PODCAST INTERVIEW with:
Mark Schofield, Senior Manager, R&D, Pall Corporation

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Empty/full separation: gene therapy's hidden challenge

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Q Could you set the scene for us in terms of why adeno-associated virus (AAV) is such an important viral vector for the gene therapy sector?

MS: We have now seen two approvals for AAV-based drugs in the US: Zolgensma® for spinal muscular atrophy and Luxturna® for inherited retinal disease. These

serve as proof principle to the industry, and we can imagine that this is going to become a very important vector for the industry to treat a widespread range of diseases.

There are orphan diseases we have been looking at from the start, but with this proof of principle, we can now see this going into mainstream gene therapies and being used in mainstream diseases. It is a very exciting time, and we can see the promise of the molecular biology revolution. The potential that the sequencing of the human genome gave us can really be delved into deeper now. We can look at replacing genes or replacing gene function using AAV as a vector, and this going to be incredibly powerful in the future.

Q What are some of the characteristics of the various AAV serotypes that translate into challenges in downstream purification?

MS: There are a series of serotypes for AAV, and we look at 1 through 10. People are also making hybrid serotypes to target particular areas of the body, whether that is the eye, the liver, or elsewhere. It is going to be very interesting in the future to see how well that targeting goes.

However, along with this comes some challenges. The different AAV serotypes behave differently in a purification and a chromatography setting, so it is hard to platform that purification, and more process development around purification is still needed.

The chromatography used in purification has settled in now, often to a couple of steps. For example, affinity chromatography first to give a bulk clean up, and then a second chromatography step, which is usually anion exchange chromatography. The challenge with the anion exchange chromatography step is to remove the empty from the full capsids.

AAV looks like a great vector, but the downside is that not all of the capsids that are produced are full – there are empty capsids present that do not have the gene therapy payload, and it is difficult to know what they do and what function they have. A lot of people consider them to be in-process contaminants, and think they have to be removed. But they might have a function, and it is hard to understand what the balance of empty and full needs to be.

Q Could you go into more depth on the importance and significant of the fully to empty capsid ratio for quality control of AAV-based gene therapy products in particular?

MS: If we dive into the literature, the impact of these empty capsids is still not clear. Are they really just a process-related impurity, or could they have some function in the treatment itself?

Looking at some published papers, the immune response here is quite complicated. There may be a balance between what is happening with neutralizing antibodies that could neutralize AAV, prevent the infection and withhold the gene therapy effect, versus a T-cell response that could come after infection. The empty capsids could play a role in evading the neutralizing antibodies without causing such a large gene therapy response as to cause a large T-cell

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response, that kills a lot of the cells that have been infected and have the effect we are looking for.

It is really hard to understand how that will play out. What is certain is that to have a controlled drug, and have a drug that is effective, the empty/full ratio has to be controlled at all times. You can't go from a few percent full in one batch to a really high percent full in another batch. It would not be a consistent drug product, and we could probably expect some very variable results.

"What is certain is that to have a controlled drug, and have a drug that is effective, the empty/full ratio has to be controlled at all times."

It is clear that the percent of full capsids has to be somewhat controlled to have a consistent drug and therapy. That is going to be the challenge, because it looks like that can't be entirely controlled during upstream processing. Perhaps, as our understanding of the biology improves and we gain more experience and practice, this will be controlled more in the upstream side. But right now, that control has to come at the downstream point. We have to be able to separate the empty and full capsids, through chromatography, to make sure we have a consistent amount of full capsids at the end.

Q For such an important topic, why has empty/full capsid separation traditionally been such an underserved element of downstream vector bioprocessing?

MS: This is an interesting question, as it is hard to know how underserved it truly is. There are very few publications or presentations on this subject, and there is a surprising gap here.

This is because there is so much competition in this space; there are so many companies charging towards their therapies, and they really don't want to give anything away. They see this as their secret sauce, their competitive edge that they do not want to give up, especially as multiple companies go after the same indications. And so, there is very little in the literature right now. That poses a problem in itself, as it is hard to know the extent of the challenge when companies aren't revealing it.

There are the different serotypes that can make the empty/full separation different from one serotype to the next. Looking at what literature there is, it seems that different payloads can affect the separation as well. This makes it hard to platform the process and to take one serotype, or one insert, and be able to purify the same way for different serotypes and inserts.

The crux of the challenge is that we are trying to separate two things which are very similar. Looking at the empty and the full capsids, it seems that they have very much the same physical-chemical properties, and they have the same size. They have very slightly different densities, so that gives one path to purification through ultra-centrifugation. When they have their DNA payload the viruses become slightly more negatively charged, and that also lets us separate them through anion exchange chromatography.

Q The issues you have described here represent a lot of challenges for the sector. In your opinion, what is at the cutting edge in terms of current technological solutions for empty/full separation?

MS: We see in academia and at the small scale that ultra-centrifugation is a great tool, but bringing that to the large scale is hard to envision, as making 10^{20} viruses through ultra-centrifugation is going to be a massive challenge. We don't see that as a scalable manufacturing platform.

Right now, it looks like the state of the art is to employ chromatography for the empty/full step. We see different chromatography approaches; people are using resins or monoliths. Looking at our own data, hopefully we can start convincing people to convert to using membranes as well.

Q What data can you share with us in terms of the Mustang® Q membrane's performance in this practical application?

MS: We started off wanting to understand our product and how it might fit in this space to do this empty/full separation.

To do that, we wanted to look at a positive control; look at that separation against a competitor product in order to be able to compare our own performance. When we started out on that path we tried a lot of different things: different resins, different chromatography approaches, and used the linear gradients that are prescribed in the literature and in other companies' application notes. When we were using linear gradients, we struggled to get good separation. We could never get the two clearly defined peaks that are the canonical separation we see in some of the literature and in some of the application notes.

Instead, we would get perhaps one peak with a bit of a shoulder. We tried lots of different linear gradients and different pHs, but we could never get good separation with our own product, or any of the other products out there.

We thought about it some more, and considered how we might break that linear gradient up to try and tease out more separation. Instead of a linear gradient that is nice and smooth, we brought in some small conductivity steps, where we held the conductivity for 10 column volumes, or 10 membrane volumes, at a certain conductivity. Then we took a step up of one or so mS/cm, held for another 10 membrane volumes, and so on.

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In this way, we tease out the gradient to be a bit longer, and we have discrete steps in the gradient. When we take this approach we can get some very distinct elution chromatography peaks. We can see peaks coming on early in that salt gradient step which have a much higher 260/280 nanometer UV ratio. It doesn't look like they have a lot of DNA in them, because the 260 is relatively low.

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When we get peaks coming out later in the salt gradient, they have 260/280 ratios that are much higher. It looks like those peaks have a much higher 260 relative to 280, and contain DNA. By bringing in these small conductivity steps, we can break up the elution peak and really see that separation.

Q Do you have any recommendations in terms of bioprocess design and complementary tools and material selection that can bring out the best in Mustang® Q membrane?

MS: Mustang® Q membrane can work really well for this separation, and is well-suited to working with viruses because it is not diffusion limited and it doesn't have pores. All the binding is on the surface, so you can have relatively high capacities. That is a drawback we see with resins, where the resins are porous and the viruses cannot access those pores, which can be a bit of a challenge for the resins and capacities. We don't see that as a challenge for Mustang® Q membrane.

Mustang® Q membrane can also be operated at very high flow rates. When we are doing our separation of empty and full capsids with those 1 mS/cm steps, we can operate at 10 membrane volumes per minute, so we can operate that step very quickly. This may not be so important for AAV because it is relatively stable, but if we get to working with some less stable viruses like lentivirus, it is a great advantage.

Going from there, these 1 mS/cm steps are really effective at getting the separation, but it is hard to imagine bringing these steps into the manufacturing suite, so we have used them as a process development tool. We can look at where we are getting empty peaks and where it looks like we get full peaks, look at the conductivities to do that, and bring that down to two salt steps. Right now, we are working at around 12 or 13 mS/cm for our first step, and then around 15 mS/cm as our salt step. When we do those two steps, we can get primarily the empty capsids eluted in the low conductivity, and primarily the full capsids eluted in that higher conductivity. This gives us a pathway to bring the approach into the manufacturing suite.

Q What would be the next steps for the development of Mustang® Q membrane in terms of application in additional serotypes, for example?

MS: We have been focusing internally on AAV5, and we had the good fortune in the last few weeks to work with a customer on a different serotype, and we got very comparable results to what we had with AAV5. We are encouraged by that, and believe we will be able to bring in some kind of platform to address lots of different serotypes.

We plan to carry on and look at more serotypes in the future, and hopefully cover the main serotypes we see people using, likely AAV2, 5, 8, and 9. We would like to generate data for all of those.

I am hoping that through the next year we will be able to collaborate with more customers, and generate more customer data with Mustang® Q membrane on different serotypes.

Q How does Mustang® Q membrane fit into the wider array of Pall's solutions and services?

MS: We have been working at Pall to develop a complete platform, and a complete range of products, for the whole AAV growth and purification process.

We already have some success on the upstream side, because the AveXis process uses the iCELLis® bioreactor system. We are very pleased to have the Pall iCELLis® bioreactor embedded in that process.

On the downstream side, we have a whole range of products that are ready to go for AAV purification, and customers are already starting to implement those. That ranges from clarification, depth filtration, sterile filtration, through tangential-flow filtration (TFF), chromatography with Mustang® Q membrane, and then towards the end of the process again with TFF to get to the final concentration formulation.

Q What support is available to adopters of Mustang® Q membrane?

MS: At Pall we make great efforts in developing customer support, and we have a huge team of people that go out and help our customers. Getting our associates into facilities is a challenge right now because of the restrictions we have. However, we still have people going in and helping people with their separations, and it is great for us to have that depth and capability.

Additionally my team will collaborate with people, generate data that way, and do beta site testing. Through these collaborations we generate more help for customers as well as more data for our own products.

We also have a process development services team that can professionally bring these solutions to the foreward, and drive those separations and purifications for customers. If a customer can get to a certain point in the process but wants to improve yield, improve purity, and wants to be able to bring that to a scalable approach, Pall's Accelerator℠ process development services team can come in and address all of that.

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