CELL & GENE THERAPY INSIGHTS

VECTOR CHANNEL:
SUSPENSION CULTURE SYSTEMS

CHANNEL CONTENT

INTERVIEW with **Rénald Gilbert,** Senior Researcher at the Human Health Therapeutics Research Centre at the National Research Council Canada

"I would advise starting to optimize your process at small-scale, because it's cheaper and faster to make changes at that point."

Key considerations for the use of suspension culture systems for viral vector manufacturing

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Q Ensuring the consistency, quality, and performance of viral vector production is a long-standing challenge for the space – where do you see real progress being made in this area?

RG: When looking specifically at viral vectors such as adeno-associated virus (AAV), or lentivirus, these vectors are produced by transient transfection. Recent developments in relation to their manufacturing have centered around the development of efficient, robust, scalable processes for large-scale transient transfection, ideally with chemically defined medium. In addition to this, there has been a push to ensure that these processes are, of course, cGMP-compliant to enable commercial manufacture.

We've also seen promising progress in the development of improved resins or membranes for the concentration and purification of viral vectors such as AAV and lentivirus, which ultimately has a positive impact on final recovery and purity.

Q Can you speak to the challenges relating to making the transition from adherent to suspension cultures during scale-up? What are some of the key considerations for making this as seamless and efficient as possible, for you?

RG: Right now, this transition is not quite seamless or pain-free. At the National Research Council Canada (NRC), strategically we made the decision to use cells in suspension culture, rather than adherent, for the production of viral vectors, due to the complexity and labor intensive issues around scale-up of adherent cells.

If you are working with adherent cells and wish to transition to scale-up in suspension culture, you need to adapt the cells, and this can take many months and not be always successful. One of the key aspects of this transition is that the adherent cells are cultured using serum and when you decide to move to suspension culture, the cells may have to be adapted to grow in serum-free medium first.

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Then you have to adapt the cells to grow in suspension conditions. Often you may observe a loss of productivity, a slower growth rate and potentially genetic instability. It's also not uncommon to see a change in the cells' gene expression pattern.

Because this transition to suspension is challenging, I would advise starting this process early, factoring in around six months before you are able to adapt your cells to the new culture conditions. Additional time will be required to characterize your cells again, and for the possibility of creating another cell bank. For these reasons, at NCR, we decided from the outset to work with cells that are already adapted to suspension culture and using serum-free medium which has simplified our production process considerably.

Of course there are options for scale-up whilst maintaining adherent culture – using roller bottles, cell factories, or fixed bed bioreactors, for example. I would advise that you either use a production process that is anchorage dependent all the way from small scale to larger scale, or start from the outset with cells already adapted to suspension culture because the transition step as outlined is currently not challenge free.

> "...either use a production process that is anchorage dependent all the way from small scale to larger scale; or start from the outset with cells already adapted to suspension culture."

Q What or where are the chief lingering safety concerns in viral vector manufacture in your view?

RG: In terms of the manufacturing process, one of the issues in working with replication incompetent lentivirus vector is that, for many countries such as Canada, it's considered as a risk group 2 agent, therefore, meaning it must be used in a BSL-2 laboratory. If you then consider manufacturing in large volumes and at higher virus concentrations, that can of course present a safety issue as well as presenting challenges regarding disposal of potentially hazardous waste. We also have to be cognizant of the possibility of generating replication-competent virus and putting in place measures to address that.

It's also important to mitigate the risk of cross-contamination of products by working in a closed environment, with minimal opportunities for contamination with bacteria or mycoplasma.

Looking at safety concerns from the final product perspective, of course it's essential that you ensure your final product for the patient is pure and that there is batch-to-batch consistency. Furthermore, if you are using plasmid to produce your viral vector, such as you do with AAV and lentivirus, you should use plasmid with a low level of endotoxins.

To help mitigate these safety concerns and optimize your end product, there's a great deal of effort to develop relevant assays, such as for example one to detect the presence of empty or non-functional virus particles, which can affect the efficacy of your product. And of course during purification you have to make sure you have a low level of contamination of genomic DNA, as well as RNA and host cell proteins.

Q What are the most important considerations for you when choosing the right cell culture platform and media, particularly in the suspension systems realm?

RG: For anyone producing material for clinical application it is important to ensure that media are serum-free and also free of animal-derived components. With serum in particular, there's a great deal of lot-to-lot variation, and it could be contaminated with adventitious agents.

Ideally, you would want to work with a medium that is chemically defined, as this would reduce lot-to-lot variation and also minimize the risk of adventitious agent contamination. And because lentiviral/retroviral vectors or AAV are produced by transient transfection, it's important your medium allows transient transfection (with adenovirus vector production this is not an issue as you do not use transient transfection).

It is also important that the medium should allow cell growth to high cell density, and support the production of viral vectors at high cell density, with rapid doubling time of around 24 hours.

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Q Can you speak more to the benefits of having a chemically defined system from the early stages of bioprocess development? And what is the related impact on raw materials and the supply chain in general?

RG: The issue of lot-to-lot variability really can be a limitation in your manufacturing process for viral vectors. If you choose a serum-free, but not necessarily chemically defined medium, such as one made using plant hydolysates for example, whilst it is free from animal-derived products, it's not a well characterized product and therefore can be prone to variation from lot-to-lot.

By having a chemically defined system you are ultimately improving the safety and purity of your end product, because all components that enter into its manufacture or final composition are well characterized.

As such, I would advise looking at optimizing your manufacturing process early on, at small-scale, ideally by choosing the medium formulation that you will want to use as you progress to large-scale manufacture. In this respect it's important to, where possible, have more than one supplier of this critical component to reduce possible supply continuity issues as you scale up, or ensure your sole provider has risk mitigation plans in place to minimize any potential delays or problems with media supply.

Q Can you talk to us through your approach to identifying suspension culture system-based bioprocess parameters, highlighting any key learnings from your experience in this area?

RG: As mentioned, selection of a cell line that will meet your requirements in terms of scalability in suspension culture within serum-free medium with good doubling time, is critical as you move to scale up. It's also important to understand if the cell line has a good history and whether there is a cGMP cell bank available, because if you have to create a cGMP bank yourself, you will need to factor in additional time until that material meets the requirements to be used for clinical applications.

Once you've identified your optimal cell line, you can start to assess which media works best with your cells. If, for example you want to produce a third-generation lentivirus vector, this involves transfection with four plasmids and therefore an important early step is to identify which ratio of plasmid will give you the highest titer of vectors. At the NRC our preferred transfection agent is PEIpro (PolyPlus Transfection); because this is GMP ready and in our hands gives highly reproducible titers. At the start of a project, we will determine the best concentration of PEIpro and the ratio to plasmid concentration, which can take several weeks as you have to test several conditions. But once you have optimized your conditions, you can then start to scale up.

We initially work with 20 ml in shaker flasks, before scaling up to 300–500 ml working volume in larger shaker flasks, to ensure the process we develop is robust, scalable, and reproducible, delivering the same titer at each step. Following the shaker flask scale-up, we move to a small bioreactor, of up to 3 L. We perform several runs at this scale before moving to different sizes of bioreactor, such as 50 L, 200 L, or even 500 L, again to assess that the yield and productivity are maintained at each scale-up step.

This approach has worked well for us when producing lentiviral vectors and AAV, and we've found that if you start with a robust cell line, a good medium formulation, normally what you observe in the shaker flasks in terms of yield, you will then replicate in the bioreactor.

Q Can you summarize the chief pros and cons in suspension culture terms of the four viral vectors most extensively used in clinical trials (adenoviral, adeno-associated, retroviral and lentiviral)?

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RG: Let's look at adenovirus, which I would say is probably the easiest viral vector to produce, namely because you don't have to perform transient transfection. It's also a well understood virus, having been used in gene therapy applications for over 20 years. It's also a very stable virus, unlike the envelope viruses, so you can use chromatographic methods to purify it.

However, one of the challenges in using adenovirus produced through HEK293 cells – which is a very common method of adenovirus production – is that you are likely to generate replication competent virus particles.

Similar to adenovirus, AAV is a fairly stable virus, and when considering its manufacturing "strengths," there are

effective methods and commercially available resins to purify AAV via affinity chromatography. Therefore, the purification step is particularly strong. However, with AAV you can be impacted by the presence of empty or partially filled capsids, which reduces potency of your end product, and removing these empty capsids is not a straightforward process at present.

In addition, as AAV is produced via transient transfection, often with three plasmids, the process is more complex and you must ensure you don't have residual plasmid in your final product.

A current limitation with transient transfection, which impacts the production of AAV, retroviruses, and lentiviruses, is the lack of efficient process to transfect at higher cell density. Presently, most of the transfection processes are conducted at cell densities not higher than 2 million cells per milliliter, which still is a challenge to overcome.

The added complexity of utilizing retroviruses and lentiviruses, is that in contrast to AAV they are not stable and this complicates downstream processes, in particular purification, because if your process lasts too long the fragility of the virus can lead to loss of infectious particles. Furthermore, these viruses are produced in the presence of exosomes or extracellular vesicles, and these are very difficult to purify and separate from your end product.

That said, a big advantage of retroviruses and lentiviruses is the availability of packaging cell lines, which removes the need for transfection, thus simplifying the production process. Whilst the yield is often lower than that achieved with transient transfection, if you have packaging cell lines you could produce the vector using perfusion mode which will allow you to perform continuous-harvest at high cell density. This is a very important point for lentivirus, or retrovirus, because the virus is not stable, by performing continuous-harvests this enables you to use the optimal amount of virus that your system would be able to make. So you may harvest the virus via perfusion during the period of three to four days. Because this process is complex however, you will need specialized equipment and trained personnel.

Furthermore, due to the aforementioned fragility of these viruses, it's more difficult to sterilize them using ultrafiltration, often with losses of up to 50% of your virus during this step. Perfusion is a less viable option for AAV, given that most of the virus is intracellular, whereas, lenti- and retro-viruses are secreted into the culture medium, from where the budded virus can be harvested.

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Q What is your view on the current prevalence of HEK293 use? What other cell types should people consider for larger-scale suspension system production?

RG: We, and other groups around the world, have developed HEK293 cells that were adapted to suspension culture with a serum-free medium, and they provide a very good yield for AAV and lentivirus. Owing to this good yield, HEK293 is currently the most popular cell line used for the production of AAV, retrovirus, and lentivirus.

In the past, HEK293 cells were widely used to produce adenovirus, but one of the issues that arose, is that due to homology between the HEK293 cells, previously transformed using sequence derived from adenovirus, and the adenovirus vector, that cell line generates replication-competent adenovirus by the process of homologous recombination. For this reason, many groups, including ours, have developed cells to specifically produce adenovirus. Some cell lines, such as the one developed by NRC are derived from A549 cells. Other cell lines include PER.C6 (Crucell) and CAP-GT cells (Cevec).

Another option for AAV is to use insect cells, a process that involves first producing the baculovirus stocks to supply different components of AAV production by infecting the insect cells. But if you want to keep with mammalian cells, I think HEK293 cells are ideal for the production of AAV as well as lentivirus.

Q What for you have been the key technological advances that have contributed to the current state-of-the-art in viral vector production platforms?

RG: Certainly, the development of chemically defined media that allow efficient transient transections and supports cell growth at high cell density over 4 million cells/mL is a big step forward. About twenty years ago, we were using serum and undefined medium components which undoubtedly complicated the process, with challenges arising around reproducibility because the composition of the medium would vary from batch to batch.

Another development would be the marked improvement in filtration and separation membranes to remove the cells and cell debris from the virus.

Q And what should be, or will be, the next steps for innovation in this field?

RG: Where the field needs to make advances is in the ability to culture cells at a higher cell density. For example, if you are working with CHO cells to produce recombinant proteins, you can work at a cell density of say 20 to 30 million cells/ml.

Unfortunately, in the field of viral vector production, this is not yet possible. It would be a key advancement if we could develop a process whereby you could transfect cells at high cell density while maintaining the cell specific yield as this would greatly improve volumetric yield.

Further to this, I feel that innovation to improve our analytical capabilities is also key to advancing this field – such as in-line measurement of metabolites and cell growth, which we expect to be developed in the near future.

Q Lastly, can you summarize what for you are the key elements for a successful approach to viral vector bioprocess scale-up?

RG: You need to start with a good cell line, that is GMP compatible, to set yourself in a strong position for future scale-up of your processes.

In parallel to optimizing your cell lines and manufacturing processes, from the outset you need to develop good analytical assays that enable you to measure the purity, integrity, and functionality of your vector. Whilst you need assays for your final process, they are also essential in the scale-up process, allowing you to assess whether a process change or improvement, for example, impacts the final product.

And finally, I would advise starting to optimize your process at small-scale, because it's cheaper and faster to make changes at that point and once you have developed the process at small scale, you have to make sure it is robust and can be scaled up.

BIO

***Rénald Gilbert** is a senior researcher at the Human Health Therapeutics Research Centre of National Research Council Canada (NRC) in Montreal. Throughout his career at the NRC, he has held various leadership roles including the Program leader of the Vaccines Program, which delivered on the Government of Canada's mandate on innovation, fostering Small Medium Enterprise (SMEs) and public well-being. Currently as the lead of the NRC Biomanufacturing Research Initiative, he manages a portfolio of projects aimed at developing new and improved proprietary platforms enabling biomanufacturing of scalable, cost-effective and safe complex biological products. Dr Gilbert is also an adjunct professor in the department of Bioengineering at McGill University. His research interests include the optimization of viral vectors for the development of vaccines and for cell and gene therapy applications, as well as their methods of production. He is currently directing research projects aiming at increasing the yield and efficacy of vectors derived from adeno-associated virus (AAV) and lentivirus using suspension cultures of mammalian cells. Dr Gilbert completed his PhD in Biochemistry at McMaster University (Hamilton Ontario) in 1994. Before starting his career as a research officer at NRC in 2002, he got trained as a postdoctoral fellow in the department of Cell Biology of the Weill Cornell Medical College (New York City) and in the department of Neurology in Neurosurgery of McGill University.*

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