining a new standard for production of viral vectors that maintains patient safety, without compromising commercial viability and accessibility to these cutting-edge advanced therapies.

While the challenges today remain great, the gene and cell therapy field must prepare itself for an increasing number of engineered and novel viral vectors currently in development, which hold great promise in increasing potency and improving tropism. Availability of specific and selective purification reagents alongside rapid, high-throughput screening techniques will be essential for development of high recovery downstream processes. Alongside increasing expression system productivity and developing a suite of robust analytical tools, overcoming these crucial hurdles will be essential to ensure viral vector-based therapies are widely available to the patients that need them.

Abdullah Sufan

Abdullah Sufan, Head of Cell and Gene Therapy Applications, joined Tozaro with over 6 years of experience in downstream purification of viral vectors and complex proteins. Abdullah has focused his career on the development and scale up of CGT processes for pre-clinical and clinical manufacturing. He is currently leading the Vector Production function at Tozaro, where he is responsible for production and purification of viral vector using novel synthetic affinity reagents in newly acquired laboratory space.