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Optimizing AAV Filtration: Comparative Analysis of Membrane Performance and Recovery Rates

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Adeno-associated viruses (AAVs) have proven to be effective tools for gene therapy due to their ability to be engineered to deliver genetic material to target cells. This study investigates the performance of three different filtration membrane packs—Lepure, Cobetter, and Merck—in purifying the AAV8 serotype. We assessed the turbidity and AAV titer before and after filtration to evaluate the efficiency of each membrane. Before filtration, the AAV8 sample exhibited a turbidity of 173.6 nephelometric turbidity units (NTU) and a titer of 1.01×10^{11} viral genomes per mL (vg/mL). Post-filtration, it is observed that the Lepure membrane achieved a turbidity of 6.65 NTU and an AAV titer of 4.48×10^{9} vg/mL, while Cobetter resulted in a turbidity of 3.44 NTU and a titer of 3.80×10^{9} vg/mL. Merck demonstrated the lowest performance with a turbidity of 0.49 NTU and an AAV titer of 9.70×10^{9} vg/mL. Notably, Lepure demonstrated the highest recovery rate at 13.3%, despite its higher turbidity, indicating minimal viral adsorption. These findings highlight the importance of selecting appropriate filtration systems to optimize AAV recovery while maintaining low turbidity levels, ultimately enhancing the efficiency of AAV as a vector for therapeutic applications. Further research is recommended to refine these filtration methods to improve the purification of AAV.

Keywords: AAV (adeno-associated virus), AAV vector purification, gene therapy, member pack filtration, recovery optimization, serotype-specific purification, transduction efficiency.

Adeno-associated viruses (AAVs) have emerged as one of the most promising vectors for gene therapy due to their favorable safety profile, capacity for long-term gene expression, and minimal immunogenicity[16,18,19,21]. AAV is a non-enveloped virus with a protein capsid that encases a small, single-stranded DNA genome—hundreds of unique strains has been identified across various species[10,24]. Recombinant AAV (rAAV), lacking viral DNA, has proven to be an effective vehicle for certain gene therapy applications. As a protein-based nanoparticle[4,24], rAAV can penetrate the cellular membrane and deliver its genetic cargo directly to the control center of the cell, the nucleus[27,28].

However, the production of AAVs at a scale sufficient for clinical applications continues to pose significant challenges[8,18,24,28]. Traditional methods often resulted in low yields, making the optimization of AAV production crucial for advancing gene therapy technologies[7,9,14]. Recent advancements in bioprocessing techniques have focused on enhancing AAV yield through various strategies, including the optimization of cell culture conditions, vector design, and purification methods[3,12]. One innovative approach

that has gained traction in recent years is the use of specialized filtration systems during the purification process. Filtration can effectively remove impurities while concentrating the viral particles, leading to improved overall yields as a pivotal step in the manufacturing of biotherapeutic product[2]. The implementation of specialized filters, such as tangential flow filtration (TFF) and depth filtration, has been shown to improve the clarity and purity of AAV preparations in the laboratory. These filtration techniques can selectively retain viral particles based on size and molecular characteristics, while allowing smaller contaminants, such as host cell proteins and DNA, to pass through[13]. By optimizing the pore size and surface properties of the filters, it is possible to enhance the recovery of infectious viral particles, thereby increasing the yield of AAV production[1,5]. Moreover, recent studies have indicated that the use of filtration can significantly streamline the purification process, reducing the need for extensive chromatographic methodologies that are often time-consuming and resource-intensive[1,6]. The combination of increased efficiency and yield makes specialized filtration systems a promising avenue for enhancing AAV production, optimizing both consistency and scalability. Such advancements will not only improve the manufacturing process, but contribute to the development of cost-effective and accessible gene therapies, ultimately expanding therapeutic opportunities for a wide range of genetic disorders[20,22]. In this study, we aim to investigate the application of a novel filtration system in AAV production and its impact on yield enhancement. By examining the effects of filtration parameters on the recovery of AAV particles, we hope to provide valuable insights that could inform best practices for rAAV viral vector productions and contribute to the advancement of gene therapy.

MATERIALS AND METHODS

The materials used in this study included filtration membrane packs, rubber tubing, a peristaltic pump, anhydrous ethanol, and 50 mL syringes—all purchased from Lepure (China). The primary filtration membrane packs included Lepure, Cobetter, and Merck (Table 1).

The experimental procedure was as follows. The rubber tubing was first rinsed with sterile ultrapure water on both the inner and outer surfaces, dried, and then immersed in anhydrous ethanol[15]. Using a syringe, the tubing was filled with anhydrous ethanol and allowed to soak for 15 minutes to ensure complete disinfection. Next, the filters were connected in series, and the rubber tubing was attached to the peristaltic pump, which was set to a rotation speed of 10 revolutions per minute (rpm). The air inside the membrane pack was then expelled, and the dead volume of the system was calculated based on the first drop of liquid that flowed out from the end, along with the flow rate at the same pump speed. The AAV sample to be filtered was then introduced into the system, and the collection of the filtered AAV sample began once half of the dead volume was expelled from the end[17]. After filtering 150 mL of the sample, the system was flushed with phosphate-buffered saline (PBS) at a fixed volume of 300 mL, approximately equivalent to four times the system's dead volume. Finally, the turbidity and titer of the samples were measured before and after filtration to assess the filtration efficiency of the membrane[23]. The recovery efficiency was calculated by the ratio of the number of AAV particles recovered to the number of AAV particles before recovery, thereby quantifying the effectiveness of the filtration process.

RESULTS AND DISCUSSION

The experimental results demonstrated significant differences in the performance of the various filtration membrane packs. Before filtration, the AAV8 serotype exhibited a turbidity of 173.6 NTU and an AAV titer of 1.01×10^{11} vg/mL. After filtration, the results varied among the three brands assessed (Table 2). Filter 1 had a dead volume of 78.24 mL, a flow rate of 15.5 mL/min, the highest turbidity of 6.65 NTU, and an AAV titer of 4.48×10^{9} vg/mL. Filter 2 showed a dead volume of 77.34 mL, a flow rate of 23.5 mL/min, a turbidity of 3.44 NTU, and an AAV titer of 3.80×10^{9} vg/mL. In contrast, Filter 3 had the highest dead volume of 81.37 mL, a flow rate of 23.8 ml/min, a significantly lower turbidity of 0.49 NTU, and an AAV titer of 9.70×10^{5} vg/mL.

Interestingly, while filter 1 exhibited the highest turbidity post-filtration, it also demonstrated

the lowest viral adsorption, indicating that such filtration parameter allowed more particulate matter, such as cellular debris or residual host cell DNA, to pass through while retaining a higher number of AAV particles. The recovery rates calculated from the experiments revealed that filter 1 achieved a statistical significant recovery rate of 13.3 ± 0.3% when compare to recovery rate of 11.3 ± 0.2% of filter 2 (p-value < 0.05, Studet's t-test); and the recovery rate of filter 3 is statistically

Table 1. Filtration membrane packs examined for comparative analysis of membrane performance and recovery rates

Brand	Primary filtration membrane packs
filter 1: Lepure	catalog No. CMND01C1C5G4, China (5 ~ 20 μm, 20 cm^2)
filter 2: Cobetter	catalog No. CDFCDCSD4070PCP, Germany (4 ~ 18 μm, 23 cm^2)
filter 3: Merck	catalog No. MD0HC23CL3, China (4 ~ 18 μm, 23 cm^2)

Table 2. Post-filtration quantitative measurements of the filter membrane packs examined for comparative analysis of membrane performance and recovery rates

Brand	Dead volume (mL)	Flow rate (mL/min)	Turbidity (NTU)	AAV titer post-filtration (vg/mL)
filter 1: Lepure	78.24	15.5	6.65	4.48×10^9
filter 2: Cobetter	77.34	23.5	3.44	3.80×10^9
filter 3: Merck	81.37	23.8	0.49	9.70×10^5

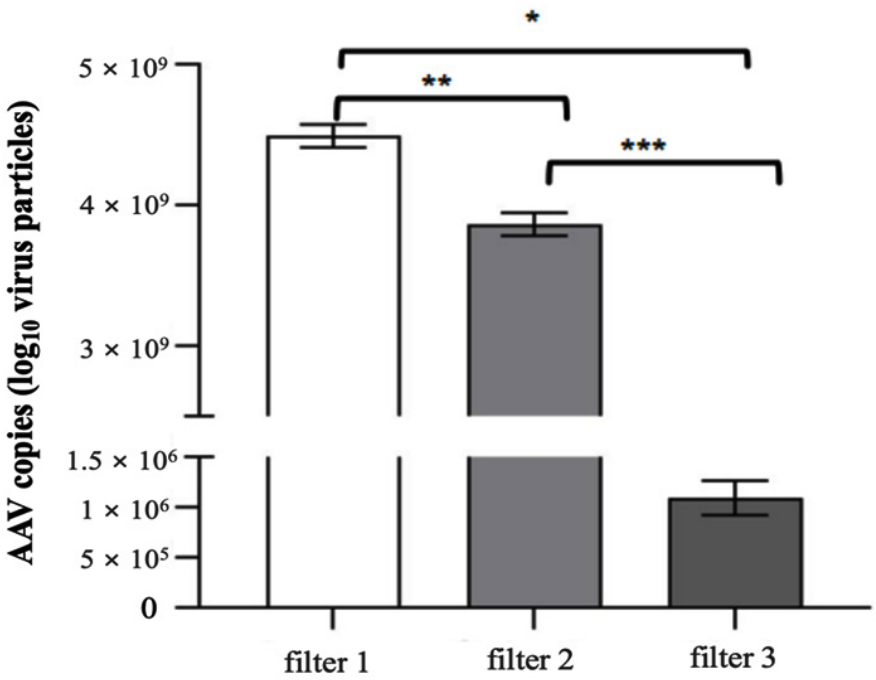

Fig. 1. Comparison of filter membranes for different commercial filters. The recovery rates calculated from the experiments revealed that filter 1 achieved a statistical significantly higher recovery rate of 13.3 ± 0.3% when compared to the recovery rate of filter 2 at 11.3 ± 0.2% (p-value < 0.05), and the recovery rate of filter 3 is statistical significantly lower to that of filter 1 and filter 2.
* = p < 0.005; ** = p < 0.05, *** = p < 0.005

significant when compare to that of filter 1 and fiilter 2 (Fig 1). The minimal viral adsorption observed with filter 1, Lepure is significant as it implies a more efficient recovery of viral vectors, despite an increased presence of impurities. These findings suggest that the choice of filtration membrane plays a crucial role for optimizing AAV recovery and minimizing loss during the purification process[1,13,26]. Additionally, the images taken during the filtration process revealed the visual differences between the filtered products to be subtle. however, the products obtained via filter 1 appeared darker compared to the nearly colorless product obtained via filter 3. This observation agrees with the turbidity measurements, where filter 1's higher turbidity suggests a greater presence of particulate matter post-filtration.

CONCLUSION

Overall, these results underscore the importance of selecting appropriate filtration systems to maximize AAV recovery while maintaining an acceptable level of turbidity[25], playing an essential role for effective gene therapy applications. The examination on the effects of filtration parameters on the retention of AAV particles in this study has proven to yield more promising results, as opposed to traditional methods such as using chromatographic filtration[11,12,16]. Further optimization and analysis of these filtration methods could enhance the purification processes for AAV production, building the foundation for clinical success in the treatment of various human diseases. It is also pivotal to conduct further research on the compatibility of filtration membrane packs across various AAV serotypes, in order to support the development of universal vectors that meet the high standards of purity, efficacy, and safety[12,18,22,28].

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Conflicts of Interest

The authors do not have any conflict of interest.

Data Availability Statement

This statement does not apply to this article.

Ethics Statement

This research did not involve human participants, animal subjects, or any material that requires ethical approval.

Informed Consent Statement

This study did not involve human participants, and therefore, informed consent was not required.

Clinical Trial Registration

This research does not involve any clinical trials.

Authors' Contributions

YeQing Wu: Conceptualization, Methodology, Data Collection, Writing – Final Approval of the Manuscript; GuoHao Xie: Conceptualization, Methodology, Data Collection, Writing – Final Approval of the Manuscript; WeiXian Xu: Data Collection, Analysis, Writing – Final Approval of the Manuscript; Jun Yuan: Data Collection, Analysis, Writing – Final Approval of the Manuscript; Wayne Wenyan Xu: Data Collection, Analysis, Writing – Final Approval of the Manuscript; Henry Xiaoyu Yu: Analysis, Writing – Final Approval of the Manuscript; Lothar Yulin Jiang: Analysis, Writing – Final Approval of the Manuscript; Jeremy Junxi Ruan: Analysis, Writing – Final Approval of the Manuscript; H. Fai Poon: Conceptualization, Methodology, Data Collection, Writing – Final Approval of the Manuscript

REFERENCES

1. Andari J. E., Grimm D. Production, Processing, and Characterization of Synthetic AAV Gene Therapy Vectors. *Wiley*. 2020;16(1).
2. Besnard L., Fabre V., Fettig M., Gousseinov E., Kawakami Y., Laroudie N., Scanlan C., Pattnaik P. Clarification of vaccines: An overview of filter based technology trends and best practices. *Elsevier BV*. 2016;34(1):1-13.
3. Binny C. J., Nathwani, A. C. Vector Systems for

Prenatal Gene Therapy: Principles of Adeno-Associated Virus Vector Design and Production. *Humana Press*. 2012;109-131.
4. Büning H., Srivastava A. Capsid Modifications for Targeting and Improving the Efficacy of AaV Vectors. *Cell Press*. 2019;12:248-265.
5. Carvalho W., Carlson J. O., Wickramasinghe S. R. Tangential flow filtration for virus purification. *Elsevier BV*. 2008;321(2):373-380.
6. Chen S.-H., Papaneri A., Walker M., Scappini E., Keys R. D., Martin N. P. A Simple, Two-Step Small Scale Purification of Recombinant Adeno-Associated Viruses. *J Virol Methods*. 2020;281:113863
7. Clément N., Grieger J. C. Manufacturing of recombinant adeno-associated viral vectors for clinical trials. *Cell Press*. 2016;3:16002-16002.
8. Conlon T. J., Flotte T. R. Recombinant adeno-associated virus vectors for gene therapy. *Taylor & Francis*. 2004;4(7):1093-1101.
9. Davidsson M., Heuer A. Small scale adeno-associated virus-vector production for preclinical gene delivery based on chloroform precipitation. *Medknow*. 2022;17(1):99.
10. Daya S., Berns K. I. Gene Therapy Using Adeno-Associated Virus Vectors. *American Society for Microbiology*. 2008;21(4):583-593.
11. Fripont S., Marneffe C., Marino M., Rincón M. Y., Holt M. G. Production, Purification, and Quality Control for Adeno-associated Virus-based Vectors. *MyJOVE*. 2019.
12. Grieger J. C., Soltys S., Samulski, R. J. Production of Recombinant Adeno-associated Virus Vectors Using Suspension HEK293 Cells and Continuous Harvest of Vector From the Culture Media for GMP FIX and FLT1 Clinical Vector. *Elsevier BV*. 2016;24(2):287-297.
13. Guo P., El Gohary Y., Prasadan K., Shiota C., Xiao X., Wiersch J., Paredes J., Tulachan S., Gittes, G. K. Rapid and simplified purification of recombinant adeno-associated virus. *Elsevier BV*. 2012;183(2): 139-146.
14. Halbert C. L., Allen J. M., Chamberlain J. S. AAV6 Vector Production and Purification for Muscle Gene Therapy. *Springer Science+Business Media*. 2017;257-266.
15. Hapoñska M., Clavero E., Salvadó J., Farriol X., Torras C. Pilot scale dewatering of Chlorella sorokiniana and Dunaliella tertiolecta by sedimentation followed by dynamic filtration. *Elsevier BV*. 2018;33:118-124.
16. Hauck B., Bajaj J., Joyce S., Qu G., Zelenaia O., High K. A., Wright J. F. 508. Development of Optimized AAV2 Vector Biosynthesis and Purification Processes To Support High Capacity Production of Clinical Vectors of High Purity and Potency. *Elsevier BV*. 2006;13:S196-S196.
17. Kim K., Park J., Jung J., Lee R., Park J., Yuk J. M., Hwang H., Yeon J. H. Cyclic tangential flow filtration system for isolation of extracellular vesicles. *AIP Publishing*. 2021;5(1).
18. Koerber J. T., Jang J. J., Yu J., Kane R., Schaffer D. V. 507. Engineering a Novel AAV Variant for Purification Via Immobilized Metal Affinity Chromatography. *Elsevier BV*. 2006;13:S196-S196.
19. Lieshout L. P. V., Domm J. M., Wootton S. K. AAV-Mediated Gene Delivery to the Lung. *Springer Science+Business Media*. 2019;361-372.
20. Marks P., Witten C. Toward a new framework for the development of individualized therapies. *Springer Nature*. 2020;28(10-11):615-617.
21. McNally D. J., Piras B. A., Willis C., Lockey T., Meagher M. M. Development and Optimization of a Hydrophobic Interaction Chromatography-Based Method of AAV Harvest, Capture, and Recovery. *Cell Press*. 2020;19:275-284.
22. Merten O., Gény-Fiamma C., Douar A. Current issues in adeno-associated viral vector production. *Springer Nature*. 2005;12(S1):S51-S61.
23. Naismith J. H. Membrane integrity — direct turbidity measurement of filtrate from MF membrane modules at an operating potable water treatment plant. *Elsevier BV*. 2005;179(1-3):25-30.
24. Naso M., Tomkowicz B., Perry W. L., Strohl W. R. Adeno-Associated Virus (AAV) as a Vector for Gene Therapy. *Adis, Springer Healthcare*. 2017;31(4):317-334.
25. Shi H., Xagoraraki I., Parent K. N., Bruening M. L., Tarabara V. V. Elution Is a Critical Step for Recovering Human Adenovirus 40 from Tap Water and Surface Water by Cross-Flow Ultrafiltration. *American Society for Microbiology*. 2016;82(16):4982-4993.
26. Shoaebargh S., Gough I. A., Medina M. F. C., Smith A., Heijden J. V. D., Lichty B. D., Bell J. C., Latulippe D. R. Sterile filtration of oncolytic viruses: An analysis of effects of membrane morphology on fouling and product recovery. *Elsevier BV*. 2018;548:239-246.
27. Vliet K. V., Blouin V., Brument N., Agbandje McKenna M., Snyder R. O. The Role of the Adeno-Associated Virus Capsid in Gene Transfer. *Springer Science+Business Media*. 2008;51-91.
28. Wang D., Tai P. W., Gao G. Adeno-associated virus vector as a platform for gene therapy delivery. *Nature Portfolio*. 2019;18(5):358-378.