CELL & GENE THERAPY INSIGHTS

DOWNSTREAM BIOPROCESSING


INTERVIEW

# Improvement of viral vector purification using Mustang® Q membrane chromatography

**David McCall**, Editor, *Cell & Gene Therapy Insights*, talks to (pictured from left to right) **Saadia Zakai,** Product Development Manager, Gene Therapy, Pall Corporation, **Mark Schofield,** Senior R&D Manager, Pall Corporation and **Hélène Lebas,** Process Development Manager, Yposkesi, an SK pharmteco company

*Cell & Gene Therapy Insights* 2022; 8(9), 1551–1560

DOI: 10.18609/cgti.2022.226

## Q Can you briefly introduce yourselves, and tell us about your respective roles?

**HL:** As a process scientist at Yposkesi, my role is to develop lentiviral vector and adeno-associated viral (AAV) vector production and purification processes from lab to industrial scale for different clients as well as internal projects.

Yposkesi is a French Contract Development and Manufacturing Organization (CDMO) for cell and gene therapy viral vector manufacturing, located in the south of Paris. We are specialists in the manufacturing of lentiviral vectors and AAV with more than 30 years of experience in that domain. We are a one-stop-shop for biotech and pharmaceutical companies seeking to advance clinical trials and commercialize advanced therapy medicinal products (ATMPs). We offer services from feasibility up to cGMP grade for clinical trials and commercialization batches. We have around 200 employees, but this number will increase next year as we are currently building an expansion of our state-of-the-art facility from 5000 to 10000 $m^2$.

**SZ:** I work in product management at Pall, and I'm focused on viral vector technologies. I look at the workflow for viral vectors and what gaps we have in our portfolio. I work out how to fill in those gaps with new or existing technologies.

It is exciting to talk about membrane chromatography because this is one of the technologies we have had for a long time, and we have been able to utilize it for viral vector applications.

**MS:** I have worked for Pall Corporation for around 12 years. I get to fulfill my ambition of being a scientist. I have a talented team of folks which gives us a lot of capability to perform complex experiments.

We focus on bioprocess R&D and at our core, we are an applications group. We want to understand the challenges of bioprocessing that customers have experienced in doing viral vector purification and come up with solutions for them either through our current products, or by developing new products to better meet those challenges.

My team is working in two different areas. Half of my team focuses on process intensification, looking after our continuous downstream purification lab and focusing on process intensification for monoclonal antibodies (mAbs). The other half of my team looks at gene therapy applications and focuses on the challenging chromatography purifications we see in that field.

## Q What are the key current trends and challenges in AAV and lentiviral vector manufacturing?

**SZ:** This is an exciting time for gene therapy. There are 1000+ cell and gene therapy trials ongoing according to clinicaltrials.gov. There are recent approvals to celebrate – for example, BioMarin's AAV-based drug, Roctavian, is now approved in the European Union


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and is expected to receive US FDA approval in March of next year. Meanwhile bluebird bio has two approved lentiviral vector-transduced cell therapies – Skysona and Zynteglo. We are going to start to see more viral vector-based drugs being approved over the next couple of years.

> "It is exciting to talk about membrane chromatography because this is one of the technologies we have had for a long time, and we have been able to utilize it for viral vector applications."
> - Saadia Zakai

**MS:** The human aspect of being able to treat so many diseases that were previously considered untreatable is mind-blowing to me. It is like science fiction come to life. There are 5000 monogenic human diseases that could all be treated by an AAV or a lentivirus gene therapy. The potential is massive.

However, we also see massive challenges with AAV in particular. The two drug approvals we have seen for AAV in 2017 and 2019 sparked huge excitement for the field. We thought that anybody could take their gene of interest, make an AAV vector, and get the therapy to work - that we would be seeing hundreds of approved therapies by 2022. That has not happened.

One of the big challenges is the tropism of AAV. Much of the AAV vector ends up in the liver, which then becomes toxic. Getting AAV directed to where we want in the body is a big challenge. There is a lot of work on new AAV serotypes to improve that tropism.

It also seems that AAV is perhaps not as infective as we hoped, so we require high doses. This provides a challenge with the immune response, as well as in manufacture, because a lot of AAV must be made to dose patients. Alongside that, we have the challenge of empty capsids that add to the undesirable vector immunogenicity without providing the desired therapeutic effect. There are many different manufacturing methods for AAV in the upstream process, but regardless of the option used, we still see 90–95% empty capsids entering downstream processing. If we are to lower the overall dose in order to reduce toxicity, we must get rid of those empty capsids.

**HL:** Today, the global cell and gene therapy industry is still immature at the regulatory level. However, this area is evolving very rapidly to ensure product safety and an appropriate risk-benefit balance for patients.

As Mark mentioned, one key current trend is the implementation of methods to separate empty and full particles in AAV processes. Meeting the associated regulatory requirements here is important because the presence of too many empty particles can lead to a less effective drug product and higher immunogenicity in patients.

As a CDMO, we also try to get ahead of new regulations – for example, by reducing the other types of impurities, such as host-cell protein and residual DNA, to have the purist drug substance possible. We always try to be at the cutting edge of technology and innovate in our domain to ensure safe products for patients.

**Q** How would you characterize the currently available viral vector purification toolkits for both lentivirus and AAV? Can you summarize the limitations of the currently available options?

**HL:** The purification of viral vectors is complicated. Today, we have affinity technology that allows us to provide as much of our AAV product (which is around 20–25 nm in size) as possible. For larger lentiviral vectors (100–150 nm), however, this technology is still in its infancy and is not currently applicable at the industrial level, as it is non-GMP. The Mustang Q is used in our lentiviral purification processes because it allows us to purify lentivirus better than other technologies on the market.

**SZ:** When I think about the purification technologies that are available for viral vectors, I look at three different categories. One is the traditional resins, another is membranes and monoliths, and the third is analytical centrifugation.

Resins have limitations including their need to be packed, whereas membrane technologies come pre-packed. Resins also tend to have longer loading and processing times than membranes. Analytical centrifugation is a technology that is used more commonly at the research level. Many gene therapy customers are using it, although it is not a very scalable method of processing.

**MS:** I see many of the same technologies that were used for mAbs being applied to gene therapy. Sometimes that works well, and sometimes it doesn't.

For AAV, there are now some great solutions for affinity purification. For example, Cytiva was the first to launch the AVB resin for AAV affinity purification. Those affinity resins are a great initial step for purification. There is normally another chromatography step for AAV to perform empty-full separation, which is where we see some more flexibility.

The mAb industry is dominated by resins. The gene therapy industries are less conservative and are exploring a whole new field without being bound by what has been done before. Here, there is some more interest in other chromatography formats going beyond resins.

The membranes and the monoliths behave differently to the resins. They behave convectively, without diffusive pores like the resins have. Large viruses of more than 20 nm will not enter the small pores of the resin. This gives the membranes and convective formats an advantage in gene therapy where we can load them and achieve high capacities.

**Q** What advantages do membrane chromatography and the Mustang Q system offer?

**HL:** As an industrialist,this system is very practical to set-up: it is a plug-and-play sterile and GMP-compliant system. We can work at a fast flow rate, allowing chromatography to be carried out in less than 3 hours from its preparation to the recovery of the product. This is an important advantage from an industrial point of view to save manufacturing time,


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especially with lentiviral vectors since they must be purified quickly due to their lack of stability during purification.

**SZ:** Mustang Q typically operates between 5–10 membrane volumes per minute, which is fast in terms of processing time and preparation. It has a high binding capacity and it comes pre-packed, meaning no packing of columns is required. In terms of purifying AAV and lentivirus, it has large pore sizes, which offers the benefits of good separation of empty-full capsids, and good purification of lentivirus.

> "The speed of operation for Mustang Q is great. Compared to running a column of 4 minute residence time, we are going 40-times quicker with Mustang Q. In the lab, we can do a purification in 10–15 minutes, so we can iterate very quickly."
> - Mark Schofield

**MS:** The speed of operation for Mustang Q is great. Compared to running a column of 4 minute residence time, we are going 40-times quicker with Mustang Q. In the lab, we can do a purification in 10–15 minutes, so we can iterate very quickly. This makes it great for process development work because we can perform 20 or 30 chromatography runs in a single day, giving us many opportunities for exploration. The pre-packed format is also desirable for customers, as it removes a step from the process.

**Q** What can you tell us about the considerations for Mustang Q's application and performance relative to alternative technologies in recent studies for lentiviral purification?

**HL:** At Yposkesi, we mainly use Mustang Q on our lentiviral platform for its performance compared to other technologies on the market. For me, the disadvantage of plug-and-play technologies is the lack of scalability. We are currently producing lentivirus from 10–200 L through 50L. We have linear scales so that each scale-up is as linear as possible.

Recently, to have a wider choice of Mustang volume and save process time to the benefit of lentivirus stability, I firstly tested 2×5 mL of Mustang in parallel on our 10 L scale. We associated two chromatography membranes side-by-side and tested this configuration rigorously to develop Mustang Q's capacity to purify more product while maintaining a set processing time.

We found we were able to purify the lentivirus twice as fast. The primary risk of this was the pressure generated on the chromatography system and having an elution between the two membranes that differed to the preferential path that could be taken. Fortunately, this was not the case here – the Mustang Q allowed us to achieve a single and beautiful peak of elution.

Next, we tested the paralleling of these membranes in a 50 L batch. On this scale, we achieved the same results as at the smaller scale. These results are promising, because we keep the same yield, the same product recovery titer, and the same volume as on a single membrane, while saving process time.

**SZ:** I think that the Mustang Q is one of the best technologies on the market for lentivirus purification. Lentivirus is a finicky molecule – it's sensitive to salt, temperature, pH, and shear. Anything you can do to improve the process helps with your overall process yield. The Mustang is a great tool for that.

**MS:** We are focused on the scalability of the Mustang range. All Mustang devices have 16 layers of membrane, so they all have the same bed height and the same ratio of membrane volume to hold-up volume. We focused on understanding the flow path to ensure we have scalable performance going from the 0.86 mL device up to the 5 L device used for full-scale processing.

**Q** What sort of results have you seen with AAV, particularly for full-empty capsid separation?

**MS:** This is still a big challenge for customers, but we are now seeing some good solutions. At small-scale, in academic labs, ultracentrifugation is still a useful approach. However, it is challenging to scale ultracentrifugation, and pulling bands out of a cesium chloride or iodixanol gradient reliably at a large scale is not a very appealing approach.

In bioprocessing, attention has turned to chromatography. Many of our customers are taking the two-step chromatography approach with an affinity step first, and then an anion exchange step second. The anion exchange step is the only step in the whole process where we can separate empty and full capsids and it has been effective in doing so.

When we started our work a couple of years ago, we looked at linear gradients. There was a misconception at that time that membrane chromatography would not be able to perform difficult separations, and that it is only suited to working in flow-through mode. However, through our recent work, we have shown that this is not the case. On the other hand, linear gradients did not work for us with any of the formats. We tried resins, monoliths, and the Mustang, and we could never get linear gradients to give us good separation at all.

Our team came up with a novel method, using 1 mS/cm small conductivity steps, which let us see the purification. This would give a series of peaks to follow by UV, looking at the 260–280 nm ratio to gauge the relative contribution of DNA and to better understand separation. Those small steps give us the chance to try lots of different buffer conditions. We can do that quickly with Mustang Q due to its fast run time.

We are achieving good purity with the Mustang Q. We can now look at bringing in a two- or three-step elution, which we can imagine being much easier to bring to a manufacturing scenario than one involving lots of steps or a linear gradient. It is great to have that strategy as an option.


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Our colleagues at Cytiva have also been taking the approach of small conductivity steps, followed by a two-step elution method. They have also been having success and gathering great data with Capto Q. It is great to see that approach as part of a comprehensive Danaher solutions toolbox that we can bring to customers. If they want to go quick and have the pre-packed format with Mustang Q they can do that; if they want to pack columns and have the traditional format of a resin, then Capto Q works amazingly well, too.

**Q** What are your thoughts on the regulators potentially setting a minimum specification for the percentage of full AAV capsids?

**MS:** Getting specifications from the FDA is always an interesting challenge. The balance of risk for any treatment is dependent on the life-saving potential of the treatment versus the risk it could cause to patients. That balance changes for every treatment.

Some treatments are truly transformational and life-changing, and the balance of risk is weighted towards having the treatment, even if some empty capsids do remain. As we go into more mainstream treatments – for hemophilia or diabetes, for instance – the balance changes. There are already treatments for those diseases, and the prognosis is relatively good. For those treatments, there is a tighter regulatory focus. Without any new approvals in the last three years, the regulators are going to be very stringent with more mainstream applications. This means removing empty capsids is going to be important.

It is hard to set specifications, even for judging the number of empty capsids. We spend a lot of time looking at our empty-to-full ratio. We use analytics to look at the number of total capsids with ELISA versus genome content using ddPCR. By looking at the ratio of ELISA versus ddPCR, you can get an idea of empty capsid number, but it is not particularly accurate. Analytical ultracentrifugation (AUC) gives a better answer for the number of empty capsids. However, that takes a lot of time, and is expensive and difficult. Even the analytics are a challenge with AUC.

**Q** Do you have any closing remarks relating to the application of membrane chromatography systems such as the Mustang Q in viral vector downstream processing?

**SZ:** Mustang is a great technology. Membrane chromatography in general has a lot of benefits. It is a proven technology for AAV and lentiviral purification, which is worth evaluating in process development. We offer free services to help our customers with their process development work, so our scientific and laboratory services group, as well as Mark's team, can offer support.

**HL:** My advice is to choose the right elution buffer to carefully select the vector of interest that you wish to purify. We must not forget that the Mustang Q is not selective,

so impurities in the product can be significant if a good elution technique is not chosen to recover the vector of interest.

This technology is beneficial for its plug-and-play aspect and the possibility of fast flow rates which reduce process times. Membrane technology is practical to set up compared to resin chromatography where an additional preparation request is necessary.

Finally, the Mustang Q is scalable, with the possibility of putting the membranes side-by-side to double the volume of purification. This makes it possible to offer a wider choice of scales.

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- Hélène Lebas

## BIOGRAPHIES

**SAADIA ZAKAI** is the Product Development Manager, Gene Therapy at Pall Corporation and is based in Boston, MA, USA. She is responsible for driving the product strategy of Gene Therapy related technologies at Pall and Cytiva. Saadia joined Pall in 2016 and has worked in field applications and sales in her previous roles. Saadia received her Bachelor of Science in Bioengineering from the University of Maryland and is completing an MBA part-time at Boston University.

**MARK SCHOFIELD** earned his degrees in Scotland, he received his bachelor's degree from the University of Edinburgh and his molecular biology PhD from the University of Dundee. For the last 11 years he has been an employee of Pall life sciences focusing on chromatography applications. Currently he holds the position of Senior R&D manager, his team works on chromatography solutions for continuous bioprocessing and gene therapy modalities.

**HÉLÈNE LEBAS** joined Yposkesi as Process Scientist for gene therapy in 2019. With over 6 years of experience in protein purification for process development, she starts her career in the purification of molecules derived from blood (antibodies (IgG, IgM, IgE), coagulation factors) and monoclonal antibodies (MAbs) during 3 years at LFB (The Fractionation and Biotechnology Laboratory). Today, she develops at Yposkesi the production and purification processes of lentiviral vectors and AAV from laboratory to industrial scale. She holds a master's degree in Bioprocess, Microorganisms, and Biomolecules from the University of South Brittany.

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DOI: 10.18609/cgti.2022.226

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## AUTHORSHIP & CONFLICT OF INTEREST

**Contributions:** All named authors take responsibility for the integrity of the work as a whole, and have given their approval for this version to be published.

**Acknowledgements:** None.

**Disclosure and potential conflicts of interest:** The authors disclose that Pall Corporation owns patents relevant to the Biotech Industry. Schofield M discloses he is part of a patent which has been filed on the 1mS/cm steps for AAV full capsid enrichment and has stocks in Danaher. The other authors have no conflicts of interest.

**Funding declaration:** The authors received no financial support for the research, authorship and/or publication of this article.

## ARTICLE & COPYRIGHT INFORMATION



**Article source:** Invited. This article is based on Podcast which took place on Nov 3 2022.

**Podcast recorded:** Nov 3 2022; **Revised manuscript received:** Dec 14 2022; **Publication date:** Jan 4 2023


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