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Innovative approaches that address current challenges in gene therapy viral vector manufacturing

INTRODUCTION

Since the first successful gene therapy in humans in 1989[1], the industry has rapidly expanded both conceptually and technically, yet manufacturing processes have lagged behind the science. This includes the production of viral vectors — primarily adeno-associated virus (AAV) and lentivirus — which scientists use as a delivery vehicle for treatment due to their infectious nature.[2] Their ability to deliver therapeutic genetic material that works by replacing genes, inactivating defective genes, or through the introduction of new genes to treat or cure a disease shows great promise despite production challenges.

Originally identified as a contaminant in adenovirus virus cultures, AAV was later revealed to have the ability to integrate into the host genome, and thus the potential of AAV as therapy vector was discovered.[3] AAV are versatile vectors, due in large part to their large variant numbers (>5,000), their ability to target different organs depending on serotype, and low pathogenicity.[4]

Lentiviruses are conversely much larger than AAV and are derived from human immunodeficiency virus 1 (HIV-1) that have been engineered to be non-replicating. Unlike other retroviruses which require active cell division, lentiviruses can also infect and integrate into typically non-proliferative cells, thus emphasizing their potential for use in gene therapy.[5]

Production of these viral vectors is a complex process and requires innovative approaches to meet safety and efficacy requirements, clinical and market demands and cost of goods targets. Preparing stable viral vectors, preventing their degradation during manufacturing, handling and storage, and maintaining their long-term stability and efficacy are all major challenges to be overcome at each stage of development, including upstream, downstream and fill/finish.

In particular, the issues of low titer and yield, and physical and chemical degradation such as unfolding, aggregation and precipitation, oxidation etc., must be mitigated to achieve desired product efficacy and shelf-life and to control undesirable immune responses.

Environmental obstacles that cause viral vectors degradation and efficacy loss need to be better understood and managed by carefully optimizing buffer, pH and excipients.[6] Early consideration of scalability is also critical; for example, where the upstream reactor scale may be as small as 1L in pre-clinical studies, the process must be iteratively developed to support the commercial scale of 500L or more.

Although each manufacturing step comes with inherent hurdles, significant improvements have been developed.

Upstream: maximizing titer output at scale

Scaling up plasmid expansion using either adherent cell culture or bioreactors presents difficulties. Cell culture surface area can be increased for adherent cell culture, as long as commensurate contamination risks are mitigated through careful adherence to culture protocols.[6,7] Common methods of scaling up adherent cell culture include multilayer systems and cell factories, which can drastically reduce the manual labor time and contamination risks by allowing culture seeding to take place simultaneously across plates at up to 40 layers.[8,9]

Bioreactors are growing in popularity as they are closed systems that also improve large-scale suspension yield. They reduce the risk of contamination and eliminate the need of dedicated clean rooms resulting in markedly reduced manufacturing costs. Although the scale for bioreactors remains low, early success with scaling from 50L to 500L AAV culturing is promising, and some manufacturers are scaling up to 5000L.[10]

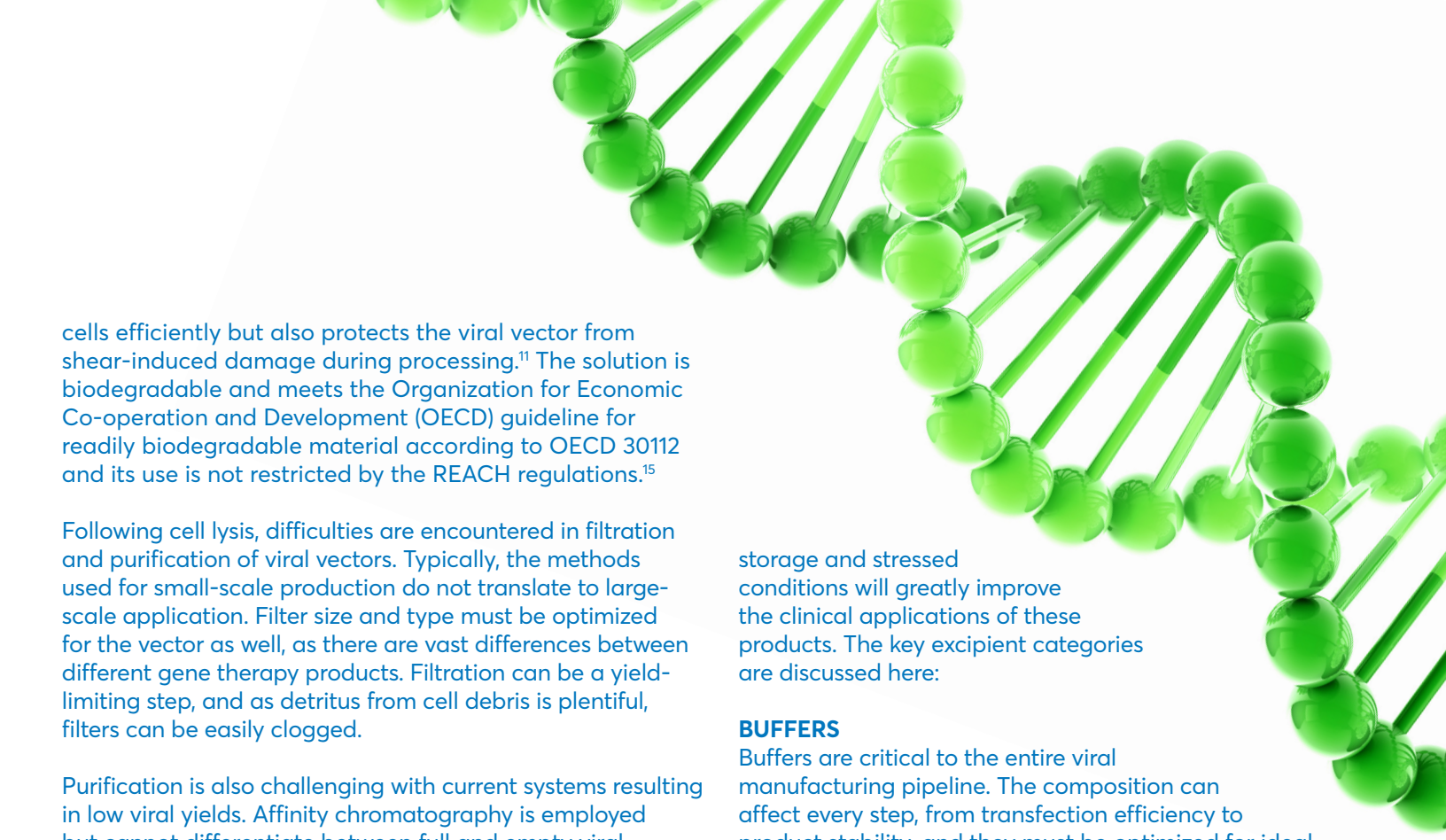
Partial automation in both approaches using single-use components and an aseptic transfer device can further streamline and insulate manufacturing by eliminating the need of cleaning validation, though each pipeline must be tailored to the application, virus, cell type, compatibility and performance necessary.

Downstream: improving harvest yield and purification efficiency

After transfection, when the desired genetic sequence has been inserted into the virus, further obstacles must be cleared to achieve desired viral yield and quality. These steps include cell lysis (AAV production), filtration and purification, where there is a lack of an effective and reproducible platform to adequately remove cells and cell debris from vectors without removing active viral particles.

While there are several traditional cell lysis methods (both physical and chemical), each has drawbacks and not all are appropriate for large-scale production. For example, repeated cell freeze-and-thaw cycling followed by gentle centrifugation is difficult to scale up, and mechanical French press homogenization is scalable but results in undesirable levels of product loss. Chemical lysis with Triton™ X-100 has served as an industry standard in terms of scalability and yield, however, research has shown that Triton X-100 causes acute oral toxicity, eye damage, skin irritation, and chronic aquatic toxicity. As a result, it was placed on the "substance of very high concern list" by European Chemicals Agency under REACH regulations.[15]

Recent advancements in cell lysis technique paved the way for a novel cell lysis solution that not only lyses the



cells efficiently but also protects the viral vector from shear-induced damage during processing.[11] The solution is biodegradable and meets the Organization for Economic Co-operation and Development (OECD) guideline for readily biodegradable material according to OECD 30112 and its use is not restricted by the REACH regulations.[15]

Following cell lysis, difficulties are encountered in filtration and purification of viral vectors. Typically, the methods used for small-scale production do not translate to large-scale application. Filter size and type must be optimized for the vector as well, as there are vast differences between different gene therapy products. Filtration can be a yield-limiting step, and as detritus from cell debris is plentiful, filters can be easily clogged.

Purification is also challenging with current systems resulting in low viral yields. Affinity chromatography is employed but cannot differentiate between full and empty viral capsids. Separation of empty capsid from full by cesium chloride gradient method is not scalable. Alternative ion exchange chromatography method, using high buffer salt concentrations necessary for viral elution, are damaging to virus activity. Aggregation, oxidation, deamidation, and proteolysis are all risk factors in the purification process as well, further contributing to the low viral yields produced by current methodologies.[6,13]

Formulation: improving virus stability and quality

Ensuring the stability of viruses also poses a significant challenge in the field of gene therapy and viral vector manufacturing. Viruses can undergo various physical and chemical degradations during manufacturing, storage and use, leading to reduced efficacy and the risk of undesired safety consequences. Excipients that increase virus stability, extend shelf life and reduce cold dependency will also benefit both production yields and product storage. Excipients range widely in form, and can include salts, sugars, surfactants, polymers and amino acids. Thus, the type of excipient necessary strongly depends on the need, and excipients can be used to stabilize vectors both in solution and in lyophilized form.

For clinical applications, excipients must be well characterized with low impurities and endotoxins to mitigate the risk of product degradation and improve overall quality. Viral products are often stored frozen, and therefore frequently undergo freeze-thaw cycles, possible pH changes and shear stress which can lead to product degradation. Better characterization and optimization of excipients under storage and stressed conditions will greatly improve the clinical applications of these products. The key excipient categories are discussed here:

BUFFERS

Buffers are critical to the entire viral manufacturing pipeline. The composition can affect every step, from transfection efficiency to product stability, and they must be optimized for ideal purified material recovery. Buffers must be formulated to reduce salinity to avoid loss of viral activity, particularly as the addition of desalting steps further reduces yield.

The goal is to move towards fewer, more universal buffers that can be used for multiple steps of production, and to which various excipients can be added to maintain or improve performance for the product in interest. Quality of buffer component is critical, for example free radical impurities in buffer can oxidize and alter the safety and efficacy of the product.

SALTS

Although reduced salinity is necessary to prevent viral activity loss, addition of some salts can improve viral titers and stability. However, increased salt content can also result in increased impurities, and thus necessitate the need for additional purification. As mentioned above, salts can have a destabilizing effect as well, and these effects are all dependent on the solution pH, concentration and type of salt. Therefore, there is a balance to be navigated when modulating salt conditions and should be carefully considered with respect to the specifics of the viral product and manufacturing process.

SURFACTANTS

Surfactants also play a critical role in the AAV manufacturing process. The surfactant, a key component in the novel cell lysis solution described above, prevents product loss due to shear stress during the cell lysis process.[11] Surfactant also prevents vector surface absorption,

aggregation and precipitation due to shear stress during manufacturing, transport and use.[6]

Fill/finish: ensuring integrity and quality

Viral vectors may aggregate and form particulates as a result of shear stress during fill/finish operations. Formulation compositions must be carefully optimized to the vector for the packaged final product to be freeze-thaw resistant, to prevent degradation in transit between storage locations and to remain stable onsite for usage. The primary causes of viral damage in storage are aggregation, genome leak from the capsid and free-radical oxidation which should be considered when formulating the final virus solution.[14]

CONCLUSION

While the challenges facing viral vector manufacturers are significant, they should not be surprising as they parallel those of the early days of mAbs. Collaboration between viral vector manufacturers and their suppliers and increased experience will facilitate a better understanding of production and how the different components affect the end product. This evolving knowledge base will enable scalable production that is more efficient cost effective, and safer to bring therapies to the patients who need them.

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