CELL & GENE THERAPY INSIGHTS

LATEST DEVELOPMENTS IN VIRAL & NON-VIRAL VECTOR MANUFACTURING

SPOTLIGHT

INNOVATOR INSIGHT

Overcoming downstream purification challenges for viral vector manufacturing: enabling advancement of gene therapies in the clinic

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The adeno-associated vector (AAV) has shown great potential for the delivery of therapeutic genes in the gene therapy field and has become a vector of choice for many therapies. Although excellent clinical outcomes have been reported, the current potential of viral vectors in the clinic is limited due to manufacturing challenges including the absence of an efficient and scalable platform purification process. Affinity chromatography has proven to be a viable solution for the purification of viral vectors and to date, a small number of, mostly single serotype-targeted affinity resins exist. However, to make these treatments economically viable, the ability to use one resin to purify multiple serotypes is vital. The POROS™ CaptureSelect™ AAVX chromatography resin can purify multiple AAV subclasses, including recombinant and chimeric vectors, with high binding efficiency. By reducing purification steps and maximizing productivity, this true pan-tropic AAV affinity resin offers a cost-effective purification solution for commercial AAV manufacturing.

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Over the last decade, significant advancements have been made in the gene therapy field by delivering the therapeutic gene to the target cells [1,2]. The main focus of gene therapy clinical applications is on the treatment of diseases caused by single-gene defects such as cystic fibrosis, hemophilia, muscular dystrophy and sickle cell anemia. Due to promising results, the clinical applications have expanded to include the treatment of cancers and neurological, cardiovascular and infectious diseases [1–3].

Currently there are two approved gene therapies in Europe: Glybera™ by uniQure and Strimvelis™ by GlaxoSmithKline. Recently, Spark Therapeutics obtained positive feedback from FDA for Luxturna, and may potentially have the first gene therapy into the US market for the treatment of inherited blindness [4]. In addition to these therapies, there is a healthy pipeline of clinical trials for gene therapy products in development that involve various types of viruses for delivering therapeutic genetic material to target cells [5]. Recombinant adeno-associated virus (AAV)-based vectors have reported excellent clinical outcomes and AAV sub-classes have shown great potential for gene delivery due to several features. Their ability to infect non-dividing and dividing cells, to mediate long-term tissue-specific gene expression, and their low level of immunogenicity, have made AAV the vector of choice in many therapies [6–8].

Even though the majority of AAV-based therapy trials are in early to mid-phase development, positive outcomes are being seen in the clinic. Recent clinical successes have underpinned major investment by both Contract Manufacturing Organizations and large Biopharmaceutical companies in the drive to commercialize these therapies, which has increased demand for clinical-grade (Good Manufacturing Practice [GMP]) viral vector products [9].

CHALLENGES IN VECTOR MANUFACTURING

The progress of these gene therapy programs from early phase through to full commercialization presents various manufacturing challenges. One major challenge is the availability of viral vector manufacturing capacity, which currently will need to be increased by an estimated 1–2 orders of magnitude to deliver the volumes required for the therapies currently under development [9–14]. Extensive optimization of upstream production of viral vectors has led to additional challenges in the downstream process of viral vector manufacturing. After production, vectors need to be recovered from large volumes of cell lysate or medium, which has proven to be a bottleneck due to the lack of scalable platform technologies for purification. Efficient downstream purification to generate clinical product of high titer, high potency and high purity, is vital to ensure stable and effective transgene expression and for preventing transmission of infectious disease. Besides that, it is important to maintain AAV vector biological activity when removing impurities and contaminates present in a feedstock that originates from host cells or culture media. This is critical to comply with strict regulatory guidelines. Multiple processes to purify viral vectors have been reported but either lack scalability or

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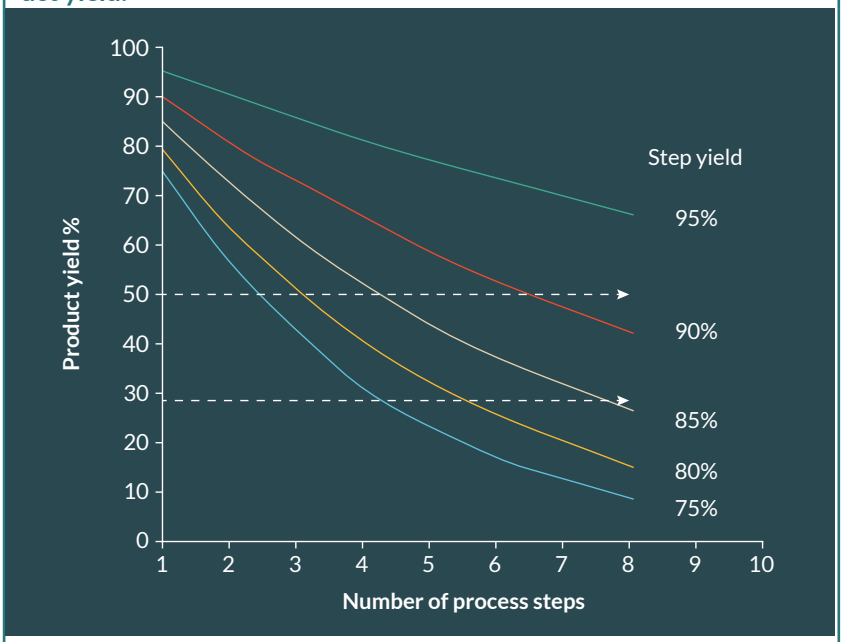
require a multitude of purification steps with low efficiency, for example, cesium chloride (CsCl) and iodixenol gradient centrifugation, or several chromatography steps that include size exclusion chromatography, ion-exchange, hydrophobic interaction, and heparin or IMAC affinity [9–14]. Even though the outcome of such a purification approach will result in pure product, the lengthy processing time in manufacturing and the complexity of such a process results in a more expensive downstream purification procedure and ultimately in cumulative yield losses (Figure 1). Next to this, the vast majority of described AAV purification processes rely on the specific serotype produced. This requires the design of a new process for each different serotype [13]. To overcome this challenge, establishing a single downstream platform for all AAV vectors is critical to increase productivity and meet industry needs for these unique therapies.

FIGURE 1

Relationship between the number of process steps and final product yield.



Even at higher step yields, the overall product yield decreases rapidly with the increase of process steps.

AFFINITY CHROMATOGRAPHY, A VIABLE SOLUTION TO ENABLE VIRAL VECTOR PURIFICATION

A recent article published by Dismuke and Kotin, discussed the benefits of affinity resins in terms of providing a scalable capture step, with a high degree of purity and recovery, for the purification of AAV serotypes [15]. The use of affinity chromatography could help overcome the current challenges in viral vector purification and increase overall product yields [16]. Due to the advantages of highly specific separation and high fold purification, affinity chromatography is a well-established platformable technique for the purification of biomolecules. It provides a robust methodology with less process optimizations. It delivers significant improvements to the downstream process by reducing the number of purification steps and therefore maximizing productivity, whilst offering scalability and process consistency. The benefits of such a platform approach have been seen in the industry with the use of protein A for purification of monoclonal antibodies, and more recently with specific affinity purification products for therapeutic compounds [17–21].

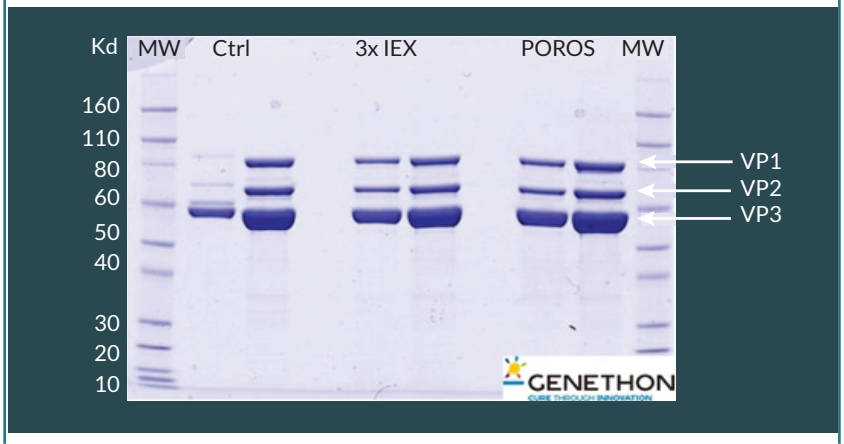
Although the use of biological affinity ligands offers many advantages, potential low stability of the ligand to sanitizing agents and non-animal-free production systems could be potential drawbacks for implementation in commercial processes [13,22]. Affinity purification solutions that would overcome

these limitations are of true value in the AAV commercial downstream process. One technology that addresses many of these limitations is for example the single-domain Camelid ligands. These ligands are highly specific and robust under various chromatographic conditions and easily produced in an animal free production system. For instance, the POROS™ CaptureSelect™ (Thermo Fisher Scientific) AAV8 and AAV9 affinity resins were developed for purification of AAV8 and AAV9 serotypes in a single capture step [23].

Both AAV8 and AAV9 resins show specific affinity for the AAV capsid as well as high dynamic binding capacity, thereby effectively eliminating process impurities, resulting in a high degree of AAV vector purity and recovery. Figure 2 shows the SDS-PAGE comparing purity of AAV9 viral vectors purified by two downstream processing methods; one using three ion exchange (IEX) steps and the other with POROS™ CaptureSelect™ AAV9 resin as a one-step capture process. The data shows that the purity profile of the AAV9 is equivalent for both downstream processing approaches. The gel also reveals similar purity, and the capsid viral protein (VP) topology for viral vector AAV9 is confirmed showing the bands corresponding to the viral structural proteins VP1, VP2 and VP3. The use of these affinity resins allowed for fewer unit operations leading to higher product yield, increasing it from 20 to 60%, whilst helping to reduce cost of goods by a factor of 6 (Figure 3).

FIGURE 2

SDS-PAGE comparing purity of AAV9 viral vector, purified by two downstream processing methods; one is using 3x ion exchange (IEX) steps and 1x affinity using POROS™ CaptureSelect™ AAV9 resin as a one-step capture process.



No residual proteins were detected. Image courtesy of Genethon.

DEVELOPMENT OF AAV AFFINITY CHROMATOGRAPHY RESINS

The AAV affinity resins are based on highly selective ligands developed with the CaptureSelect™ technology. This technology has been validated in numerous commercial and late-phase development biotherapeutic downstream purification processes, including blood coagulation factors, hormones and antibody-derived therapeutics [21].

The technology is based on single domain heavy chain (V_HH) antibody fragments, which represent the smallest fully functional antigen-binding domain of Camelid heavy chain-only antibodies (Figure 4). Those domains are compact and highly specific, and also robust under various chromatographic conditions. During ligand discovery, the final ligand is selected by screening V_HH libraries and then produced in an animal-origin-free (AOF) system in *Saccharomyces cerevisiae* at any scale [17–20].

The resulting ligands are immobilized on POROS™ 50 micron polymeric beads, (Figure 5),

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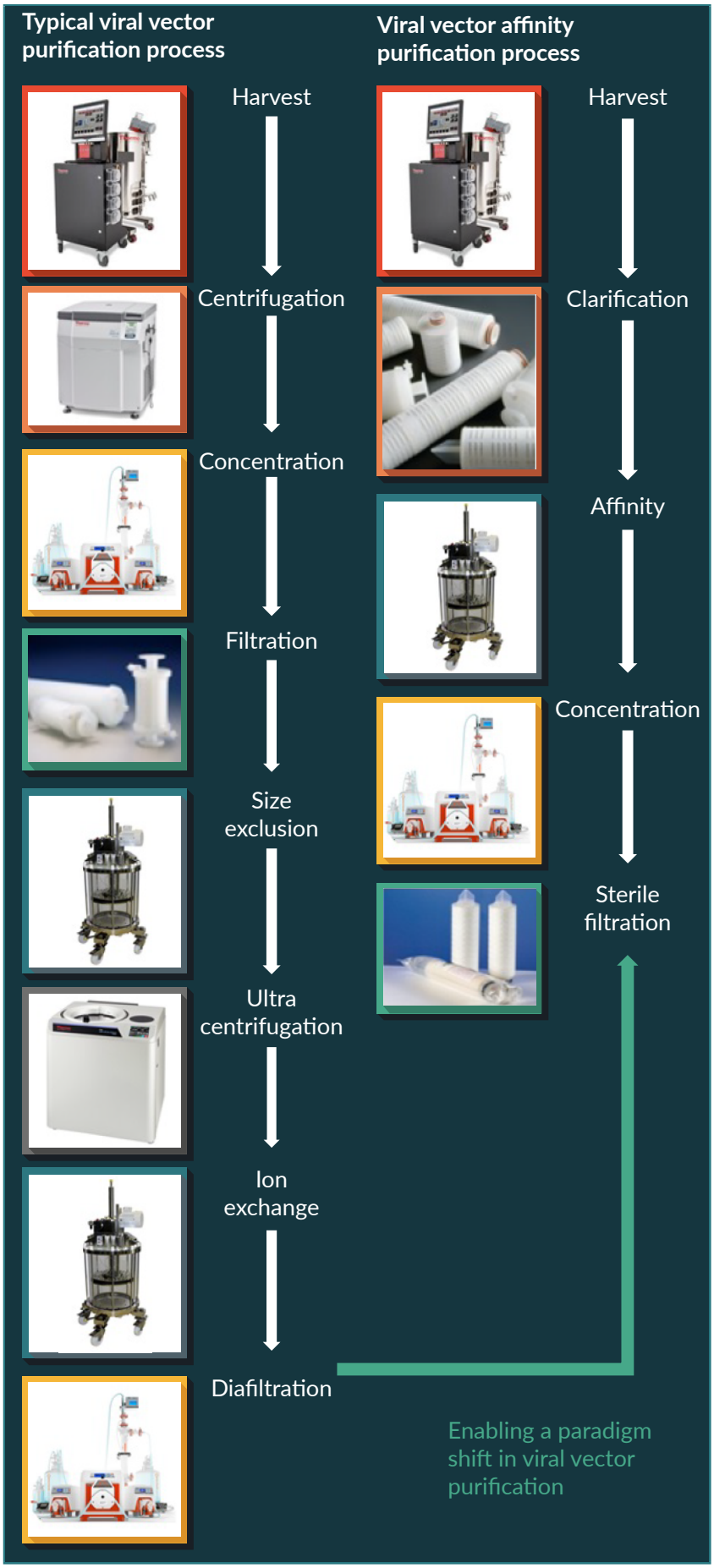
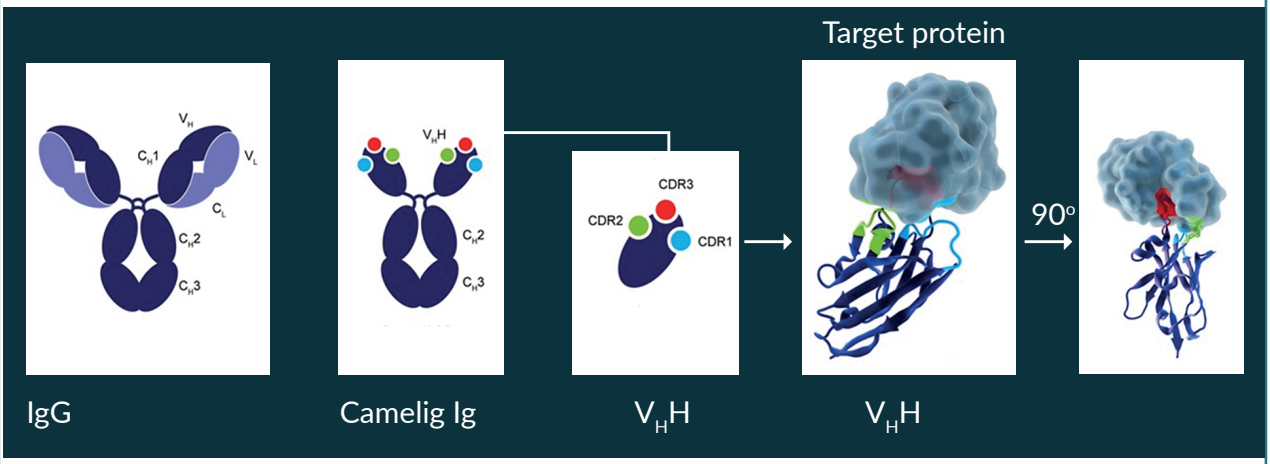


FIGURE 3

A paradigm shift in viral vector purification to increase yields and decrease process development timelines.
The use of affinity resins allows for fewer unit operations leading to higher product yield and improved productivity

FIGURE 4

The CaptureSelect™ technology is based on Camelid-derived single domain antibody fragments (V_HH).

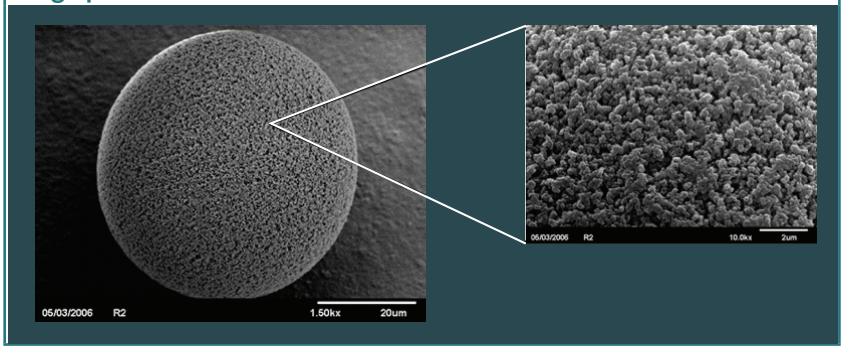


Schematic representation of a regular antibody (IgG) compared to a Camelid antibody (Ig). The V_HH fragments are the smallest (15kD) fully functional antigen binding domain of Camelid heavy chain-only antibodies.

characterized by large 'through-pores' making it suitable for the capture of large biomolecules such as viral vectors, where dynamic binding capacities of >1 × 10^{13} viral genome per mL have been obtained [23]. The large throughpores of the beads lead to improved mass transfer capability that enhances efficiency resulting in a higher capacity of the resin. In addition, the smaller particle size provides superior resolution, which can be maintained independent of the operating flow rate due to the rigidness of the beads [24].

FIGURE 5

Scanning Electron Microscope image of a POROS bead showing the large pore structure of the bead



The combination of POROS™ and CaptureSelect™ technologies has resulted in powerful affinity resins that can be utilized for immunoaffinity chromatography in a single capture step, thus significantly simplifying viral vector purification process.

ESTABLISHING A SINGLE PURIFICATION PLATFORM FOR MULTIPLE AAV SEROTYPES

As described by Dismuke and Kotin, a pan-tropic resin that can be used for the purification of all AAV serotypes would be very beneficial to the industry [16]. To that end, the AVB Sepharose™ High Performance resin (GE Healthcare) has been shown to exhibit selectivity for a small variety of AAV serotypes, whilst the most recent POROS™ CaptureSelect™ AAVX affinity resin has been specifically developed to purify a wide range of AAV serotypes including AAV1-AAV9 as well as recombinant and chimeric vectors (Table 1).

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Specificity of the resin

POROS™ CaptureSelect™ AAVX affinity resin has been engineered to bind all naturally occurring as well as synthetic AAV serotypes. In the following study, various AAV subtypes were evaluated to assess the selectivity to natural and synthetic capsids. Table 1 gives the static binding data for a number of naturally occurring and recombinant AAV serotypes. The broad specificity for AAV viral vector subclasses was confirmed.

Comparative study of AAV resins

In addition, Genethon performed a study to assess the performance of the AAVX affinity resin for various AAV serotypes, including synthetic vectors. Figure 6 shows the selectivity of the POROS™ CaptureSelect™ AAV resins utilizing a 96 well plate format to assess percentage binding for AAV8, AAV9 and AAV10 as well as 4 synthetic serotypes. Next to a selectivity study, a comparative study of AAVX and AVB Sepharose™ was performed, showing superior performance of AAVX over AVB Sepharose™ in all serotypes tested. The data reveals that the AAVX affinity resin has high potential to be a platformable affinity purification solution for various AAV serotypes due to the high affinity binding for all serotypes.

TABLE 1

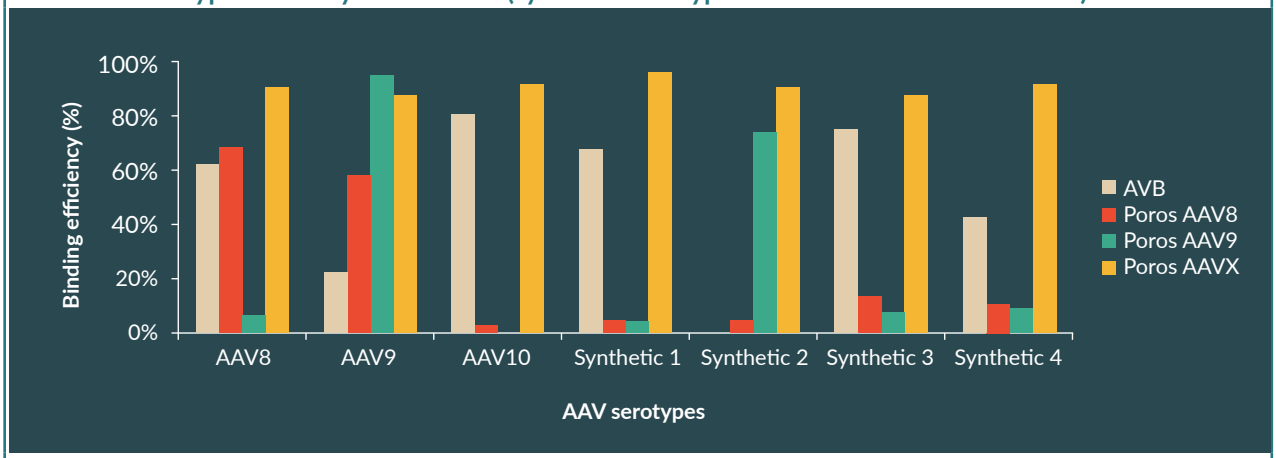
POROS™ CaptureSelect™ AAVX resin

AAV serotype	Binding % (in eluate)
AAV1	99.63
AAV2	97.8
AAV2_HSPG	98.33
AAV4	98.05
AAV5	97.88
AAV6	97.45
AAV6.2	98.93
AAV7	98.37
AAV8	97.76
AAV9	98.43
AAVrh10	96.28
AAVrh32.33	99.29
AAV9PHPB	98.51
AAV7m8	98.39

The AAVX resin serotype specificity was tested using a static binding assay with a wide variety of serotypes. Resin was mixed with AAV (from clarified lysate) in a tube for 10 min. Specificity determined based on elution recovery; acidic elution buffer, pH 2 (0.1M citric acid). VG was determined by qPCR.

FIGURE 6

Data from a comparative % binding study using AAVX and AVB Sepharose™ affinity resins for AAV8, 9, and rh10 serotypes and 4 synthetic ones (synthetic serotypes are a result of chimerization).



POROS™ CaptureSelect™ AAVX resin is the only resin to have high affinity binding for all these serotypes (more than 85% of the loaded material was captured). The experiments were performed in 96 well plate format with 25µl of resin. The vectors titers were measured in the Flow Through.

General considerations of resin usage

POROS™ CaptureSelect™ AAV affinity resins are easily adopted in a downstream purification process.

Typical affinity chromatography optimization approaches for these resins, including binding, washing and elution conditions can be applied. For example, in terms of binding considerations for equilibration, standard neutral buffers (pH 6 to 8) such as 10–50 mM sodium phosphate or Tris can be used. Elution conditions may vary as target molecules differ in their binding/elution behavior. However, to elute most target molecules reducing the pH to pH 2–3 is generally successful [23].

SEPARATION OF EMPTY CAPSIDS & FULL AAV VECTOR PARTICLES

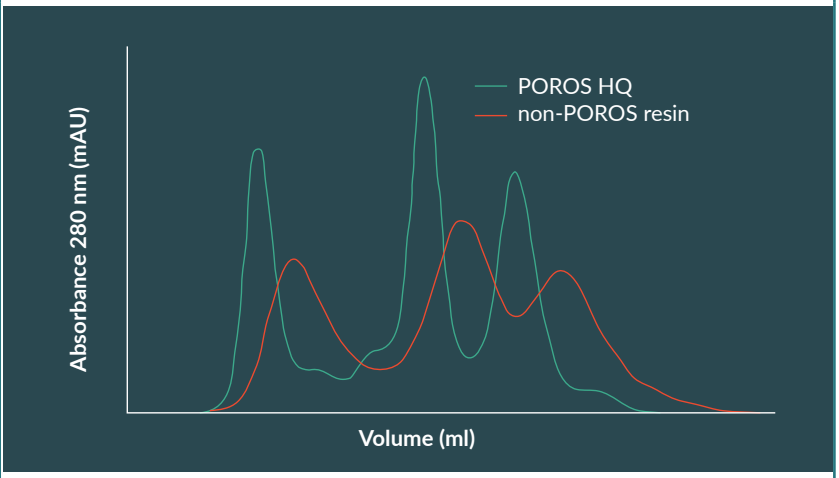
Empty capsids are a byproduct of vector biosynthesis and may cause reverse effects in patients receiving therapy. Therefore, the clinical use of AAV vectors might need the removal of empty AAV capsids from the final product [24].

The utilization of an affinity column requires a polishing step after capture to separate empty capsids from full ones. Ion exchange (IEX) chromatography has shown to be most applicable for this application, making use of the difference in pI of full and empty AAV particles. Since the difference in pI is only 0.4 units, a high resolution IEX resin with superior selectivity such as POROS™ HQ50 is required (Figure 7) [13].

For example, Qu *et al.* have shown efficient separation of empty from full capsids by optimizing POROS™ HQ50 in a polishing step presented in Figures 8 & 9 [25]. The use of POROS™ HQ for this purpose resulted in a good yield and was successfully at large scale, making it a suitable polish step after affinity capture.

FIGURE 7

Resolution graph of POROS™ HQ, 50 micron, strong anion exchange resin.



The graph reveals the successful separation of a mix of three proteins where two of them only differ by 0.1 units in isoelectric point whilst a lower resolution non-POROS resin is not successful in separating the protein mixture. Sample: Transferrin 5mg/mL, pI 5.6, Chicken Ovalbumin 10mg/mL, pI 4.6, Soybean Trypsin Inhibitor 4mg/ml, pI 4.5; total protein loaded: 4.4 mg, 1.3 mg/ml.

CONCLUSION

Gene therapy shows great potential to treat a variety of diseases. The field is establishing efficient commercial manufacturing capabilities for these unique therapies to increase process productivity in order to meet market needs. Affinity chromatography is a powerful tool to address the unmet market need in viral vector downstream purification.

Thermo Fisher Scientific has developed a portfolio of affinity resins, including POROS™ CaptureSelect™ AAVX, AAV8 and AAV9 (Table 2) that have been designed to enhance the purification of AAV viral vectors.

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These resins offer important benefits:

- One-step AAV purification from crude material with high purity and yield
- High specificity and capacity, reducing the process volume significantly for subsequent steps and maximizing yield
- Basis of platform purification (reproducible and scalable)
- Robust with less process optimization steps

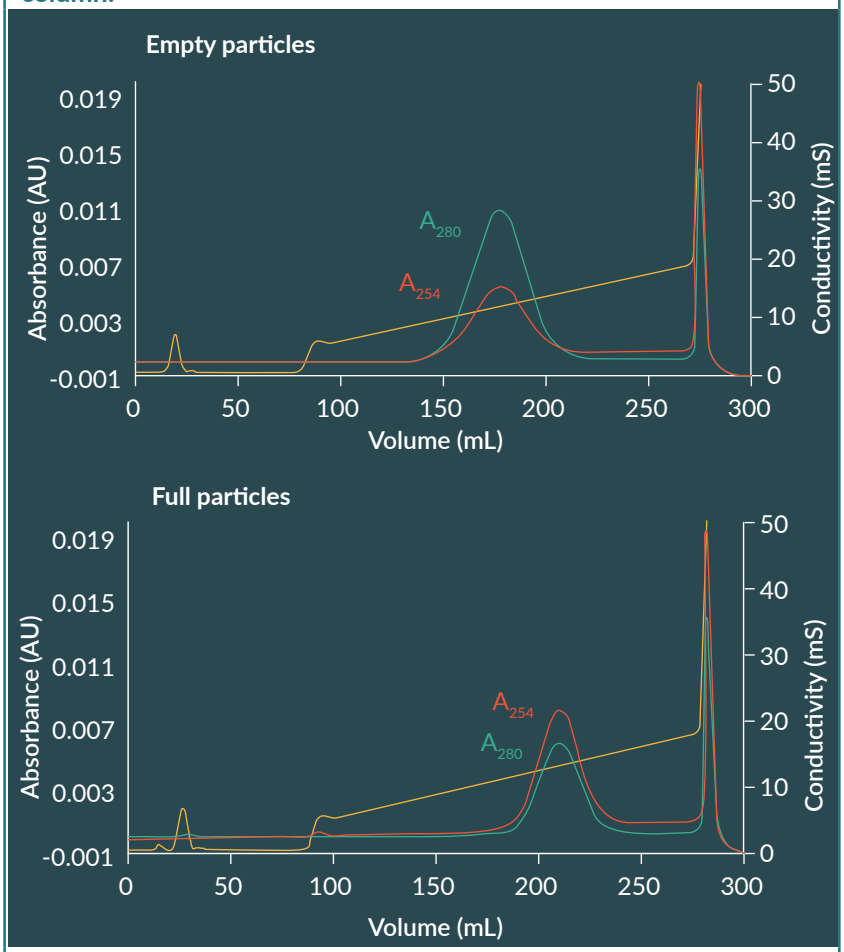
The utilization of affinity chromatography will be a significant improvement to the downstream processing of viral vectors, by reducing the purification steps and maximizing productivity. The availability of such highly selective resins will enable the biomanufacturing industry to overcome current production challenges in the gene therapy field.

FINANCIAL & COMPETING INTERESTS DISCLOSURE

TOT, SS, PH, JDR and FD are employees of ThermoFisher. ThermoFisher is the manufacturer of all AAV resins mentioned in the article. No writing assistance was utilized in the production of this manuscript.

FIGURE 8

Elution profiles of empty particles and full particles, separated by CsCl gradient ultracentrifugation, applied onto a POROS™ HQ50 column.



The empty capsids elute in earlier fractions (A) than the full AAV particles (B). [25]

TABLE 2

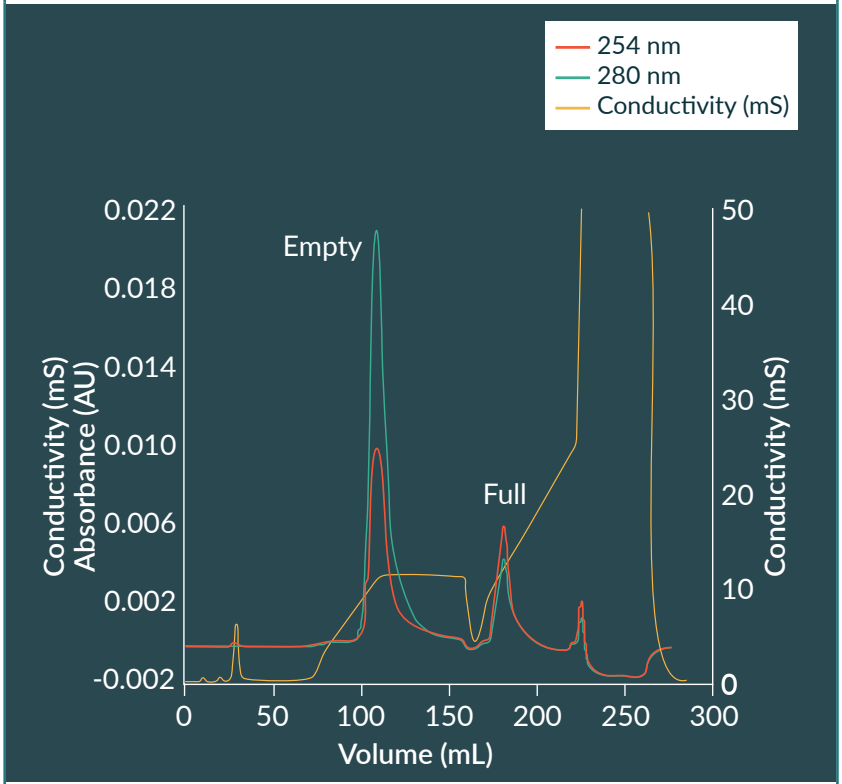
POROS™ CaptureSelect™ AAV affinity resins describing binding capacities and serotype affinity.

Thermo Scientific™ resin	Binding capacity (vg/mL)	Serotype affinity
POROS CaptureSelect AAV8	$>10^{13}$	AAV8
POROS CaptureSelect AAV9	$>10^{14}$	AAV9
POROS CaptureSelect AAVX	$>10^{14*}$	AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8*, AAV9, recombinant and chimeric vectors**

* viral genomes per milliliter (vg)/mL, binding capacity will vary based on serotype, feed stream, additives, and mutations to parent serotypes. ** To date, the AAVX ligand has shown affinity towards all serotypes tested.

FIGURE 9

Chromatogram of an AAV mixture of empty and full particles.



Capsids were separated by CsCl gradient ultrafiltration and mixed together in a ratio of 16:1. The AAV mixture was applied onto a POROS™ HQ50 column showing excellent separation of the empty from the full AAV particles. [25]

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