



Scaling Up Gene Therapy Manufacturing with Single-Use Technology

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In recent years, the biopharmaceutical industry has increasingly placed special focus on the development of sophisticated gene therapies to treat, prevent, or potentially cure a broad range of life-threatening and life-changing diseases. Nevertheless, implementing traditional scale-up processes in the gene therapy manufacturing environment can be challenging. With product integrity and safety remaining the highest priorities, gene therapy manufacturers are actively looking for ways to optimize their processes to allow for capacity scalability.

Single-use technology (SUT) offers several benefits, which gives it the potential to play a fundamental role across the full spectrum of gene therapy manufacturing workflows, from liquid handling to use with bioreactors and purification systems. However, the abundance of single-use technologies available means gene therapy manufacturers face a pressing dilemma over choosing the technology that will best meet their needs and allow them to scale to their target operation sizes.

This article will discuss the top five key considerations gene therapy manufacturers need to take into account to make the best possible choice for their SUT equipment and supplier: (1) the target cell culture, (2) the transfection process, (3) the product purification process, (4) the plasmid and reagent supply, and (5) the quality and performance of single-use consumables.

Selecting the right SUT equipment for your process needs

In traditional biomanufacturing, genes for the target product are stably encoded into a production cell. Process developers and manufacturers can then design and develop optimal manufacturing conditions to ensure consistency and quality during commercial scale-up. Innovative new treatments born from advancements in science and technology, such as the revolutionary field of gene therapy, do not readily fit this model, based on various unique factors, and therefore require specialized skill sets, development processes, and manufacturing strategies to bring them to market.

Specifically, gene transfer requires testing of many variables to identify the best final recipe for production and delivery, increasing the pressure on process development scientists who are already facing a high demand for speed. Producing the yield necessary while maintaining quality and efficacy requires equipment that is flexible and scalable, leading to the focus on SUT in gene therapy manufacturing. The overall increased adoption of SUT in today's industry is due to the inherent flexibility of disposable technology, which allows for faster product changeover, better sterility assurance, lower capital costs, and many more benefits that facilitate smaller batch sizes for smaller patient indications and niche products.

To reap the advantages of SUT for your gene therapy, though, you must take into consideration the following five areas during selection of the equipment and supplier:

1. Target cell culture
2. Transfection
3. Product purification
4. Plasmid and reagent supply
5. Quality, performance, and supply of single-use consumables

Target cell culture

Historically, many gene therapies have been produced using adherent cell culture, where flasks or cell factory systems were able to yield enough product to meet demand. As batch sizes increase, materials often cannot be produced efficiently and quickly enough with these methods, thus leading to the need for commercial-scale stirred-tank bioreactors. This transition in cell culture types requires significant process development in either establishing microcarrier-based processes for adherent cells or adapting or transitioning to suspension cell lines.

It is not always straightforward, though, as the dynamic environment of the bioreactor can present new stimuli, including mixing shear as well as aeration and bubble impacts. These can all potentially alter cell viability and even cell physiology and metabolism. When moving to a stirred system, there are several strategies you can use to protect your cell culture, such as adding surfactants to the media to protect the cells during suspension, or customizing the bioreactor to reduce the amount of agitation and, therefore, shear force. However, this is not usually a one-size-fits-all situation. It is important to not necessarily assume that methods used for other biologics can be directly applied to gene therapy manufacturing. Find a supplier who can partner with you through your SUT bioreactor options and help identify the equipment you need to be successful.

Transfection

As you identify the target cell culture and its needs, you must consider how you will deliver the target plasmid DNA material and modified genes to the cells. The strategy will also depend on the choice of the viral vectors, such as lentivirus (LV) or adeno-associated virus (AAV). That choice is perhaps driven by the desired length of DNA or target patient cell types. As you consider how you will get the DNA past the cell membrane, reagent selection (such as lipid- or polymer-based), plasmid count, and delivery timing are important for understanding how to maximize product yield and minimize empty or ineffective vectors. The more capable your SUT equipment, such as in mixing power, programmable control, and customization of inlets and outlets, the more tools you will have at your disposal for transfection optimization.

Product purification

Purification is always a critical part of bioprocessing, regardless of the drug you are producing. The type of viral vectors you produce will dictate the purification methods you will employ. Key considerations include the need for cell lysis, impurity removal, and desired yield. Scale-up is also important, as certain tools available at small scale, such as for physical cell lysis, are not readily adaptable to large volumes. As with cell culture, building on the shoulders of biopharma and bioprocessing predecessors can provide tools to create your purification process. In particular, advancements in chromatography resin development for viral vectors have demonstrated that the purification of these vectors can be significantly improved. Implementing affinity chromatography

in the downstream process reduces the number of purification steps and offers scalability at the same time, thereby maximizing productivity. Nevertheless, each viral vector is different and will require process optimization to maximize productivity of the downstream process. SUT options for buffer mixing, storage, and fluid transfers can all aid in the speed and adaptability of your processes.

Plasmid and reagent supply

Gene therapy is a rapidly growing market with an expected value of over \$6 billion by 2027 [1]. Depending on the therapy, producers will need to secure their transfection reagent supply from reliable and validated vendors. As a critical component of viral vector production, high-quality plasmid DNA is in high demand, and there is a need for increased global production. Gene therapy manufacturers are then confronted with a potential bottleneck. If using constrained plasmid CDMO partners, users may face long waiting times for these materials. Strategic partnerships can help alleviate this to some degree but require available partners and excellent forecasts for planning future needs. The most control over a supply chain comes with building plasmid production in-house but at the cost of building that infrastructure. SUT can again be advantageous here, as lower capital costs and operating expenses that directly correlate with production rates can benefit facility construction and planning. Specifically, single-use fermentation systems capable of producing plasmid DNA from *E. coli* cultures are at the core of allowing plasmid producers to gain the advantages of using SUT across their processes.

Quality, performance, and supply of single-use consumables

Many of today's single-use bioreactors were introduced over a decade ago and have grown with the demands of traditional bioprocessing. While many tools initially designed to serve the biopharma sector will be directly translatable to gene therapy production, a flexible supplier who can customize and adapt their SUT equipment and products to meet the needs of your processes will be highly advantageous. While your process will determine what equipment you will need, remember to choose robust equipment and avoid pitfalls of scale-up with well-defined processes and scale parameters. Bioreactor controller capabilities can come to the forefront in this regard, as they must be adaptable as your needs change.

Beyond the equipment, you must also ensure your consumables supplier has a secure supply chain that can deliver reliable single-use products at a rapid pace while maintaining the quality expected by customers, regulators, and patients. Inquire about your supplier's supply chain, including available components, manufacturing footprint, and redundancy of supply. Patients cannot afford batches lost to failure of consumables, or time lost to unreliable deliveries.

As demand for SUT equipment has increased, so too have expectations, especially around availability. Look for a supplier with an expanded single-use network that will help bring manufacturing closer to you and allow for timely and in-full delivery for the long term.

In addition, harmonized quality systems and processes help ensure all equipment is manufactured using the same quality standards across every site; a global standard component library enables the use of consistent, high-quality products regardless of the manufacturing location.

Polymer film for bioprocessing containers is a key component of flexible, single-use consumables, so ensuring film availability is also critical. The supplier you choose must have safety stock and redundancy plans with their own suppliers. Through a strong manufacturing infrastructure and reliable supply chain for your operations, you can take advantage of the cost savings and risk-reducing benefits of SUT while trusting that partner will support you toward success throughout the life cycle of your gene therapy product.

Reference

1. Grand View Research (February 2020). Gene Therapy Market Size Worth \$6.6 Billion By 2027. <https://www.grandviewresearch.com/press-release/global-gene-therapy-market>