The future of AAV manufacturing: a multidisciplinary perspective

The incredible pace of progression seen over recent years has made gene therapy one of the most exciting areas of modern medicine, and it is showing no signs of slowing down. In particular, gene therapies utilizing adeno-associated virus (AAV) vectors have shown significant promise.

One of the main reasons for this is their encouraging characteristics such as low immunogenicity. Additionally, the DNA carried by modified AAV cannot integrate into the host genome. Further interest has also been driven by the successful regulatory approval of two AAV-based therapies—LUXTURNA® and ZOLGENSMA® in 2017 and 2019, respectively—which has gained AAV-based therapy a reputation as a proven platform. However, this progress looks set to bring with it challenges for AAV manufacturers. For example, increased demand for gene therapies will make streamlining and expanding AAV production capacity vital. Moreover, as the industry itself continues to mature, there will also be a need for manufacturers to have a pathway to successfully navigate evolving regulatory environments.

To find out more about the challenges on the horizon for AAV manufacturers, as well as the solutions that could ease their transition into the future, we spoke to a panel of Thermo Fisher Scientific AAV manufacturing professionals with experience in cell culture media, analytics, and viral vector production.

Panel

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To start us off, Céline, could you begin by outlining what you think are the biggest changes that we are likely to see in the gene therapy field within the next 5–10 years?

CM: That's a big question. I think one of my biggest hopes is that gene therapy will become more accessible and mature, so that we can begin to address rare genetic diseases with a clear etiology more systematically. Right now, there is a lot of research being undertaken, but there is currently no clear path for clinical development. Once standardized clinical pathways are established, it will drive bioprocessing forward as it will become easier to see where improvements need to be made within manufacturing processes.

Focusing specifically on bioprocessing itself, there remains a widespread need for a better understanding of viral vector production. There is also clearly interest in different gene therapy approaches. For example, currently around 80% of gene therapies use viral vectors—usually either AAV or lentivirus (LV)—and a lot of work is going into improving their performance. However, other vectors are being considered from both clinical and manufacturing perspectives. Even gene editing approaches such as CRISPR-Cas9 are still in the early stages of development and are likely to become more widely adopted over the next 5–10 years.

From what's been mentioned, it is certain that the next few years will be a transformative period for the gene therapy industry. Looking specifically at AAV manufacturing within your specific areas, what are some of the key challenges that manufacturers will come up against?

EJH: As the gene therapy field moves toward addressing diseases that affect larger patient populations, inevitably we are going to see a need for increased AAV titers to meet the growth in demand. This is going to be a particular challenge for AAV manufacturers, as it is really a two-fold task—requiring them to not only increase titers, but also maintain these increased titers as the process is scaled up. Another layer of complexity is added by the individualized nature of gene therapies. It is becoming increasingly clear that each serotype and gene of interest has its own optimal process, which impedes the development of one-size-fits-all solutions. Lastly, the need for an increased focus on scalability is going to be essential. Many existing processes have been developed within academia and optimized at that scale, which makes further scale-up difficult. To enable manufacturers to maximize titers, the scalability of the process will need to be prioritized from the early stages of process development.

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JB: Besides total titers, gene therapy manufacturers also need to address several analytical challenges regarding AAV quality attributes: most importantly, empty capsids and oncogenic host cell DNA. Controlling these impurities is key, as they contribute to the effectiveness, safety, and applicability of gene therapies. The ultimate goal of a gene therapy is to deliver the right amount of the desired genetic sequence into the patient's cells via the AAV capsid. Therefore, a robust process that delivers a consistent concentration of full versus empty capsids is central to maintaining drug product batch-to-batch consistency and enabling the desired therapeutic result to be achieved in the clinic.

The presence of these impurities can negatively affect the safety profile of the therapy by increasing the risk of immunogenicity, so it needs to be accurately controlled. In addition, inconsistencies in the empty-to-full capsid ratio and the resulting inconsistencies in the concentration of the desired genetic sequence per therapeutic dose can alter potency. As a result, higher delivery volumes would be required, which can lead to two directly related challenges: larger volumes can be prohibitive to deliver into certain parts of the body, and they can also increase the impurities being administered into the patient. This can increase the risk for adverse reactions, especially in the presence of oncogenic host cell DNA.

Looking at the move toward suspension cell cultures for AAV production, new single-use bioreactors (S.U.B.s) have shown a lot of promise in streamlining process scale-up.

Optimizing the empty-to-full capsid ratio is particularly important during the upstream manufacturing process, as downstream purification can only remove empty capsids and has no effect on the overall full capsid yield.

CM: Related to both AAV quality and titers, analytics is also a key challenge. Firstly, we need to better understand the target quality profile of the AAV vector to be able to optimize the process. Secondly, we also need to be able to identify the links between specific process elements such as raw materials and the quality attributes of the manufactured AAV. Coming back to titers, some existing assays require large amounts of AAV to deliver accurate results which, if manufacturers are already struggling with low titers, can be prohibitive. Many assays are also currently low-throughput, which can cause delays and further complicate production processes.

EJH: Looking more specifically at individual challenges with increasing titers and improving quality, a major challenge is improving transfection efficiency. Many HEK293 cell–based AAV manufacturing processes currently use transient transfection using a triple plasmid transfection protocol. The need to transfect the cells with three separate plasmids adds complexity, as not all cells will receive the optimal ratio of the plasmids. This affects both titers and quality and particularly impacts the empty-to-full capsid ratio. There are also questions around the scalability of some transfection techniques, such as with calcium phosphate–based transfection of adherent cell cultures, which has a highly variable transfection efficiency depending on process conditions.

PD: One thing to pick up on from Emily's answer is the incompatibility of adherent cell culture processes with large-scale AAV production. In adherent cultures, scaling up is only achievable through increasing the surface area which, with methods like adherent cell chambers, would require huge amounts of facility space. Even if microcarriers are being used, the amount required and the sterilization procedures that need to be undertaken result in additional time-consuming steps. Given the importance of scalability in increasing the volumes of AAV produced across the industry, adherent methods are not going to be suitable in the long term. As a result, suspension cell cultures, which can be more easily scaled, are likely to become the industry standard, and manufacturers will need to adapt both new and existing processes to this change.

GM: In my area, which is plasmid production, the main challenges are similar to other areas with respect to increasing consistency and streamlining workflows. In particular, it is becoming clear that traditional peptone-containing *E. coli* media may not be ideally suited to large-scale plasmid production. This is due to the variability that could be introduced by non-chemically defined products such as peptones, which can reduce batch-to-batch consistency. Additionally, peptones from both animal origin and animal origin–free sources also pose a potential viral contamination risk, which needs to be mitigated.

Clearly, overcoming these challenges is vital for developing an economically sustainable AAV production process. What solutions are available to AAV manufacturers to help them adapt to future demands?

CM: As we've heard already, improving AAV titers and quality is critical. One process parameter that can hugely influence both of these is the choice of cell culture media used. The issue in this area is that optimizing HEK293 media for AAV production is more challenging and time-consuming compared to other types of cell culture media development. Specifically, as the quality attributes of AAV can be greatly affected by many different factors, finding an appropriate starting point is challenging. In addition, due to the expedited regulatory timelines being implemented for gene therapies by many regulatory bodies, cGMP-compliant

workflows need to be established sooner, making efficient media development and optimization vital.

To help manufacturers accelerate this process and develop a highly optimized medium, Thermo Fisher released the Gibco™ Viral Vector HEK Media Panel in 2021. The panel is comprised of five nutritionally diverse media formulations and provides access to Thermo Fisher's team of field application scientists. Together, this can help manufacturers streamline the identification and optimization of an ideal medium for their specific process. The formulations used in the panel are also all available at a larger scale, manufactured in a cGMP-compliant facility. This means they can be used once the process has been scaled up to commercial volumes without introducing process variability.

EJH: In addition to panels for improving media optimization, Thermo Fisher has also developed system-based solutions to help manufacturers boost AAV titers and quality. These include the Gibco™ AAV-MAX Helper-Free AAV Production System Kit, which is a complete system comprising pre-optimized components formulated to achieve high titers across multiple AAV serotypes. Crucially, the system contains a transfection kit that is designed to maximize transfection efficiency while using 25% less plasmid DNA on a per-cell basis compared to alternative methods—ultimately resulting in a lower cost per viral particle produced. As a fully scalable solution, the kit also enables AAV manufacturers to reach commercial production volumes using the same process established during early-phase development.

PD: Looking at the move toward suspension cell cultures for AAV production, new single-use bioreactors (S.U.B.s) have shown a lot of promise in streamlining process scale-up. For example, the Thermo Scientific™ DynaDrive™ S.U.B has been developed to be linearly scalable from 50 L up to 5,000 L, enabling manufacturers to easily scale their process in line with increasing demand. Other features such as the unique impeller drive train design and drilled hole sparger, which have been developed to lower shear stress, can also improve AAV productivity by reducing cell loss due to shear damage.

The need for larger production volumes is also being supported by the development of existing AAV production media into new formats that are more conducive for large-scale manufacture. In response to this demand, Thermo Fisher has made both Gibco™ AAV-MAX Viral Production Medium and Gibco™ FreeStyle™ F17 Expression Medium available in the proprietary Gibco™ Advanced Granulation Technology (AGT™) format. By providing an advanced format option that is fully scalable and enables faster reconstitution, manufacturers will be able to transition to large-scale AAV production more seamlessly.

GM: Related to the need to improve the consistency of plasmid production and reduce contamination risks, using a chemically defined (CD) medium with a known and consistent formulation can help. In response to the industry demand for this solution, Thermo Fisher released a CD alternative to traditional *E. coli* media, Gibco™ Bacto™ CD Supreme Fermentation Production Medium (FPM). When designing this medium, flexibility and simplicity were key considerations. As a result, Bacto CD Supreme medium is formulated to be either filter-sterilized or autoclaved (depending on the end-user's requirements) and is available as a single-part solution, eliminating the need for time-consuming reconstitution and blending of multiple components.

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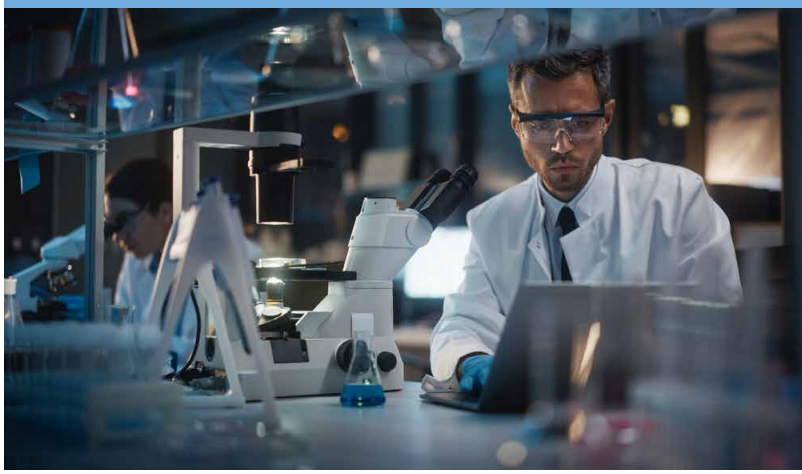

Are there any specific areas in which you foresee regulatory changes, and are there any steps that AAV manufacturers can take now to help them adapt?

GM: Focusing on the plasmid development stage, there are some changes we expect to see. Currently, it is only "recommended" that raw materials used for plasmid production comply with cGMP manufacturing quality standards. As the industry continues to grow though, it is likely that we will start to see harmonization around the requirements for plasmid production and standardized global regulations, rather than recommendations.

In terms of preparing for this, if manufacturers are producing plasmids in-house, we already advise them to choose a vendor that can provide high-quality cGMP-compliant raw materials, as well as appropriate regulatory guidance and documentation. Alternatively, if plasmids are being sourced externally, it is important to have line of sight and information related to the manufacturing process, particularly the raw materials used, to validate that the plasmids meet their requirements.

CM: I think one thing that's certain is that changes are going to happen—whether that's because of raw material changes, facility upgrades, or regulatory requests. What can manufacturers do about this? It is vital to develop a coherent and extensive data set clearly defining their process and product and to ensure that this is properly documented and stored from an IT perspective. This is important as they will be required to prove that, although their process has changed, their therapeutic product is the same. Having this data set allows manufacturers to identify what is critical to their process and where there are areas of flexibility if changes cannot be avoided. By always referring to these data, AAV manufacturers can check that any changes are not causing any deviations to the initial product profile.

Of course, many vendors, including Thermo Fisher, have very active internal R&D departments and are already working to help manufacturers adapt to future demands. Could you talk a little about the future solutions and technologies that are at the cutting edge of your portfolio areas?

CM: To go back to my earlier point around analytics, at Thermo Fisher we are continually innovating solutions to meet emerging analytical challenges. In addition to providing the tools and equipment required for manufacturers to develop their own assays, we also offer specific services, kits, and protocols for certain applications.

EJH: In general, I think we are always developing new ways to help manufacturers improve their AAV manufacturing process. This involves developing solutions to enable them to optimize all areas of their process, from the overall cell culture and transfection conditions, down to individual reagents. Looking more long-term, I think at some point we may find an AAV titer limit due to the inherent productivity limits of the cells themselves, so we will need to also look at complementary strategies.

PD: To come back again to transfection, the development of solutions for stable transfection (in particular, packaging and producer cell lines) could have a big impact on the industry. Although they are currently costly and time-consuming to develop, they have the potential to address many of the challenges experienced by AAV manufacturers, including improving scalability and minimizing batch-to-batch variation. There is still a lot of work needed in this area to develop commercially viable solutions, but it is something that we are closely monitoring at Thermo Fisher.

Going forward, it is undeniable that new technologies are going to be instrumental in helping AAV manufacturers meet future demands.

So, finally, how important is it for manufacturers to already be working with a vendor who is future-facing, and what kinds of activities should they be looking for to confirm this?

CM: The pace of evolution within the gene therapy industry is rapid, so it's important to choose a vendor that is planning for the future. Crucially, manufacturers should be looking for a vendor that they can have a productive long-term relationship with. One of the things we do at Thermo Fisher is to work closely with developers at the early stages of their process and then continue to support them as they evolve their process and address any challenges that arise.

In terms of what to look for to confirm this, it is always a positive sign when vendors are actively playing a role in shaping the future of the industry through membership to industry organizations. Optimized and fit-for-purpose solutions also demonstrate a high level of industry awareness. Finally, looking at the quality of the literature the vendor is publishing, such as peer-reviewed research, product app notes, or protocols, is another way to identify whether they are at the cutting-edge of the industry.