Scalable Purification of Viral Vectors for Gene Therapy

An Appraisal of Downstream Processing Approaches

Maya Fuerstenau-Sharp, David Pettitt, Kim Bure, James Smith, Jamie Ware, and David Brindley

Gene therapy is the transfer of genetic material to a patient's cells to achieve a therapeutic effect. Therapeutic DNA is largely delivered using viral vector systems based on adenoviruses (Ad), adenoassociated viruses (AAV), and lentiviruses (LV). With the application of such viral vectors as clinical therapeutics growing, scalable commercial processes (particularly for purification) are being investigated and optimized to best ensure that critical quality attributes (CQAs) are retained. Herein we review viral vector purification techniques and the effect of different characteristics of vector classes on the selection of optimal unit operations.

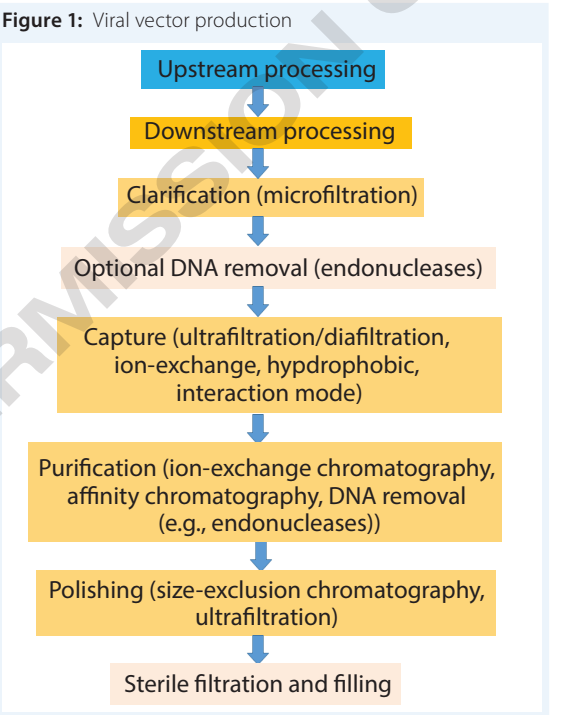
Viral Vector Classes

Each class of viral vector has its own challenges and opportunities for gene therapeutics (Table 1). Adenoviruses were initially proposed for gene therapy over 20 years ago, but they are now less popular for clinical applications because of significant complexities such as their challenging vector design and systematic administration, large genome size, and (most important) their high immunogenicity (**1**).

Lentiviruses already have shown promise in clinical trials, for which they have been used in the treatment of Wiskott–Aldrich syndrome, X-linked adrenoleukodystrophy, and metachromatic leukodystrophy (**2–4**). The central mechanisam of action (MoA) for lentiviruses relates to their ability to transduce both dividing and nondividing cells, resulting in life-long transgene expression and potential therapeutic effect.

AAVs also are considered a promising tool for gene therapy because of their lack of pathogenicity and immunogenicity paired with long-term transgene expression and broad cell tropism (**5**). Currently, the only commercialized gene therapy in the Western world is Glybera (alipogene tiparvovec) from uniQure, which is based on AAV1 and has been used to treat hereditary lipoprotein lipase deficiency (LPLD).

Figure 1: Viral vector production



Biomanufacturing Considerations

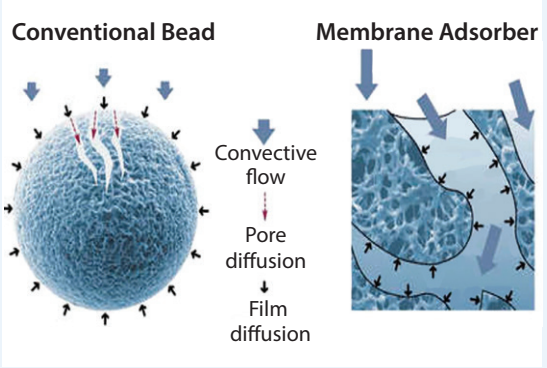
Gene therapy applications require large-scale processes that can generate highly pure and biologically active vectors that fulfill regulatory chemistry, manufacturing, and controls (CMC) requirements. They should be robust, scalable, cost-effective, and ideally applicable to a large variety of viral vectors (**6**). As Figure 1 shows, viral vector production consists of upstream and

Table 1: Viral vector overview (**46–50**)

Viral Vector	Example	Advantages	Disadvantages	Manufacturing Considerations
Retroviruses	Oncoretroviruses, spumaviruses, VSV	Effective integration into target cell chromatin, clinical phase	Limited ex vivo application, Insertional mutagenesis	Amenable to RCR-free scale-up and banking
Lentiviruses	VSV-pseudotyped, HFV	Efficiently transduce CNS cells, long-term transgene expression, application in adoptive T-cell platforms, clinical phase	Insertional mutagenesis, preclinical phase, limited cassette size	Amenable to large-scale clinical production
Adenoviruses	Human adenoviral serotypes (**47, 50**)	Clinical phase, demonstrable efficacy	Pre-existing immunity and inflammatory response, complexity for manipulation	10-L to 50-L clinical-grade production achievable
AAV	Human parvovirus, AAV-1 – AAV-6	No known disease associated with AAV infection, low toxicity, long-term transgene expression, clinical phase	T-cell response, antibody neutralization, low DNA packaging capacity	DSP complexity
HSV	HSV-1	Efficient infectivity for multiple cell types	Difficulties with long term gene expression, lack of patient experience, preclinical	Low titers

*AAV = adenoassociated virus, HSV = herpes simplex virus, RCR = replication-competent retrovirus, CNS = central nervous system (including neurons and glial cells), VSV = vesicular stomatitis virus, HFV = human foamy virus, DSP = downstream processing

Figure 2: Conventional chromatography compared with membrane adsorbers



downstream processes comprising several steps, depending on the viral properties of a product.

Upstream processing of virus particles for gene therapy vectors entails virus growth and harvesting, whereas downstream processing is focused at viral vector purification. Notably, downstream purification of viral vectors accounts for the majority of the overall manufacturing cost, and it represents a bottleneck in viral vector manufacturing (**7**). Scalable downstream processing contains several steps: clarification (microfiltration), capture (ultrafiltration/diafiltration), purification (ion-exchange chromatography (IEX) and affinity chromatography (AF)), and polishing (size-exclusion chromatography (SEC) and ultrafiltration).

Because each virus has different biochemical and physical properties, viral gene-therapy vector purification must be tailored accordingly. This process requires optimization to preserve virus infectivity (closely related to product efficacy and a typical release assay) and maximize recovery. The decision regarding purification method should take characteristics such as virus particle size and stability, charge at neutral pH, and relative particle stability into consideration (**8**). You must thoroughly understand your purification process to identify critical steps that affect final-product quality.

Viral Vector Purification Methods

Several approaches can be taken for viral gene therapy vector purification, including ultracentrifugation and membrane and column chromatography methods. The latter two are amenable to scale-up.

Ultracentrifugation is used mainly at laboratory scale and is not scalable because available rotors usually have only small-volume capacities. It frequently results in a loss of active viral particles, which could be attributed to viral aggregation and shear forces among other possible explanations (**8**). Compared with membrane and column chromatography, ultracentrifugation is challenging to automate. Its use can increase processing times and the risk of product degradation.

Although **column chromatography** tools are scalable and routinely used for purifying

Table 2: Vector purification chromatography products

Viral Vector	Purification	Phase	Proprietary Chromatography Product	Ref.
Ad5	IEX	Column resin	Fractogel DEAE (EMD Millipore); Q Sepharose XL, Source Q15, Streamline Q XL (GE Healthcare); CHT ceramic hydroxyapatite (Bio-Rad)	17–24
	SEC	Column resin	Toyopearl HW-75F (Tosoh); Superdex 200, Sephacryl,S-400HR, and Sephacryl S-500 (GE Healthcare Life Sciences)	
	AF	Column resin	TosoHaas chelate Zinc	
AAV1	IEX	Column resin	POROS HQ (Thermo Fisher), HiTrap Q (GE Healthcare Life Sciences)	25–27
	AF	Column resin	AVB Sepharose HP (GE Healthcare Life Sciences)	
AAV2	IEX	Column resin	Q-Sepharose, Source 15Q, POROS HQ, HiTrap Q, POROS PI, MacroPrep DEAE (Bio-Rad); UNO S1(Bio-Rad), Fractogel SO3, POROS 50HS, SP Sepharose HP, CHT ceramic hydroxyapatite	27–39
	AF	Column resin	A20 Mab coupled to HiTrap-Separose and AVB, Sepharose HP POROS HE/P, POROS HE1/M, HiTrap Heparin, Cellufine sulfate resin (JNC)	
AAV4	IEX	Column resin	Sepharose HP	29
AAV5	IEX	Column resin	Source 15Q, Mono Q HR, POROS HQ, HiTrap Q, POROS PI, SP Sepharose HP; Sartobind Q, Mustang Q (Pall), Sartobind S, Mustang S	27, 29, 37–40
	AF	Membrane	Mucin-Sepharose	
	SEC	Column resin	Superdex 200 (GE Healthcare Life Sciences)	
AAV6	IEX	Column resin	HiTrap Q HP	41
AAV8	IEX	Membrane	Mustang Q, Sartobind Q, Sartobind STIC, Mustang S, Sartobind S,	42
Baculovirus	IEX	Membrane	Sartobind D, Sartobind S, Mustang S	43, 44
	SEC	Column resin	Sepharose CL-4B	
Lentivirus	IEX	Column resin	HiTrap Q and Fractogel TMAE	45–47
	IEX	Membrane	Sartobind Q, Sartobind STIC, Mustang Q, Sartobind D	
	AF	Column resin	Fractogel Heparin	

IEX = ion-exchange chromatography; SEC = size-exclusion chromatography, AF = affinity chromatography

biomolecules, they are not well suited for purification of larger molecules such as DNA and viruses. In such cases, purification typically is achieved by diffusion through a 0.1-µm matrix.

With membrane pores >3 µm, **membrane adsorbers** have become popular tools for purifying viruses. They adsorb virus particles to a solid phase. Because of their large pore size, membrane adsorbers can be operated at significantly faster flow rates, therein resulting in significant time and cost of goods (CoG) savings (**9**). Another advantage is their possibility of applying mild elution conditions, which increases the likelihood of preserving virus infectivity (**6**). Figure 2 illustrates how virus purification is achieved with membrane adsorbers as opposed to using beads.

Modes of Purification Chromatography

Chromatography can be divided further into four modes, each achieving virus purification by using a different mechanism. Purification through IEX is based on the net charge of proteins located on the outside of the viral capsid. AF exploits interactions between a matrix-coupled receptor or ligand with the viral capsid. SEC (gel filtration) separates viruses from DNA, capsids, and proteins according to size. And hydrophobic-interaction chromatography (HIC) binds viral capsid proteins to a matrix through hydrophobic interaction using an aqueous solvent (**10**).

Modes of Choice Based On Vector Class

Because each vector class has specific characteristics, certain purification modes are apt to function more effectively (Table 2). For the purification of adenoviruses, namely Ad5, all aforementioned chromatographic methods can be used. Because Ad viruses are negatively charged at a neutral pH, various anion-exchange adsorbents can be used for their purification.

IEX using anion exchangers also has been reported for several AAVs (**11**). Immobilized-metal affinity chromatography (IMAC) is another method by which Ad can be purified. That approach is based on binding Ad particles to charged zinc ions bound to a column. That method provides several options for purifying AAVs, exemplified by heparin affinity chromatography, which is particularly suited for AAV2 purification (**12**). SEC is a suitable method for polishing Ad and AAV5, and HIC purifies Ad particles with high concentrations of ammonium sulfate among other salts (**13**). The latter method has yet to be described for the purification of AAV serotyes (**10**).

Because of their negative charge, lentiviruses are routinely purified using membrane adsorbers and AEX tools based on columns used in a capture step. This step varies the most among different viral vector purification groups. Lentiviruses also can be purified by using heparin affinity chromatography, but it introduces an animal-derived reagent that should be elminated in large-scale gene-therapy manufacturing (**14**). Finally, baculoviruses are commonly purified using membrane-based anion exchangers in the capture step, anion-exchange membrane adsorbers, and SEC.

Progress, But Work Remains

The selection of purification technologies in gene therapy bioprocessing is critical to end-product quality and CoG and very much a multifactorial process. Although platform bioprocesses are emerging for gene therapy bioprocessing, they are not yet established as such. Each vector requires thorough characterization and process development. However, classical large-scale purification technologies (particularly chromatographic approaches) are highly amenable to gene therapy bioprocessing. As such, this emerging industry would be well served to seek to unite emerging basic science expertise in genetic engineering approaches with established bioprocessing skills and technologies.

Tools and technologies required to build a sustainable and profitable gene-therapy industry are within our grasp. But the challenge at hand is determining how to configure them optimally to accelerate development of life-saving and efficacious gene therapies to the clinic, with data packages that pass regulatory muster and a cost that providers can afford.

References

1 Lukashev AN, Zamyatnin AA Jr. Viral Vectors for Gene Therapy: Current State and Clinical Perspectives. *Biochemistry* (Mosc). 81(7) 2016: 700–708; doi: 10.1134/S0006297916070063.

2 Aiuti A, et al. Lentiviral Hematopoietic Stem Cell Gene Therapy in Patients with Wiskott–Aldrich Syndrome. *Science* 341(6148) 2013: 1233151; doi: 10.1126/science.1233151.

3 Cartier N, et al. Hematopoietic Stem Cell Gene Therapy with a Lentiviral Vector in X-Linked Adrenoleukodystrophy. *Science* 326(5954) 2009: 818–823; doi: 10.1126/science.1171242.

4 Biffi A, et al. Lentiviral Hematopoietic Stem Cell Gene Therapy Benefits Metachromatic Leukodystrophy. *Science* 341(6148) 2013: 1233158; doi: 10.1126/science.1233158.

5 dos Santos Coura R, Beyer Nardi N. Genetics A Role for Adenoassociated Viral Vectors in Gene Therapy. *Genetics Mol. Bio.* 31(1) 2008: 1–11.

6 Morenweiser R. Downstream Processing of Viral Vectors and Vaccines. *Gene Ther.* Oct. 2005: S103–S110.

7 Lyddiatt A, O'Sullivan D. Biochemical Recovery and Purification of Gene Therapy Vectors. *Curr. Opin. Biotechnol.* 9(2) 1998: 177–185.

8 Segura MM, Kamen A, Garnier A. Downstream Processing of Oncoretroviral and Lentiviral Gene Therapy Vectors. *Biotechnol. Adv.* 24(3) 2006: 321–337.

9 Ball PR, Gyepi-Garbrah I. Membrane Adsorbers as a Tool for Rapid Purificationof Gene Therapy Products.

10 Burova E, Ioffe E. Chromatographic Purification of Recombinant Adenoviral and Adenoassociated Viral Vectors: Methods and Implications. *Gene Ther.* Oct. 2005: S5–S17.

11 Gao G, Vandenberghe LH, Wilson JM. New Recombinant Serotypes of AAV Vectors. *Curr. Gene Ther.* 5(3) 2005: 285–297.

12 Clark K, et al. Highly Purified Recombinant Adeno-Associated Virus Vectors are Biologically Active and Free of Detectable Helper and Wild-Type Viruses. *Hum. Gene Ther.* 10(6) 1999: 1031–1039.

13 Huyghe BG, et al. Purification of a Type 5 Recombinant Adenovirus Encoding Human p53 By Column Chromatography. *Hum. Gene Ther.* 6(11) 1995: 1403–1416.

14 Merten OW, Hebben M, Bovolenta C. Production of Lentiviral Vectors. *Mol. Ther. Methods Clin. Dev.* 13 (3) 2016: 16017. doi: 10.1038/mtm.2016.17.

15 Lusky M. Good Manufacturing Practice Production of Adenoviral Vectors for Clinical Trials. *Hum. Gene. Ther.* 16, 2005: 281–291.

16 Green AP, et al. A New Scalable Method for the Purification of Recombinant Adenovirus Vectors. *Hum. Gene Ther.* 13(16) 2002: 1921–1934.

17 Kamen A, Henry O. Development and Optimization of an Adenovirus Production Process. *J. Gene Med.* 6, 2004: S184–192.

18 Huyghe BG, et al. Purification of a Type 5 Recombinant Adenovirus Encoding Human p53 By Column Chromatography. *Hum. Gene Ther.* 6(11), 1995: 1403–1416.

19 Blanche F, et al. An Improved Anion-Exchange HPLC Method for the Detection and Purification of Adenoviral Particles. *Gene Ther.* 7(12) 2000: 1055–1062.

20 Peixoto C, et al. Purification of Adenoviral Vectors Using Expanded-Bed Chromatography. *J. Virol. Methods* 132(1–2) 2006: 121–126.

21 Vellekamp G, et al. Empty Capsids in Column-Purified Recombinant Adenovirus Preparations. *Hum. Gene Ther.* 12(15) 2001: 1923–1936.

22 Konz JO, et al. Development of a Purification Process for Adenovirus: Controlling Virus Aggregation to Improve the Clearance of Host Cell DNA. *Biotechnol. Prog.* 21, 2005: 466–472.

23 Smith R H, Levy JR, Kotin RM. A Simplified Baculovirus-AAV Expression Vector System Coupled with One-Step Affinity Purification Yields High-Titer rAAV Stocks from Insect Cells. *Mol. Ther.* 17(11) 2009: 1888–1896; doi: 10.1038/mt.2009.128.

24 Urabe M, et al. Removal of Empty Capsids from Type 1 Adeno-Associated Virus Vector Stocks By Anion-Exchange Chromatography Potentiates Transgene Expression. *Mol. Ther.* 13(4) 2006: 823–828.

25 Zolotukhin S, et al. Production and Purification of Serotype 1, 2, and 5 Recombinant Adenoassociated Viral Vectors. *Methods* 28(2) 2002: 158–167.

26 Qu G, et al. Separation of Adeno-Associated Virus Type 2 Empty Particles from Genome Containing Vectors By Anion-Exchange Column Chromatography. *J. Virol. Methods* 140(1–2) 2007: 183–192.

27 Kaludov N, Handelman B, Chiorini JA. Scalable Purification of Adeno-Associated Virus Type 2, 4, or 5 Using Ion-Exchange Chromatography. *Hum. Gene Ther.* 13, 2002: 1235–1243.

28 Gao G, et al. Purification of Recombinant Adenoassociated Virus Vectors By Column Chromatography and Its Performance In Vivo. *Hum. Gene Ther.* 11(15) 2000: 2079–2091.

29 O'Riordan, et al. Scalable Chromatographic Purification Process for Recombinant Adeno-Associated Virus (rAAV). *J. Gene Med.* 2, 2000: 444–454.

30 Brument N, et al. A Versatile and Scalable Two-Step Ion-Exchange Chromatography Process for the Purification of Recombinant Adeno-Associated Virus Serotypes-2 and -5, *Mol. Ther.* 6(5) 2002: 678–686.

31 Zolotukhin S, et al. Recombinant Adenoassociated Virus Purification Using Novel Methods Improves Infectious Titer and Yield. *Gene Ther.* 6(6) 1999: 973–985.

32 Chahal PS, Aucoin MG, and Kamen A. Primary Recovery and Chromatographic Purification of Adenoassociated Virus Type 2 Produced By Baculovirus/Insect Cell System. *J. Virol. Methods* 139, 2007: 61–70.

33 Grimm D, et al. Novel Tools for Production and Purification of Recombinant Adenoassociated Virus Vectors, *Hum. Gene Ther.* 9, 1998: 2745–2760.

34 Clark KR, et al. Highly Purified Recombinant Adenoassociated Virus Vectors Are Biologically Active and Free of Detectable Helper and Wild-Type Viruses. *Hum. Gene Ther.* 10, 1999: 1031–1039.

35 Anderson R, et al. A Method for the Preparation of Highly Purified Adenoassociated Virus Using Affinity Column Chromatography, Protease Digestion and Solvent Extraction. *J. Virol. Methods* 85, 2000: 23–34.

36 Harris JD, Beattie SG, Dickson JG. Novel Tools for Production and Purification of Recombinant Adenoassociated Viral Vectors. *Methods Mol. Med.* 76, 2003: 255–267.

37 Smith RH, Ding C, Kotin RM. Serum-Free Production and Column Purification of Adenoassociated Virus Type 5. *J. Virol. Methods* 114, 2003: 115–124.

38 Auricchio A, et al. A Single-Step Affinity Column for Purification of Serotype-5–Based Adenoassociated Viral Vectors. *Mol. Ther.* 4, 2001: 372–374.

39 Sayroo R, et al. Development of Novel AAV Serotype 6 based Vectors with Selective Tropism for Human Cancer Cells. *Gene Ther.* 1, 2016: 18–25.

40 Davidoff AM. Purification of Recombinant Adeno-Associated Virus Type 8 Vectors By Ion-Exchange Chromatography Generates Clinical Grade Vectorstock. *J. Virol. Methods* 121, 2004: 209–215.

41 Vicente T, et al. Purification of Recombinant Baculoviruses for Gene Therapy Using Membrane Processes, *Gene Ther.* 16, 2009: 766–775.

42 Wu C, Soh KY, Wang S. Ion-Exchange Membrane Chromatography Method for Rapid and Efficient Purification of Recombinant Baculovirus and Baculovirus gp64 Protein. *Hum. Gene Ther.* 18, 2007: 665–672.

43 Scherr M. Efficient Gene Transfer into the CNS By Lentiviral Vectors Purified By Anion Exchange Chromatography. *Gene Ther.* 9, 2002: 1708–1714.

44 Yamada K, et al. Lentivirus Vector Purification Using Anion Exchange HPLC Leads to Improved Gene Transfer. *Biotechniques* 34, 2003: 1074–1078, 1080.

45 Segura MM, et al. Production of Lentiviral Vectors By Large-Scale Transient Transfection of Suspension Cultures and Affinity Chromatography Purification. *Biotechnol. Bioeng.* 98, 2007: 789–799.

46 Pettitt D, et al. Emerging Platform Bioprocesses for Viral Vectors and Gene Therapies. *BioProcess Int.* 14(4) 2016: S8–S17.

47 Rincon MY, Vandendriessche T, Chuah MK. Gene Therapy for Cardiovascular Disease: Advances in Vector Development, Targeting, and Delivery for Clinical Translation. *Cardiovas. Res.* 108(1) 2015: 4–20.

48 Kay MA, Glorioso JC, Naldini L. Viral Vectors for Gene Therapy: The Art of Turning Infectious Agents into Vehicles of Therapeutics. *Nature Med.* 7(1) 2001: 33–40.

49 Sheu J, et al. Large-Scale Production of Lentiviral Vector in a Closed System Hollow Fiber Bioreactor. *Mol. Ther. Meth. Clin. Dev.* 2, 2015: 15020.

50 Kallel H, Kamen AA. Large-Scale Adenovirus and Poxvirus-Vectored Vaccine Manufacturing to Enable Clinical Trials. *Biotechnol. J.* 10(5) 2015: 741–747.

Maya Fuerstenau-Sharp *is a field-marketing manager at Sartorius Stedim (Göttingen, Germany).* ***David Pettitt*** *is an academic surgeon, CASMI research associate, associate at IP Asset Ventures, Ltd, and a doctoral researcher at the University of Oxford.* ***Kim Bure*** *is director of regenerative medicine at Sartorius Stedim.* ***James Smith*** *is a CASMI research associate and doctoral researcher in the University of Oxford's Nuffield Department of Orthopedics, Rheumatology, and Musculoskeletal Sciences, and an associate at IP Asset Ventures, Ltd.* ***Jamie Ware*** *is a CASMI research associate. Corresponding author* ***David Brindley*** *is founder and academic director of CASMI's Translational Stem Cell Consortium, senior healthcare translation research fellow in the medical sciences division's department of paediatrics at the University of Oxford, Cooksey-Saïd fellow in healthcare translation at the University of Oxford's Saïd Business School, honorary senior research associate at the University College London School of Pharmacy's Centre for Behavioral Medicine, a research fellow in cell therapy commercialization at the Harvard Stem Cell Institute, and regenerative medicine regulation and risk management lead at the USCF Stanford Center of Excellence in Regulatory Science and Innovation (CERSI) in Stanford, CA.*