Cell Therapy Production

A comprehensive look at key elements to success

Featuring:

- Cell Therapies Offer A Bright Future for Patients
- Make Time for Process Development
- Implementing Adherent Cell Culture Technology for Cell Therapy Bioprocess
- Media Optimization for Cell Therapy Production
- Selecting the Appropriate Scaling Strategy for Different Cell Therapy Applications
- Amplifying Adenoviral Particles in the Corning® HYPERStack® Cell Culture Vessel
- Closed and Aseptic Processes for Cell Therapy Manufacturing

Cell Culture DISH Inc.

CORNING

Author Bios

Ben Josey
Field Application Scientist
Corning Life Sciences

Dr. Ben Josey is a Field Application Scientist at Corning Life Sciences. He received his Ph.D. in Pharmaceutical and Biomedical Sciences from the Medical University of South Carolina working on small molecule and biomaterial-based treatments for cancer and neurodegenerative disease. Dr. Josey has held several positions in academia focused on translational research to overcome immunological barriers facing cell therapies. Dr. Josey works extensively with research and process development groups optimizing assay and cell culture scale-up conditions for viral production and cellular therapeutics.

Debbie King
Scientific Technical Writer

Debbie King is a freelance technical writer and a frequent contributor to The Cell Culture Dish, Inc. specializing in editorial content in the cell culture and gene therapy space. Her writing style is technical, yet approachable and engaging. She currently works with many biotechnology and pharma clients to provide her writing expertise. Her extensive cell culture experience from her previous positions at STEMCELL Technologies Inc in QC and product development has been an asset to her as a technical writer. She is proud to have been an integral member of the process development team to commercialize the mTeSR/TeSR hPSC media.

Austin Mogen, PhD,
Senior Field Application Scientist
Corning Life Sciences

Dr. Austin Mogen is a Senior Field Application Scientist at Corning Life Sciences. He received his doctorate from the University of Florida and gained industry experience as a Senior Scientist of upstream process development and manufacturing supervisor for viral vector manufacturing. In this position he focused on bioprocess development, closed system solutions for cell culture scale-up, and viral vector production. Since joining Corning, Dr. Mogen works extensively with academic researchers and process development groups, optimizing cell culture assays and cellular scale-up conditions for viral production, cellular therapeutics, and biologics.

Brandy Sargent
Editor in-chief
Cell Culture Dish & Downstream Column

Brandy Sargent is the Editor in-chief and frequent author of The Cell Culture Dish and The Downstream Column, She has worked in the biotechnology industry for over twenty years, first in corporate communications and public relations, then in technical sales and marketing, and most recently as a writer and publisher. She strives to introduce topics that are interesting, thought provoking, and possible starting points for discussion by the biomanufacturing community. She has been fascinated by the different applications of biotechnology since she first started working in the industry and continues to be fascinated as the industry evolves.

John Yoshi Shyu, Ph.D.
Director, Scientific Applications & Technical Support - Americas
Corning Life Sciences

Dr. John Yoshi Shyu (shyujy@corning.com) currently oversees the technical applications and support teams in North America at Corning Life Sciences. Dr. Shyu works with his team to support product development, application, and market adoption, especially in the fields of Bioprocess, Cell & Regenerative Therapy, Viral Vector/Vaccine production, and 3D Cell Culture.

Catherine Siler, Ph.D.
Field Application Scientist
Corning Life Sciences

Dr. Catherine Siler is an accomplished Field Application Scientist for Corning Life Sciences, with a Ph.D. in Biology from Johns Hopkins University. Dr. Siler enables scientists and researchers in the life science industry to overcome challenges with cell culture and scale-up for clinical manufacturing of advanced therapies, and utilizes her research and teaching experience to drive the adoption of an industry-leading global product portfolio of innovative single-use consumables for research, process development and bioprocessing applications.

Chris Suarez, Ph.D.
Field Application Manager, North America
Corning Life Sciences

Dr. Chris Suarez is the Manager of the Field Application Scientist team at Corning Life Sciences. He received his Ph.D.in Medicinal Chemistry and Molecular Pharmacology from Purdue University working on viral production, cell line engineering and cancer biology. Dr. Suarez has held positions in academia focusing on translational research to overcome mechanisms of therapeutic resistance in breast and prostate cancer using 3D tumor organoid models. Dr. Suarez works extensively with process development groups, optimizing production capabilities and cellular scale-up conditions from viral production to cellular therapeutics. In addition, he focuses on collaborations utilizing 3D technology like the Corning spheroid and Elplasia® microplates, Transwell® inserts and extracellular matrices to provide more predictive models for therapeutic response.

Cell Therapies Offer A Bright Future for Patients

Cell therapies offer tremendous potential to treat, and in some instances, cure diseases for which there is often no treatment available. The term cell therapy encompasses several different types of therapeutics. Cell therapies have a variety of modalities and use different cell types, have different manufacturing protocols, and treat different diseases. Thus, the production of a cell therapy is complex, with manufacturing technologies and procedures still evolving to meet these unique needs. In this publication, we aim to provide an overview of key considerations for cell therapy production, technologies that are being developed and employed, and ultimately how a therapeutic candidate moves from research scale to clinical or commercial manufacturing.

Industry Outlook

The global cell therapy market is strong and continues to increase. Market research predicts an annual growth rate of 5.4% reaching USD 8.83 billion by 2027. This unprecedented growth is attributed to new cell therapy product approvals, expansion of approved indications for current products, as well as increasing awareness, acceptance, and use of these advanced biological products globally.

Cell and gene therapies continue to progress through the clinical pipeline with the highest number of ongoing clinical trials to date, 1,220 worldwide[1]. This market growth coupled with impressive clinical results and recent regulatory approvals has driven an increase in demand for manufacturing solutions.

Indications

Cell therapies have been getting a great deal of attention as of late—most notably, gene-modified cell therapies for cancer immunology, such as CAR T therapies. In fact, the American Society of Clinical Oncology's (ASCO) Clinical Cancer Advances 2018 report named adoptive immunotherapy with chimeric antigen receptor T cells (CAR T) as the most important clinical cancer advancement of the year. The largest indication for cell therapy is oncology, followed by indications for the central nervous system[2].

Recent approvals in the CAR T space have driven both research and investment. In 2020, the FDA approved Kite's CAR-T therapy, Tecartus, for patients with relapsed or refractory (R/R) mantle cell lymphoma. Results in patients have been groundbreaking, with a nearly 90% response rate[1]. In February 2021, the FDA approved Bristol Myers Squibb's CAR-T therapy, Breyanzi, for the treatment of diffuse large B-cell lymphoma. Breyanzi was the first therapy approved under the FDA's Regenerative Medicine Advanced Therapy (RMAT) Designation. RMAT designation facilitates the expeditious development of regenerative medicine therapies intended for serious conditions

Cell Therapy Manufacturing

Efficient manufacturing is a critical aspect of determining whether a cell therapy will be commercialized or not. Including process development and manufacturing plans early in product development to ensure speed-to-market and commercial feasibility is important to success. In addition, if a cell therapy is designated under a regulatory expedited timeline, this will impact development timelines and needs to be considered during process design and scale-up.

Autologous vs. Allogeneic

Autologous therapies are examples of personalized medicine. They use a patient's own cells as the starting point and are manufactured as a single lot per patient. Autologous scale up is frequently referred to as "scaling out" because manufacturing requires the production of many small lots of individual products manufactured at the same time.

Allogeneic therapies on the other hand are manufactured in large lots from unrelated donor tissue. Allogeneic therapies are considered "off the shelf" in that it is one product used to treat many patients. These therapies require scale up by increasing the manufacturing vessel volume.

Process Development

The goal of process development is to reach a robust biomanufacturing process with efficiency gains, consistency, cost reduction, maintenance of quality and safety attributes, and overall risk reduction as additional key objectives. Although speed-to-market initiatives have condensed already tight timelines, process development is a crucial intermediate step

between research and commercial manufacturing. The success of producing a robust and cost-effective cell product at a scale that can meet patient needs is built upon the optimization achieved during process development. While the individual process steps can vary, the general cellular therapy workflow includes upstream and downstream unit operations such as cell isolation, cell culture media optimization, cell expansion, modification, purification, and characterization.

Media Optimization

In cell-based therapies, the cultured cells are the therapeutic product, which necessitates cell culture media optimization to meet growth and productivity targets for cell production. Off-the-shelf media solutions can provide a fast and efficient solution in early development, but there are some specific scale-up conditions that are difficult to meet with off-the-shelf media. Moving from small-scale, small volume static cultures to large-scale, large volume vessels trigger a host of additional requirements that cannot be easily addressed with an off-the-shelf solution. Thus, media development and optimization is important to successful clinical and commercial manufacturing. Often, partnering with a contract media manufacturer can provide the experience and support needed for a successful transition from lab to commercial scale media.

Scalability

Scale is one of the biggest considerations when designing the manufacturing process. This can be a challenge to achieve, particularly if a product's approval timeline is accelerated. Determining how to scale-up or scale-out means identifying how much material is needed for the therapeutic being manufactured. Because the cells are the therapeutic product, they need to retain their phenotype and functionality regardless of the manufacturing method.

Allogeneic therapies fit into a more traditional, centralized manufacturing model, where one therapeutic batch can treat many patients. In contrast, autologous therapies necessitate a de-centralized model, where manufacturing occurs near the point of care due to the patient-specific nature of the therapy. Here, one patient equals one manufacturing batch, which limits batch volumes and associated economies of scale at that level.

Aseptic Processing and Closed Systems

Aseptic processing is stated as "Handling sterile materials in a controlled environment, in which the air supply, facility, materials, equipment and personnel are regulated to control microbial and particulate contamination to acceptable levels."[3] Aseptic controls are important in cell therapy production as there are typically more open operations in these processes when compared to traditional protein based biologics, thus the risk of contamination is greater. In addition, with cell therapies, commonly used viral clearance and sterile filtration methods are not possible, as the cell is the therapy.[4]

A truly closed system with no environmental exposure eliminates the risk of contaminants, but the nature of certain process steps in cell therapy production may not currently be achievable in a closed system manner. As such, a "functionally closed system" allows users to perform unit operations in a realistic and logical but safe manner.

In Closing

Cell therapies offer a tremendous opportunity to increase our medicinal arsenal, but there are manufacturing challenges and solutions are evolving. Suppliers are creating innovative, fit for purpose products to solve the unique challenges that cell therapy products present. In this publication, we will discuss current solutions and also future opportunities for improvement.

References:

1. Alliance for Regenerative Medicine. Advancing gene, cell, & tissue-Based Therapies-ARM annual report & sector year in review: 2019. Accessed Oct. 11, 2020: Retrieved from www.alliancerm.org/sector-report/2019-annual-report/
2. Alliance for Regenerative Medicine. Growth & Resilience in Regenerative Medicines: 2020. Accessed May 1, 2021: Retrieved from alliancerm.org/sector-report/2020-annual-report/
3. Parenteral Drug Association (2011). Process simulation for aseptically filled products PDA task force-2011. Accessed Oct. 11, 2020: Retrieved from store. pda.org/ TableOfContents/TR2211_TOC.pdf
4. Barone, P. W. et al. Viral contamination in biologic manufacture and implications for emerging therapies. Nat Biotechnol 38, 563-572 (2020).

Cell Culture DISH Inc.

CORNING

Make Time for Process Development

Methods for growing cells in a dish have advanced at an extraordinary rate over the last 100 years. This ability to grow cells, as the therapeutic agent, has paved the way for advanced cellular therapies. For these therapies, millions, and sometimes billions of cells need to be produced under highly controlled and consistent manufacturing conditions. Given that cells are complex, require a specific growth environment, and can be directly impacted by cell culture conditions, growing cells to therapeutic levels can be extremely challenging. Many methods for culturing cells at small scale do not translate to large-scale bioproduction. This necessitates process development, or the optimization and development of small-scale cell culture methods into processes designed for large-scale bioproduction.

Developing a robust biomanufacturing process is the primary goal, with efficiency gains, consistency, cost reduction, maintenance of quality and safety attributes, and overall risk reduction as additional key objectives. Although speed-to-market initiatives have condensed already tight timelines, process development is a crucial intermediate step between research and commercial manufacturing. The success of producing a robust and cost-effective cell product at a scale that can meet patient needs is built upon the optimization achieved during process development. The individual process steps can vary, but the general cellular therapy workflow includes upstream and downstream unit operations such as cell isolation, cell culture media optimization, cell expansion, modification, purification, and characterization. In this article we will present important considerations and best practices for navigating process development for cellular therapies.

Product/Process Characterization

The question most customers ask is, where do we start? Regardless of the methodology, the form and function of the cell therapy must be retained when transitioning from lab- to large-scale. When designing a manufacturing-ready process, it is important to first determine the critical to quality attributes (or CQAs). These are often specific to the therapy and include cellular characterization and functional studies. Once these are determined, it is possible to examine the existing scaled down process and evaluate scale-up technology, all with the CQAs in mind. Lack of adequate process characterization and appropriate in-process controls along with poorly defined CQAs pose significant challenges for characterization and validation of cell therapies[1]. The ability to demonstrate reproducible manufacturing processes is compounded by the inherent biological variation of the starting material that form the basis of cellular therapies. This variability makes it even more important to understand the critical process parameters (CPPs) that contribute to product quality; where “the process is the product” is a common adage with cell therapies.

Critical Quality Attribute (CQA)[2]
– A physical, chemical, biological, or microbiological property or characteristic that should be within an appropriate limit, range, or distribution to ensure the desired product quality.

Critical Process Parameter (CPP)[2]
– A process parameter whose variability has an impact on a critical quality attribute and therefore should be monitored or controlled to ensure the process produces the desired product quality.

Those products that demonstrate candidacy for clinical safety and efficacy during clinical trials with defined and measurable CQAs are likely to garner approval from regulatory agencies. In order to evaluate and measure how any changes in process development impact these attributes, a representative, scale-down model is critical. Scale-down process models can identify when process design changes impact product performance. A robust process control strategy, supported by the appropriate in-process and final product analytic testing, enables iterative testing of multiple conditions and/or process parameters for process optimization.

Analytical Tools

Once the CQAs are determined, analytical, or assay development is typically required to ensure accurate measure of CQAs. While the FDA defines quality attributes that must be provided with any regulatory submission, there are many different types of products in development, each with its own set of unique characteristics, making it challenging to develop common assays for product characterization[3]. Having the appropriate analytical tools and validated assays to quantify CQAs as early as possible in the pre-clinical product development process streamlines decision-making. It also provides the developer with more confidence that an observed effect is reproducible in the clinical phase. As previously mentioned, the final cell product must maintain its phenotype and functionality, which can be assessed by measures of cellular morphology, karyotype, surface marker expression, and *in vitro* differentiation potential and can be separated into in-process and finished product analytics.

- In-process analytics – Designed to provide information about your process, where measurements are taken throughout the manufacturing process to ensure it is consistent and robust before it is transferred to manufacturing (i.e., cell morphology, cell count, metabolites/gas measurements).

- Product analytics – QC product release testing to confirm the safety, identity, strength, purity, and quality (SISPQ). Some example assays for CQAs are listed below:

Attribute	Analytical Assay
Identity	DNA sequencing, Western blot
Potency	Functional assay specific to the cell product
Purity	ELISA, qPCR
Sterility	Mycoplasma, endotoxin testing

This is, however, not a straightforward endeavor, since cell therapies are complex, with their own unique characteristics that may require the development and validation of bespoke analytical assays, particularly to assess the functionality of the therapy, which can take time to establish. Many of the traditional assays suffer from low throughput and resolution. Advances in analytical techniques such as digital droplet polymerase chain reaction, flow cytometry, and high-performance liquid chromatography, have enabled more consistent and accurate measurement of quality attributes[1].

Media Optimization

Another factor that can determine the process development investment is the cell type – are the cells anchorage-dependent or anchorage-independent? The technology and parameters to optimize for scale-up and to grow the cells will be very different.

The cell culture media, the formulation as well as the packaging, warrant consideration during process development. It can be worthwhile to enlist the help of media providers, like Corning, to identify and resolve formulation, packaging and regulatory issues and to accommodate the large volumes required for commercial manufacture. Often times a developer will have a media formulation that performs well for their application, but may need assistance identifying GMP-grade raw materials or have custom packaging needs for compatibility with closed system processes, such as media in bags rather than bottles.

Custom media products at Corning are manufactured under the current ISO 13485 standard and FDA Quality System Regulation 21 CRF 820, current good manufacturing practices (cGMP), FDA-registered facility for Class 1 Medical Devices, which meet all cGMP and FDA requirements for manufacturing and sterility. Leveraging Corning's expertise and broad product portfolio can support your cell therapy product from discovery to commercial manufacturing.

Ultimately, the amount of process development needed is highly dependent on the therapeutic and project timelines, but our authors emphasize the need and benefit of early process development investment to ease progress through the product pipeline. Knowing your product targets will allow you to work backwards to determine the CQAs and CPPs that need to be built into the process and prevent technology transfer to manufacturing prematurely. The wave of new products resulting from innovative technologies like CAR-T underscores how effective product and process development is tied to the commercial success of a cell therapy.

Application Considerations

Many different cell types are being studied, and through biological engineering, designed for the purpose of cellular therapies. Each cell type has its own requirements regarding medium, growth factors, and culture conditions.

Manufacturing Scale

Often the first consideration in choosing a bioproduction platform is determining the scale required to meet therapeutic dose. This is often the target volume or cell number needed to achieve dosage for a clinical trial and/or commercial therapy. There are some key differences in scale that are determined by the type of cellular therapy, autologous vs. allogeneic. Autologous cell therapy workflows involve taking a small sample from the patient, processing it, and re-treating the patient; making the scale substantially smaller than allogeneic therapies. Working volumes are typically in the 0.5 – 5 L range. This in turn promotes a scale-out approach, where the same production equipment is used in parallel, with a dedicated unit for each patient. Allogeneic therapies on the other hand have a common cell source used to treat multiple patients, requiring larger unit operations. Large-scale single use bioreactors with volumes exceeding 200 liters are often implemented in these workflows. The options for upstream and downstream manufacturing equipment, automation technology (i.e., in-line or at-line sensors) and single-use consumables vary tremendously depending on the scale of operation. Scale considerations often serve as an important limiting factor when considering which technology candidates are suitable for.

Conclusion

Fulfilling the promise of cell therapy requires that we effectively transition from proven research-scale to production-scale capable of generating safe and effective therapies to treat patients. Taking a thorough approach to process development, adhering to emerging best practices, leveraging proven technologies and the expertise and resources of preferred vendors can help ensure that large-scale production success can be achieved.

References

1. C. Challener, "Mapping a Route for Cell and Gene Therapy Process Development" BioPharm International, 33 (1) 2020.
2. ICH Q8 (R2) – Pharmaceutical Development, September 2009
3. National Academies of Sciences, Engineering, and Medicine; Health and Medicine Division; Board on Health Sciences Policy; Forum on Regenerative Medicine. Navigating the Manufacturing Process and Ensuring the Quality of Regenerative Medicine Therapies: Proceedings of a Workshop. Washington (DC): National Academies Press (US); 2017 Oct 26. 3, Identifying and Measuring Critical Quality Attributes. Available from: https://w.ncbi.nlm.nih.gov/books/NBK475684/

Implementing Adherent Cell Culture Technology for Cell Therapy Bioprocess

A panel discussion with:

Ben Josey • Austin Mogen • Brandy Sargent

Cellular therapies are therapeutics that utilize cells as the therapy. As the industry has grown, an increasing number of cell therapies have entered clinical trials, and some have achieved remarkable results. The potential of these therapeutics to treat diseases and conditions that were previously thought untreatable is inspiring. Several of the most promising candidates in the pipeline use mesenchymal stromal cells and pluripotent stem cells, both of which require an adherent substrate for native biological function. Thus, utilizing an adherent platform for production of these cell types provides several advantages, including shorter process development and optimization time, no need to adapt cells to suspension, and the ability to implement various surface modifications that promote the relevant biology.

When it comes to scale-up and commercial production, the industry typically thinks of suspension culture. This is based on the success of traditional protein-based therapeutic manufacturing. However, cell therapies are significantly more complex to manufacture and thus require more sophisticated manufacturing platforms. While suspension has traditionally been seen as the way to reach economies of scale, new adherent platform technologies such as stacked vessels, microcarriers, and fixed bed bioreactors, are making it possible for adherent cells to approach similar economies of scale while meeting the unique biological requirements of cell therapies.

We hear the term cellular therapy being used more and more frequently. Can you describe what a cellular therapy is and why it is an important therapeutic modality?

Austin Mogen

The broad term “cellular therapy” is used to describe a therapeutic modality that utilizes a cell as the therapy instead of something like a small molecule, protein or viral vector. In some ways, cells are like complex miniature machines that can be engineered by scientists to provide a therapeutic function such as killing tumor cells, reducing inflammation, or producing insulin, to name a few.

Ben Josey

Cellular therapies are important because of the impressive efficacy that some cell therapies have demonstrated in clinical trials. Diseases and conditions that were previously thought untreatable or incurable now have potential treatments and in some cases cures. Cell therapies harness the body’s own natural mechanisms by utilizing cells that are naturally found in your body. When these cells are partnered with scientific advancement, they can be programed to use your body’s natural processes to help treat and cure itself.

What kinds of cellular therapies would utilize an adherent platform?

Ben Josey

Some of the most promising front runners in the adherent cell therapy manufacturing space currently include mesenchymal stromal cells and pluripotent stem cells, both of which require an adherent substrate for native biological function. This would be in comparison to something like an immune cell that naturally exists in the blood and floats around in suspension. These stem cells can then be differentiated into a variety of other cell types (neurons for treating a brain condition, cardiomyocytes for heart diseases, islet cells for diabetes, etc.), many of which are also attachment dependent. So, utilizing an adherent platform in this context connects more closely to cells’ natural biological origin.

Can you lay out for listeners the benefits of adherent vs. suspension cell cultures and when you might want to use each in cell therapy manufacturing?

Austin Mogen

To a large extent, cell culture platforms seek to mimic the native environment for whatever type of cell is being grown. For example, cells from solid tissues (like muscle) are more amenable to adherent culture, and cells from liquid tissues (like blood) are better suited for suspension culture. While it is possible to coax some cells to shift modalities, deviating from this basic premise creates challenges. It often requires extensive adaptation and sometimes genetic modification of adherent cell lines to make them amenable to suspension culture conditions. Suspension cultures have long had the advantage of being able to be grown in traditional, large-scale stirred tank bioreactors, which reduces the processing steps and lowers the facility footprint, but most stem cell-derived therapies require an adherent substrate. Newer technologies such as stacked vessels, microcarriers, and fixed bed bioreactors, are making it possible for adherent cells to approach similar economies of scale as suspension processes.

We hear a lot about the trend of transitioning from adherent platforms during research and trials to suspension when scaling up biological therapies to commercial production. Why do you think this is happening? To me, it seems that some cellular therapies are better kept in adherent production.

Ben Josey

This is mostly driven by economics and outdated facility designs. Another component is the lack of awareness about the advances in adherent platforms currently available. For traditional adherent platforms, such as T-flasks and roller bottles, the labor and space

required for scaling up isn't feasible. While they work well for research and development, the number of flasks and bottles required for scale up to production levels makes them impractical. In addition, existing bioproduction technologies were developed with the transition from microbial fermentation to single component protein production. This process utilized large tanks to achieve economies of scale. Cell therapies are more complex and require a more complex and more adaptable or modular solution and the industry is moving towards this realization. Novel adherent technologies have been developed to try to address the need to balance economies of scale and the unique biological requirements of cell therapies.

Austin Mogen
Some of the developments in terms of technology that are making it possible to scale up the adherent cell culture workflows include microcarriers, as well as novel technologies in fixed bed bioreactor design. There has been an increase in the development of technologies that are pushing the boundaries of what we can do in terms of culturing adherent cells at large scale.

What is unique about adherent platforms for stem cell applications?

Austin Mogen
In the context of stem cell culture, one significant advantage that adherent platforms have is the ability to utilize various surface modifications. Biologically, most stem cells exist in contact-dependent niches, wherein their direct contact with other cells and extracellular matrices play major roles in the maintenance of their stemness. This is important to their ability to differentiate into different cell types, which is particularly relevant when we talk about the therapeutic value of these cells.

Anoikis is a form of programmed cell death that occurs in anchorage-dependent cells when they detach from the surrounding extra-cellular matrix (ECM). It is a well-recognized issue in stem cell cultures. When using adherent platforms, it is possible to apply coatings that mimic the relevant ECM components. This is not possible in suspension and in some ways microcarriers bridge this gap. Microcarriers are small spherical beads that can be used in a suspension system like a bioreactor and they can be coated with relevant ECM proteins or synthetic mimetics that are helpful for culturing stem cells.

Ben Josey
Microcarriers do bridge this gap to some extent and they are a great solution for many situations. However, they also introduce their own set of challenges around process optimization. Cells that are coming from a traditional 2D vessel need to be adapted when moving into a larger format. Process development time is an important consideration, so a company must decide if microcarriers are a good solution or if one of the other advanced adherent platforms would be a better fit.

It seems to me there are many adherent cell culture platforms to choose from, can you describe some of them?

Ben Josey
In the context of stem cells, most people are familiar with 2D planar vessels like T-flasks. These are widely used in research, but quickly become limited at production scales. The next level up from T-flasks would be a stacked vessel like the Corning® CellSTACK®. These are essentially just big T-flasks that are connected and stacked in layers. The top tier in the stacked vessel evolution is the Corning HYPER technology, which uses propriety gas-permeable film technology to maximize the amount of stacked growth surface area achievable in a given footprint. What all of these systems have in common is that the growth environment is static, meaning that the cells sit in a stationary media that is then exchanged at intervals.

Moving up in scale from 2D planar vessels are microcarriers, which are considered a 3D system utilizing small spherical beads paired with a suspension bioreactor. This is a hybrid adherent/suspension system.

Lastly are the structured support bioreactors such as packed bed, hollow fiber, and fixed bed bioreactors, such as the new Corning Ascent FBR. These systems combine a fixed substrate with bioreactor technology, wherein you are able to grow adherent cells attached to a surface while in some fashion flowing fresh media past the cells. These systems add in some additional process optimization challenges, but they also provide huge benefits in the ability to monitor culture conditions, such as dissolved oxygen, pH, and metabolites. This can be very useful in optimizing a process and bringing it to production capacities.

What are the considerations for manufacturing cell therapies using some of the adherent systems described above?

Austin Mogen
There are advantages and disadvantages to each platform technology. For 2D planar stacked vessels, they have the advantage of a very low barrier to entry with low up-front investment costs and a high degree of process flexibility. Typically, these are single use vessels, so the primary costs are purchasing the vessel, incubator space, and clean room space. Thus, requiring minimal capital investment. The process development is minimized because the cells behave very similarly as they did in T-flask culture. This provides a simple transition from R&D to scale up. Take the Corning® HYPERStack® vessel for example, cells typically perform the same in a T-flask as they do in a HYPERStack vessel with minimal process development required. It is a good choice when time to market and minimizing the process development time as much as possible are key considerations. Whether you use one stack or 60 stacks, your cells are consistently exposed to the same 0.22 mL of media per cm^2 growth area and you can easily add or subtract modular HYPERStack vessels based on the needs of a given production run.

On the other hand, 2D stacked vessels are limited in their compatibility with inline process monitoring and the labor required to handle these at commercial scale can become significant. One alternative is the use of microcarriers, which offer the combination of maximized surface area combined with the process control of a bioreactor. However, these systems can be difficult to optimize, and process development can be much more extensive than for a 2D process. For cell therapy research applications, a dissolvable microcarrier such as Corning's DMCs can provide substantial benefit for cell harvest. Another technology option that is becoming more popular is a structured support bioreactor, which offers a nice balance between 2D systems and 3D microcarriers. These bioreactors offer the high surface area to volumetric footprint of a microcarrier, thereby reducing space requirements and handling, as well as the process control of a bioreactor. For some applications, similar process development time to a 2D vessel system can be achieved. Historically, it has been difficult to effectively harvest cells from these systems, however, recent advances in technology are now making this possible. The new Corning Ascent™ FBR System is one example of a fixed bed reactor system designed to enable cell harvest.

What are the challenges of scaling up stem cells for clinical and commercial cell therapy manufacturing?

Ben Josey
There are many challenges facing companies in this space. The challenges are different depending on the various parts of the system. Across the board, there is a need to produce large numbers of cells (tens to hundreds of billions of cells) without altering the underlying biology of the cells that would impact the therapeutic effect. There is the need to accomplish this within a commercially viable cost of production. Many of these cells are not infinitely expandable. They differ from more traditional protein production cell line expansions, so achieving efficiency with doubling times and passage numbers becomes much more critical. Being able to achieve scale while maintaining biological function and relevance all within reasonable production costs is a big challenge that the industry faces.

Austin Mogen
Other challenges that are specific to scaling up stem cells include decreasing process variability and increasing the consistency of the bioproduction process. Cells are complex, composed of nucleic acids, proteins, and lipids; they provide quite a challenge in selecting the right production system. It is critical to have a robust production process so you can maintain consistency throughout that process both within the individual process and also between production lots. When you are producing a therapeutic you must ensure that what you produce in one lot is comparable to future lots. Automating some of the parts of the process to reduce the number of manual steps and opportunities for operator variability would greatly improve consistency. In addition, having the right analytics in place to properly measure the cellular characteristics or critical quality attributes permits insight into the process and provides parameters by which to measure whether the process is consistent throughout.

What recommendations do you have for scientists looking to scale up their stem cell culture?

Austin Mogen
In some situations, the best advice is to start at the beginning and take things one step at a time. This is not one of those situations. Start with the end goal in mind and be prepared to adapt. For example, it is important to define upfront the scale required to meet the necessary therapeutic dosage for that therapy. This can be applied to clinical trial and commercial scale-up operations and will help in choosing the scale-up platform and its attributes. Additionally, it is essential to define your critical quality attributes for the cellular therapy being developed and the methods of measuring these attributes. Maintaining these attributes throughout the scale-up process will ensure success. Ensuring that you have robust assays in place and can take accurate measurements throughout your process to maintain consistency is a good starting place.

How do you see the future of stem cell therapy manufacturing evolving and specifically adherent based manufacturing?

Ben Josey
It will probably end up going a couple different routes. Very interesting and specialized technologies are being developed to address situations that are just as unique as the cells being grown. In spite of all the technological advancements, current bioreactors are still primitive. In the body, these cells exist in a biologically relevant, anchorage-dependent context, within a constantly monitored and updated recirculation system. I see the future moving in this direction, with scalable modular bioreactor units that possess internal biocompatible scaffolding and are connected to mostly automated recirculation systems with adaptable online monitoring.

Austin Mogen
One of the future focuses of the cell therapy manufacturing space is automation and online monitoring. We are getting to a point where we have developed robust, future-looking technologies for scaling up and culturing these cells at large scale. However, many of these processes are still quite manual, and that can introduce variability into the process. So, automating many of the process steps, as well as online monitoring, will permit quantification of the cell characteristics online and in real time instead of taking days to generate that data. Lastly, the downstream process must be able to process a large amount of cells after they have been harvested from the cell culture scale up system. Work still needs to be done to increase the scale of downstream processing technology.

Do you have anything else that you would like to add for listeners?

Austin Mogen
It is a very exciting time in human medicine with advanced therapies being developed to overcome many previously untreatable diseases. The field is moving very quickly.

Research scientists and suppliers, like Corning, are moving as fast as they can to develop the best possible cellular engineering and bioprocessing tools to make the process of generating these therapies much smoother. I urge scientists to work with your supplier partners, such as Corning, and utilize their expertise to help develop and implement novel technologies into scale-up workflows.

Ben Josey
My favorite part of my job is working with scientists to try and help them meet their goals. I'd urge them to keep pushing the boundaries of science and please reach out to the Corning team and other suppliers to let them know about any trouble they are facing. By reaching out they will be able to see if suppliers have solutions or will work with them to overcome their challenges. There are so many exciting therapies in the pipeline and on the horizon that are just barely out of reach. If we all work on it together, we can push the industry forward and make them a reality.

Media Optimization for Cell Therapy Production

In cell-based therapies, the cultured cells are the therapeutic product, which necessitates different manufacturing processes as compared with other biologics productions. As a cell therapy developer, the big considerations in the scale-up process include the input cells, culture vessels, and culture media. Inadequate consideration and planning can not only impact the efficacy of the cell therapy, but also present costly regulatory roadblocks that can delay progress through the product pipeline. Since the culture media is linked to the growth and productivity of the cells, it is one of the most critical aspects of process development during scale-up.

While off-the-shelf media solutions can provide a fast and efficient solution in early development, there are some specific scale-up conditions that are difficult to meet with off-the-shelf media. Moving from small-scale, small volume static cultures to large-scale, large volume vessels trigger a host of additional requirements that cannot be easily addressed with an off-the-shelf solution. Thus, media customization by a media manufacturer is an attractive solution to the increased considerations associated with large scale; these include media stability, packaging, handling and storage, and de-risking the overall process.

Here, we look at media development and customization to provide insight into the cell culture media scale-up process. We also examine how partnering with a contract media manufacturer can provide the additional experience and support needed for a successful transition from lab to commercial scale cell therapy production.

Media Stability

Media stability can present a challenge; particularly since most media formulations used to culture specialized cell types used in cell therapies are complex. These formulations frequently contain raw material components like growth factors that can lose efficacy over time and stability must be carefully considered. In addition, during the scale-up process, the use of large culture vessels necessitates a larger volume of media and cell therapy developers may want to ensure consistency in their process by only using one lot of cell culture media for an entire production run. So, the capacity to store and manage a large inventory of cell culture media while maintaining shelf life and stability is critical. For this reason, it is important to consider batch size, storage conditions, capacity, and media availability, i.e. order to delivery times when planning process scale-up. For certain therapies like autologous CAR-T where the vein-to-vein time from collection to manufacturing to re-infusion is very short, not having enough media on-hand for production can be disastrous.

As mentioned, the raw materials themselves can impact media stability. Working with a media provider can provide the support to potentially adjust your media formulation, identifying substitutions or more consistent cGMP-grade reagents that could improve stability.

Media Packaging

Large-scale manufacturing requires the use of automated and aseptic, closed processes. During scale-up when a process moves from open to closed, bottled media is not a desirable format because of the complexity and inherent aseptic risk with handling many bottles. Therefore, moving to bagged media systems is a common transition in scale-up. Related to this is the connection technology – how will the media be transferred from the bags to culture vessels while maintaining sterility in a closed system. The type of tubing used (i.e. the type of plastic and diameter) in conjunction with the bag connectors (Table 1) will also be dependent on the specifics of your process. Having a supplier that can identify and address the needs of your program can help mitigate any bottlenecks in the transition to closed system operations.

At Corning we frequently work with customers to design media packaging that accommodates different needs that may be particular to their process – bags with specific types of connections or with dip tubes to ensure they can fully empty the bag, for example.

Table 1. Common connector technologies used with bagged media formats.

Connector Technology	Details
Open Connectors	Open connectors require a controlled environment like a BSC to make an aseptic connection with the media bag like luer fittings and MPC-type connectors.
Aseptic Connectors	Aseptic connectors can establish an aseptic connection regardless of the environment, which is advantageous over open connectors, but they can be difficult to operate and are more expensive
Tube Welding	Best for situations where only a few aseptic connections are needed because it takes time, equipment and expertise to establish a proper sterile tube weld.

In-house vs. Outsource of Media Manufacturing

Many companies will need to make the critical decision of whether to enlist the services of a contract media manufacturer for their cell therapy needs. Current Good Manufacturing Practice (cGMP) media production is complex and is governed by strict oversight by regulatory agencies, such as the U.S. Food and Drug Administration (FDA), Health Canada, and the European Medicines Agency (EMA). Extensive validation and documentation are required in order to fulfill cGMP requirements, which is time- and labor-intensive to achieve in-house if the infrastructure and capacity are not already in place. For example, having a dedicated manufacturing suite and a suitable water system (Water for Injection (WFI)) in place for biomanufacturing are necessities for cGMP manufacture and these are costly investments.

The advantages of partnering with an outsourced manufacturing company is that the necessary infrastructure and expertise are established and available to help meet specific media needs from formulation to packaging, and regulatory requirements. Moreover, it

lowers capital costs as there is no need to invest in a WFI system, which is particularly valuable in the early stages of scale-up. As well, with years of experience in media development and commercial manufacturing and as a provider of high-quality raw materials, the components are available through one supplier making it an easy and simplified tech transfer transition for a cell therapy developer. Perhaps most valuable is the knowledge and expertise that these companies can provide because of their involvement with other customers/processes so they can make recommendations based on what has or has not worked in the past to help you meet your needs.

Regulatory Considerations

Developers need to meet current regulations for cell therapy manufacturing, and choosing a media supplier can help. For example, custom media products produced at Corning are manufactured in a facility that meets the current ISO 13485 standard and FDA Quality System Regulation 21 CRF 820, also known as current good manufacturing practices (cGMP).

Media Customization Services

It is helpful to understand what the media customization process looks like when outsourcing this development. We have used the experience of working with the team at Corning to provide insight into this process. Corning routinely works with companies to produce customized, tailored media and reagents to meet unique process needs, steps include:

1) Formulation/Batch Size
Typically, discussions between team members from both the Corning and customer side occur to determine the media formulation. Batch size determination may go together with the specific media components since their stability/shelf life may impact maximum batch size.

Serum:
For serum-containing formulations, Corning has an extensive portfolio of serum products that are collected from government approved abattoirs in the United States, Australia, and New Zealand, processed, and tested in accordance with and EP (European Pharmacopeia) and USP (United States Pharmacopeia) methods. Corning holds Certificates of Suitability (CEP) from the European Directorate for the Quality of Medicines (EDQM) for US and Australian origin serum. As well, Corning facilities are ISIA traceability certified for serum.

Animal-component or Serum-free Formulations:
As the cell and gene therapy market increases, there is a drive towards minimizing or eliminating animal components in media formulations to remove the potential immunogenic risk from xenogeneic raw materials. At Corning, custom media and sterile solutions are produced in separate designated animal and non-animal manufacturing suites. For animal component free (ACF) media production, the raw material processing is also segregated from the animal-containing manufacturing stream, which allows for certification as completely animal component free.

Corning can also provide expertise to help bring a customer formulation to animal-component free state since there are recombinant proteins available to replace more traditional animal-derived components.

2) Format
Upon scale up, the format in which the custom media is packaged is also a huge consideration and primarily dependent on the customer's process needs, as well as handling/processing capabilities and storage capacity. Corning can accommodate different sizes of bottles as well as bags depending on the scale, from 30 mL to 1L in bottles and from 1L bags to 200L bags suitable for various connector technologies. Most commonly, 20-200L bag formats are utilized. Above 200L sizes can be accommodated but these can be difficult to manage and store. Additionally, there is a higher risk of losing your entire batch of media if there is a bag failure as well as the associated cost of such a failure, both components and time.

3) Quality Control
All custom media undergo compendial testing according to industry standards using USP or EP monographs to ensure it meets biological (i.e., endotoxin, mycoplasma) and physiochemical requirements (i.e., pH, osmolality, conductivity). Depending on the formulation, whether it is a salt solution or a more complex cell culture media, functional testing can be performed to demonstrate that the media can support cell proliferation.

Ultimately, quality control testing that accompanies a custom media to ensure that the product is suitable for the intended process and downstream use is driven by the customer. For example, quality samples can be taken at the beginning, middle, and end of a production run to ensure manufacturing consistency. Additional stability testing of raw material components and in the final formulation may be required as well. Depending on the media formulation, confirming the correct identity and concentration of media components can be done. This will require a dialog between Corning and the developer's quality team to identify the critical parameters for the Certificate of Analysis.

4) Small-scale Pilot batch
Once the specifications are established, a small-scale pilot batch is generated ideal for development and testing purposes prior to cGMP scale-up. Once this pilot batch has passed quality control testing and is acceptable to the customer, the final step is to manufacture a full-scale cGMP batch.

5) Large-scale cGMP batch
The overall cGMP batch sizes range from 100L to 8,000L and the options available can depend on the media formulation (Table 2).

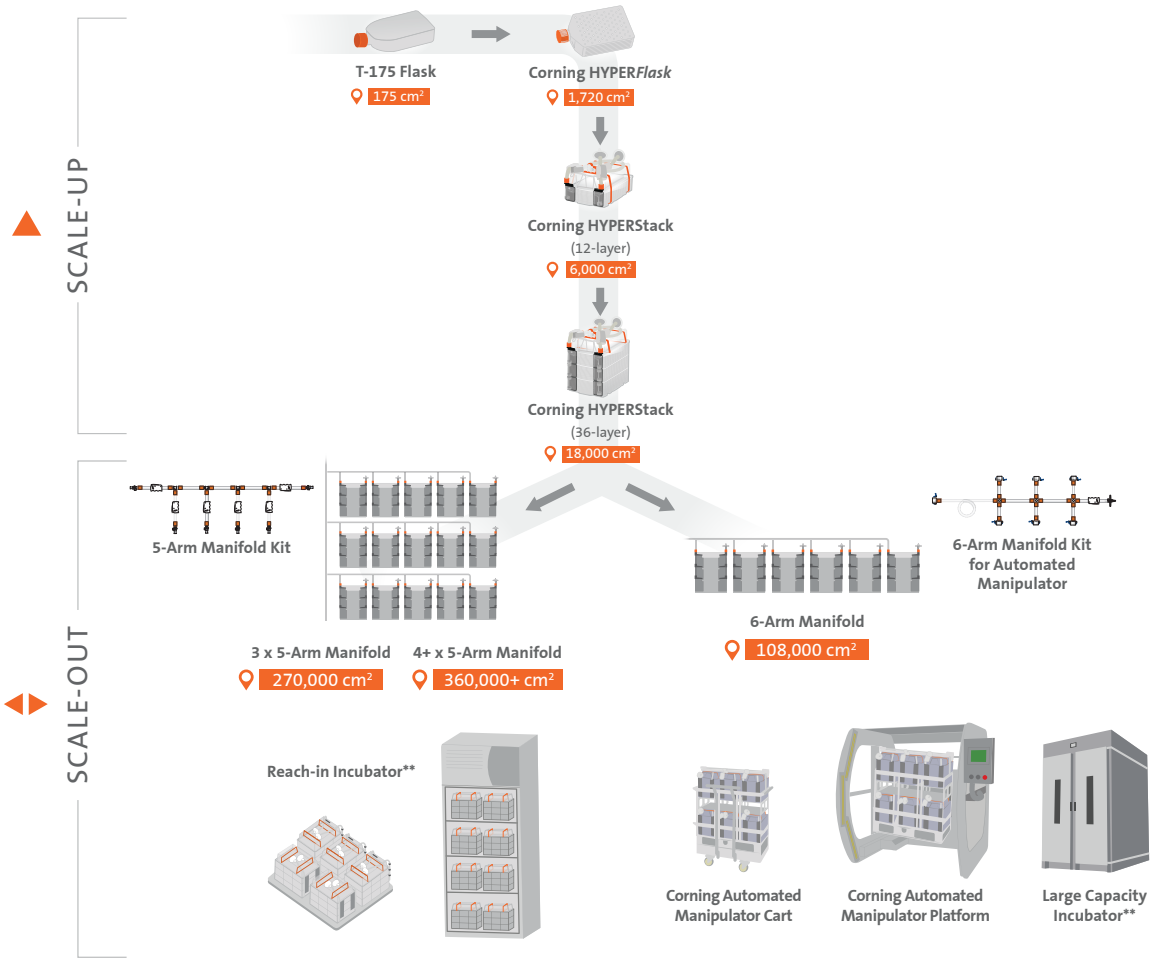
Media Type	Volume
Animal	100 – 200L 1,000 – 6,000L
Animal-component free	100 – 200L 400 – 2,000L 1,600 – 8,000L

While media customization and optimization for cell therapy manufacturing can feel daunting, there are key considerations to focus on. Working with a company to help with this work can be very beneficial in that it provides vast experience across a range of products and capabilities that is difficult and costly to replicate using in-house resources. In addition, outsourcing media customization, frees valuable labor and capital resources that cell therapy developers can utilize for other aspects of the cell therapy commercialization process.

Scaling Adherent Cell Culture with Corning® HYPER Technology

Whether your goal is adherent cell expansion for cell or gene therapy workflows or biopharmaceutical production, Corning HYPER technology enables you to scale from 1,720 cm^2 to hundreds of thousands of cm^2 of growth surface area. Using an ultra-thin gas-permeable film, HYPER technology is able to provide up to 10X the growth surface area of a traditional cell culture vessel of comparable footprint. Single-use manifolds, laboratory-standard reach-in incubators, and the Corning Automated Manipulator Platform support your scale-out.

The Road to 300,000 cm^2 and Beyond

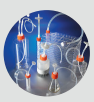


Supporting Your Workflow

Filling and Addition of Components


Inoculation Containers


Manifold Kits


Closed System Accessory Solutions

Expansion and Processing


Automated Manipulator


SUT Bags


Large Capacity Incubator**

Harvest and Cryopreservation


Collection Containers


Customizable Processing Tools


Cryo-preservation Solutions

** Products not sold by Corning

Click to download a print resolution PDF of this infographic.

Selecting the Appropriate Scaling Strategy for Different Cell Therapy Applications

Cell therapies represent a new paradigm in medicine, and the commercialization of several treatments has prompted a need for those developing these therapeutics to look at the scalability of their manufacturing platforms. The decision on how to scale-up and scale-out while choosing the appropriate platform and associated technology relies heavily on the therapeutic being manufactured. Because the cells are the therapeutic product, they need to retain their phenotype and functionality regardless of the manufacturing method. Utilization of primary cells to develop these therapeutics presents unique challenges. These include addressing issues such as donor variability, increased sensitivity to changes in their environment and downstream considerations for formulation and final fill.

In considering the different strategies of both autologous (patient-derived cells) and allogeneic (cells from a donor) cell therapies, it is recognized that a single model for scaling production is not suitable. Allogeneic therapies fit into a more traditional, centralized manufacturing model, where one therapeutic batch can treat many patients. In contrast, autologous therapies necessitate a de-centralized model, where manufacturing occurs near the point of care due to the patient-specific nature of the therapy. Here, one patient equals one manufacturing batch, which limits batch volumes and associated economies of scale at that level.

All said, there are some key considerations that should be examined when looking to scale these therapies for manufacturing. In this article, we aim to provide guidance for scaling-up and scaling-out while considering those factors that are essential to identifying the optimal platform required to manufacture a cell therapy product for clinical use.

Scale Up and Scale Out

When considering strategies to expand the number of cells being grown, considerable effort is focused on the decision to scale up and scale out. By definition, scale-up of a process involves using larger vessels to increase the volume of production. In scale out, the culture vessel remains the same, but more units are added to a system to increase production capacity. To illustrate this, Figure 1 shows an example of scaling-out where a researcher moves from using a single cell culture flask to multiple flasks of the same dimension before scaling-up to a larger format. In this scenario, scaling up would include moving to larger size flasks or other high throughput stacked vessel systems such as the Corning® CellSTACK®, HYPERFlask®, HYPERStack®, or CellCube® for attachment-dependent cells, or spinner and shaker flasks for cells in suspension.

While considerations for scaling out a production process can be completed quickly, because the unit of production remains the same, the considerations for scaling up can often involve more planning. Questions will focus on the time needed to optimize cell culture expansion, harvest and quality testing at the process development stage, through pilot-scale production, and up to large-scale manufacturing. There is a need to invest time at the process development stage to identify potential challenges that would increase difficulty in transferring the process to manufacturing scale. Therefore, it is important to proactively identify and address potential issues during process development that could be barriers to large-scale manufacturing. In cell therapy, where the functionality of the cells is paramount, scaling strategy requires significant validation to ensure cells retain the same phenotype and quality at each stage of the process.

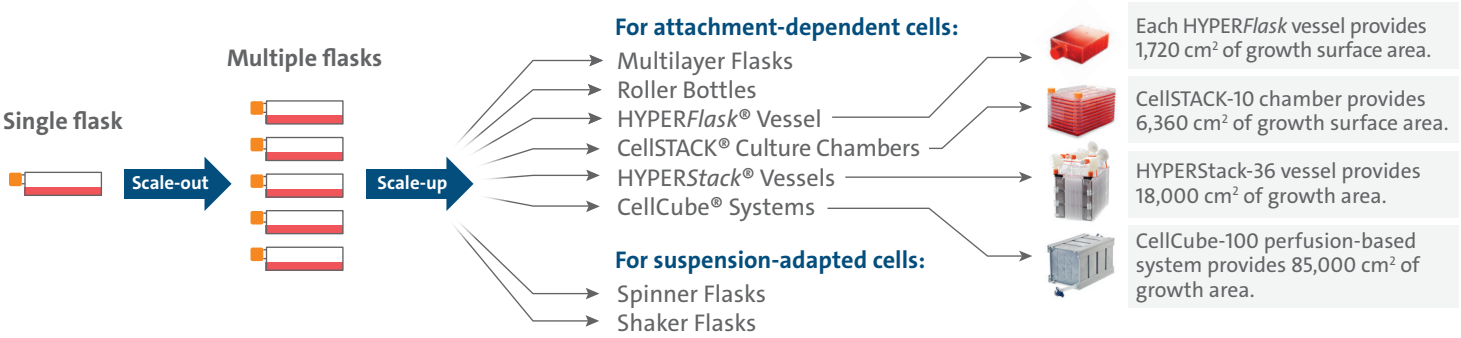


Figure 1. Scale-out strategy with cell culture flasks involves addition more of the same type of flask into your production whereas, scale-up involves moving to a larger culture vessel format such as Corning CellSTACK®, HYPERFlask® or CellCube® systems or spinner and shaker flasks depending on the type of cells.

There is certainly a place for both scale-out and scale-up approaches but the decision largely depends on factors like type of cell therapy or biology of the cells, timelines (i.e. time to market, product demand), facility space, availability of skilled technicians and capital investment/budgetary constraints. Timely and effective planning will facilitate a successful transition to increased production capabilities.

Also, depending on product demands or company needs, the decision could be made to switch to a technology different than the one used for development. For example, a developer might decide to switch from adherent, static cell culture to a non-adherent, suspension culture system to meet production demands.

1) Cell Type Considerations
In cell therapy, many of the commercially available adoptive cell therapies target primary cell types, like cytotoxic T cells to generate chimeric antigen receptor (CAR)-T cell therapies. These cells are non-adherent and can be cultured in suspension systems. Scaling up suspension-cultured cells to larger volumes/vessels is a complex operation that requires careful agitation/mixing to ensure sufficient gas and nutrient exchange while minimizing cell damage caused by shear stress. It is important to assess the flow dynamics based on the equipment (i.e., number of impellers, sparge strategies, stir speed, mix strategy) and their impacts on cell type. These issues can present unique challenges, as often times multiple vessel designs are utilized in a scale out process, but identifying and resolving them are critical to maintaining product quality. Additionally, utilization of process analytical technology, commonly used in large-scale protein production, can help with cell therapy manufacturing optimization by providing critical information about the health and behavior of the culture.

Scaling strategy requires significant validation to ensure cells retain the same phenotype and quality at each stage of the process.

As interest grows in allogeneic cell therapies approaches like CAR-T, several biopharmaceutical companies are investing in induced pluripotent stem cells (iPSC) as their T-cell source, which can be expanded and differentiated *in vitro* to meet the cell number and dosage requirements for cell therapy[1]. The expansion of iPSCs is commonly performed using two-dimensional adherent conditions in feeder-dependent systems with embryonic fibroblasts or feeder-free systems with purified matrices. Robust large-scale production of these cells is a major challenge since these cells are prone to unwanted, spontaneous differentiation and cell death in suspension culture. Intermediary microcarrier-based systems are a useful culture format for iPSCs to maintain their attachment requirements. Microcarrier-based systems allow for process scale-up to stirred suspension bioreactors compatible with process analytical technology to enable real-time monitoring. However, adapting to a new microcarrier system from static, 2D culture may require a significant investment in process development time as conditions must be validated for each vessel being considered for inclusion in the scale up strategy for manufacturing.

2) Timeline Considerations
Speed to market is an important factor to consider. In some cell therapy applications, where there is a race to be first to market, companies may opt to keep attachment dependent cell therapies in adherent manufacturing systems rather than spend time transitioning to a suspension-based system. In contrast, if demand for the product is high, companies may choose to spend the time up front to transition to a suspension based system, if they determine that they can achieve higher and/or more cost-effective production by making the switch.

Another important consideration when deciding on a scalable manufacturing strategy is the product lifecycle. How long is the anticipated market relevance and demand for the product? What scale does production need to achieve to meet demand? The investment for a two- versus a ten-year product lifecycle will be very different and factors into capital investments for upgrading space and equipment or investing in a new technology platform. It is also prudent to ensure that the supply chain for critical components can support the product duration and demand to mitigate potential delays.

3) Facility Space and Labor Considerations
The available square footage for equipment can also impact scalability options. Real estate availability in many biotech hubs in North America can be scarce and expensive, making facility space a limiting resource for many cell therapy manufacturers. This can factor heavily into the adoption of certain cell culture platforms. Some culture systems are more compact than others, while others require additional equipment and/or skilled personnel to operate. In some cases, additional staff training will be necessary to implement new production technologies.

4) Budgetary Considerations
Many of the factors in determining the approach to scaling production of a cell therapy are impacted by budgetary constraints. How much capital investment can be put towards scaling processes – space, equipment, personnel, product demand and time available for process development and validation. Their associated cost must be weighed against the potential return before a decision can be made.

Navigating a Successful Tech Transfer

As Corning has worked with many cell therapy companies, we are able to provide an overview of the process of navigating a successful technology transfer. After thoughtful consideration of their current and likely future in-house manufacturing capabilities, many cell therapy developers opt to enlist the help of a contract manufacturing organization (CMO) or contract development and manufacturing organization (CDMO) to meet their production goals and timelines. The need for a GMP-manufactured product to meet regulatory requirements is also a driver to move production to a CMO/CDMO already equipped to operate in this space. This requires a transfer of knowledge from the sponsor company to the contract organization that often includes validation of the GMP process development and manufacturing methods to be used, implementation of a quality control strategy, and collaborating to secure supplier agreement plans and scheduling.

When choosing a CMO/CDMO, the careful selection of a service provider with extensive experience in large-scale manufacturing, knowledge of regulatory requirements and a commitment to collaboration is critical and can ease the technology transfer process. At Corning, our application scientists frequently facilitate the successful technology transfer from customers to their chosen CMO/CDMO. From their experience, they have identified the following key considerations when planning the transition.

1) Know your process and product
It is important to understand the critical quality attributes at small-scale that make your product work. Having analytical assays to characterize attributes like product identity, potency, purity and functionality is critical to the success of the technology transfer

process. These parameters help establish the quality control metrics against which the impacts of changes in the production process can be measured during scaling operations.

Also, early in the process it is important to communicate the projected demand for your product to determine whether the CMO/CDMO has the capacity to meet production requirements in the allotted timeframe. Ultimately, understanding key product quality attributes and timeline to market will facilitate a more informed decision when choosing a CMO/CDMO.

2) Find commonality with your CMO/CDMO

One of the key challenges facing tech transfer is the difference in equipment and operating procedures between the sponsor company and the CMO/CDMO. Problems can arise if the CMO/CDMO selected is unfamiliar with the technology platform used by the customer, which would necessitate an adoption of processes that could cost valuable time and resources. Having some commonality with the CMO/CDMO, whether it is using a consistent cell culture surface (i.e., tissue-culture treated or Corning CellBIND® treated) and/or a consistent cell culture platform can mitigate risk in tech transfer. Having familiar touchpoints between the two sites can also be useful when problems arise and troubleshooting is required. For example, if the seed train from a T-flask to a Corning CellSTACK® worked for your process during development, keep it the same in tech transfer to the CMO/CDMO. Certain platforms, like the CellSTACK provide the opportunity to operate at varying scales depending on the vessel chosen, which can be advantageous.

After all, tech transfer is not a one-way street. It requires a dedicated team, continuous communication and collaboration for success. Enlisting the help of technology providers, like Corning, can provide significant value-add to the relationship between a customer and CMO/CDMO. The Corning team strives to unify and connect the two parties to ensure tech transfer success, such as facilitating training of technicians at both sites to ensure operational and performance consistency. Corning can also help CMOs/CDMOs identify economies between their different culture platforms to enable flexibility, decrease cost and streamline supply chain. As an example, the tubing manifold used in closed system operations with the Corning CELLStack 10-layer vessel are compatible with the Corning HYPERStack® 36-layer vessels, simplifying the number of parts that a manufacturer would need to stock, as well as negating additional staff training to operate the manifold between the two systems.

Ultimately, having a thorough understanding of the cell therapy product and leveraging the expertise of technology providers and contract organizations can streamline the scaling strategy to facilitate the transition from lab- to large-scale manufacture. Cell therapy developers looking to enter manufacturing scale with cell culture product specifications that are difficult to achieve at large scale will face costly delays. Therefore, it is prudent to keep scalability in mind as early as possible in the development pipeline to avoid this risk. The key to increasing patient accessibility to meet market demand for cell therapies will be better, scalable manufacturing processes in conjunction with automation tools that drive down cost and time-to-treatment.

References

1. Nianias A, Themeli M. Induced Pluripotent Stem Cell (iPSC)-Derived Lymphocytes for Adoptive Cell Immunotherapy: Recent Advances and Challenges. Curr Hematol Malig Rep. 2019;14(4):261-268. doi:10.1007/s11899-019-00528-6

Cell Culture DISH Inc.

CORNING

Amplifying Adenoviral Particles in the Corning® HYPERStack® Cell Culture Vessel

Introduction

Adenoviruses and other viral systems are routinely used in research and industrial applications[1,2]. Reports indicated that the use of adenoviruses for the delivery of transgenes is one of the most commonly used tools in both *in vitro* and *in vivo* research[2]. Additionally, adenovirus transductions have been recently used at both the preclinical and clinical stages in cell therapy and vaccine production leading to an increase in demand to produce more virus as efficiently as possible[1,2].

To allow researchers and vaccine manufacturers the opportunity to produce even higher yields in the same spatial footprint as stacked vessels, Corning offers the HYPERStack cell culture vessel. The Corning HYPERStack vessel features Corning's HYPER (**H**igh **Y**ield **PER**formance) technology which consists of a gas permeable film as the attachment surface, eliminating the air headspace requirement in traditional vessel types. This approach provides an increase in the number of layers and corresponding cell growth surface area compared to traditional rigid single or multi-layered culture vessels.

The focus of this study was to determine the efficacy of generating amplified virus using the unique Corning HYPER technology. Standard methodologies utilize traditional stacked vessels for virus generation. Utilizing the HYPERStack vessel, researchers can generate similar titers in a smaller spatial footprint saving both time and space. The results depicted here demonstrate that the experimental approach to generate adenovirus in the HYPERStack vessel led to similar titers but larger yields compared to a standard 2-layer stacked vessel.

Methods and Materials

Cell Culture

HEK-293AD cells (Cell BioLabs Cat. No. AD-100) were maintained in DMEM without sodium pyruvate (Corning Cat. No. 10-017-CM), 10% FBS, and 1X MEM Nonessential Amino Acids (Corning Cat. No. 25-025-Cl). Transduction of HEK-293AD Cells

Cells were seeded onto a Corning CellBIND® surface 2-layer CellSTACK® (Corning Cat. No. 3310) or Corning HYPERStack 12-layer vessel, Corning CellBIND surface treated (Corning Cat. No. 10012) at 45,000 cells/cm^2 (0.217 mL/cm^2) and incubated overnight at 37°C, 5% CO2, 98% relative humidity. The following day, the medium was removed and combined with adenovirus encoding Green Fluorescent Protein (GFP) (Multiplicity of Infection [MOI] 10). The medium was then added back to each vessel. The amount of virus (mL) added to each vessel was calculated using the following formula:

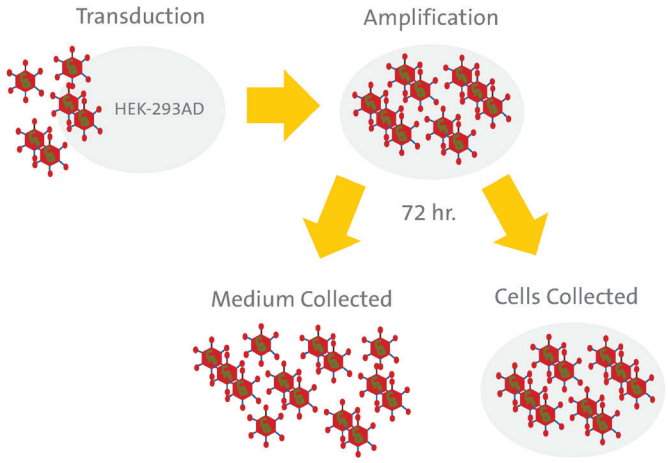
$$\frac{(\text{Cells/cm}^2)\ [\text{cm}^2\ \text{of Well}])\ \times\ (\text{MOI 10 [IFU/Cells]})}{(\text{IFU/mL})}$$

An MOI of 10 was selected to reach the desired cytotoxic effect (<50% cells remained) in 72 hours. The crude adenovirus added to each vessel was prepared as described in Generating Crude Adenoviral Particles in the Corning HYPERFlask Vessel (CLS-AN-213). GFP expression and cell morphology were monitored throughout the course of the experiment using the Olympus IMT-2 inverted fluorescence microscope. For tips on cell visualization in a HYPERStack-12 vessel, see Corning Application Note: Options to Visualize Cells in Corning HYPERStack-12 Vessels (CLS-AN-174).

Adenovirus Harvest

The cells and medium were collected 72 hours post-transduction (Figure 1). To collect the cells from the HYPERStack vessel, PBS (without Ca^{2+} and Mg^{2+}) (Corning Cat. No. 21-040-CM) was added to each vessel (0.033 mL/cm^2) and incubated at 37°C for 3 to 5 minutes. To collect cells from the stacked vessel, 2 to 3 PBS washes were necessary to remove all cells. To minimize volume during freeze-thaw cycles, the cells were pelleted in a centrifuge

Figure 1. Schematic of adenovirus production.



at 500 x g for 10 minutes at 4°C, and the cell pellet was resuspended in 10 mM Tris, pH 8.0, 100 mM NaCl (0.023 mL/cm^2). The medium was retained, aliquoted into 50 mL centrifuge tubes (Corning Cat. No. 430921), and stored at -80°C to be titered later. The total volume of the medium was also recorded. The cell suspension was then subjected to three freeze-thaw cycles (-80°C/37°C), then centrifuged at 3,000 x g for 10 minutes at 4°C to pellet the cell debris and the supernatant containing the adenoviruses released from the cell suspension was collected. The recovered adenovirus encoding GFP was also aliquoted and stored at -80°C.

Adenovirus Titer

The QuickTiter™ Adenovirus titer ELISA kit was purchased from Cell BioLabs (Cat. No. VPK-110). The ELISA assay was performed as described previously (CLS-AN-213) and the signal in the wells was measured utilizing a PerkinElmer EnVision® Multilabel Reader.

Functional Analysis of Adenovirus

MDBK and Vero cells were transduced with adenovirus obtained from both vessels and analyzed via flow cytometry as described previously (CLS-AN-213).

Results

Cell Morphology and GFP Expression

To assess adenoviral production on a 2-layer stacked vessel compared to a Corning® HYPERStack® vessel, HEK-293AD cells were transduced with adenovirus encoding GFP. GFP expression and cell morphology were monitored throughout the course of the experiment. The cells and medium were collected when less than 50% of the cell population remained attached to each vessel. Similar cell morphology and GFP expression were observed in both vessels (Figure 2) at the time of harvest.

Adenoviral Production

The viral particles obtained from either the cells or medium remained in two different fractions throughout the course of the study to (i) minimize processing and (ii) demonstrate the viral yields obtained from both fractions. For large-scale production, the cells may be lysed by either lowering the ionic strength (hypotonic shock) or with the aid of mild pressure changes that can be induced by a microfluidizer® (Microfluidics) or cross-flow filtration system[3]. Once collected, the titer of adenovirus encoding GFP from each vessel (either from cells or medium) was determined using the QuickTiter ELISA kit to quantitate infectious forming units (IFU)/mL. Similar titers were obtained from both vessels (Figure 3). The average titer obtained from the adenoviruses recovered from the cells were 2.4 x 10^9 IFU/mL and 2.2 x 10^9 IFU/mL from Corning® HYPERStack® and stacked vessels, respectively (Figure 3A). The average titer obtained from the medium was 6.3 x 10^8 IFU/mL and 5.3 x 10^8 IFU/mL from HYPERStack and stacked vessels, respectively (Figure 3A). When normalized based on surface area, similar IFU/cm^2 were observed with both vessels (Figures 3B and 3D). However, since the HYPERStack-12 vessel has a larger surface area compared to a 2-layer stacked vessel there was a significant increase in total viral yield (>5 times) (Figures 3C and 3E). These results indicate that adenovirus particles may be generated in the HYPERStack vessel with similar titers but larger yields compared to the standard 2-layer stacked vessel.

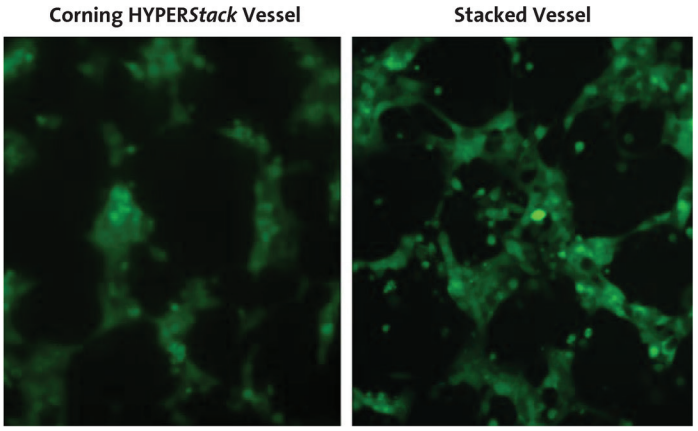


Figure 2. Similar cell morphology and GFP expression was observed between vessels.
Representative images from the same experiment demonstrating morphology/GFP expression on the day of harvest of the HEK-293AD cells. Cells and medium were collected 72 hours post-transduction. Images obtained using an Olympus IMT-2 inverted fluorescence microscope. Magnification 100X.

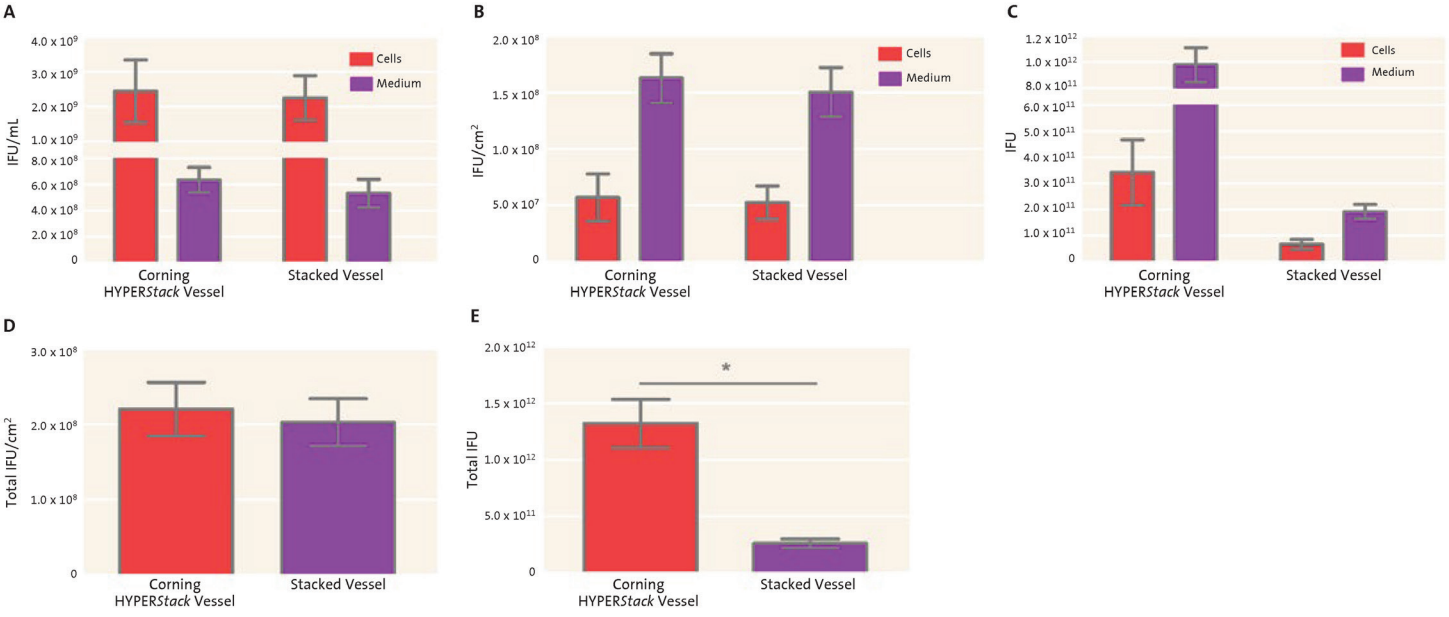


Figure 3. The Corning HYPERStack vessel leads to equivalent viral production per cm² compared to a stacked vessel.
(A) Direct comparison between the HYPERStack vessel and stacked vessel titers obtained using the QuickTiter ELISA Adeno kit. (B, D) When normalized on a per cm^2 basis, the HYPERStack yielded similar infectious adenoviral particles. (C, E) The HYPERStack vessel generated a significantly higher amount of total infectious adenoviral particles. (D, E) Total infectious adenoviral particles were calculated based on titers and the volume of each fraction (cells and medium) Paired t-test, * p <0.05, N=3.

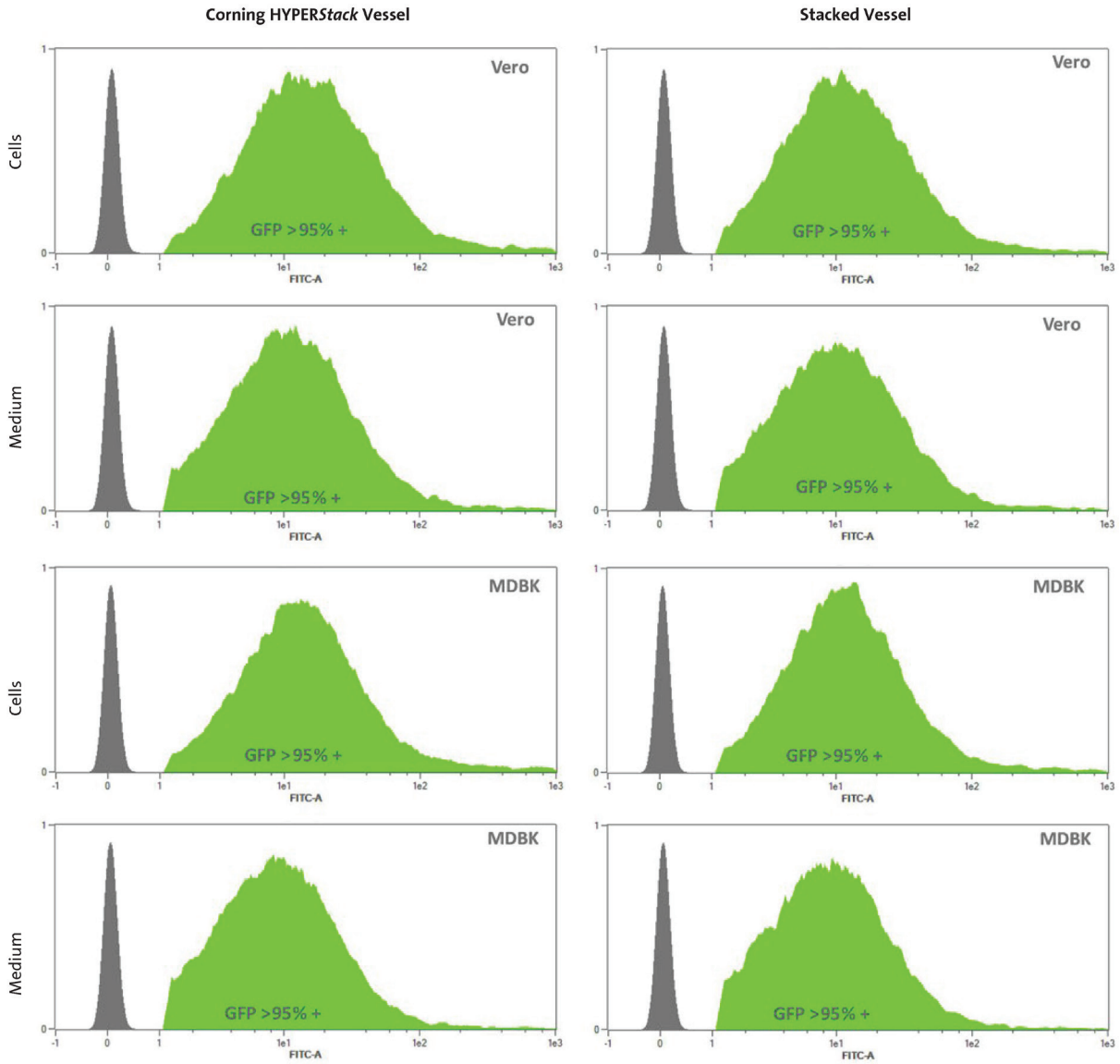


Figure 4. Vero and MDBK cells transduced with adenovirus exhibit similar GFP expression levels.
Representative flow cytometry data shows the expression of GFP (green) compared to a negative control of non-transduced cells (black). After three independent experiments, the GFP expression in both the Vero or MDBK cells was greater than 95% regardless of which vessel or fraction the virus was generated in.

GFP Expression in Vero and MDBK Cells

To verify that the virus obtained from the HYPERStack vessel was as functional as virus obtained from the 2-layer stacked vessel, Vero and MDBK cells were transduced with amplified adenovirus encoding GFP. Each cell type was transduced with virus obtained from either the HYPERStack or stacked vessel at MOI of 100. After 72 hours, the cells were collected and analyzed via flow cytometry. After three independent experiments, the average GFP fluorescence in each cell line with each adenovirus was greater than 95% (Figure 4). Cells were transduced at MOI of 100 to ensure high expression. Previous results also demonstrated equal GFP expression regardless of the vessel when transduced at lower MOIs (10, 50, and 100) for shorter time periods (24 and 48 hours). GFP expression from each experiment varied between 30% to 95% depending on a) MOI and b) time (data not shown).

These data indicate that the virus obtained from the Corning® HYPERStack®-12 vessel is as infectious as virus obtained from a 2-layer stacked vessel.

Summary

- This study demonstrates the utility of the HYPER technology in adenovirus production.
- Adenoviral particles can be amplified in the Corning HYPERStack vessel at similar titers compared to traditional tissue culture vessels, allowing for greater virus production in a smaller footprint.
- Adenoviral particles generated on the HYPER technology platforms also exhibit similar levels of infectivity as in a traditional vessel.

References

1. Hansson, M. Nygren, P-A. and Stahl, S., Biotechnol Appl Biochem 2000. 32:95-107.
2. Silva, A.C., Peixoto, C., Lucas, T., Kuppers, C., Cruz, P.E., Alves, P.M., and Kochanek, S. Current Gene Therapy. 2010. 10:437-455.
3. Segura, M. M., Kamen, A. A., and Garnier, A. Methods in Molecular Biology. 2011. 737:89-115.

Closed and Aseptic Processes for Cell Therapy Manufacturing

Over the past four years, the landscape of medical treatments has expanded beyond small compounds to include therapies composed of or derived from human cells. With the success of cellular therapies for blood cancers, the cell therapy market has expanded rapidly, with the potential to provide life-changing therapies for more widespread indications. Cell therapy manufacturers must now meet the unique challenge of producing biologically complex therapies in a robust yet cost-effective manner, all while preventing potential contamination by other biological agents. Although the development of these therapies for small patient populations happens at research laboratory scale using manual, open processes, the demands of the market require novel manufacturing processes that are consistent, robust, and scalable. Central to such processes are the use of closed-system and single-use technologies.

In traditional cell-based production systems, open process steps are common and can be compatible in a ”one-product, one-facility” format. However, autologous, patient-specific cell therapies require numerous small, individualized batches that must be protected from external microbial contamination, as well as cross-contamination from other patients. Implementation of closed systems allows parallel manufacturing of patient-specific therapies while enabling manufacturers to work efficiently and reducing chances of contamination and production loss.

In this article, we identify key strategies to help researchers successfully transition their cell therapies from the lab to commercial production. We detail the advantages of implementing closed systems as well as some of the key hurdles when transitioning from traditional bioprocesses.

There is no ”one-size-fits-all” in the design of a closed system process; A successful process will take into consideration the diversity of cellular products as well as facility layouts and capabilities.

What is a Closed System?

By definition, a closed system is one in which the manufacture of a product is isolated from both the surrounding environment and operators[1]. If gases or other reagents need to be added to the closed system, they must be pre-sterilized using validated terminal or filter sterilization methods and the addition must be done in a way that does not expose the product to an uncontrolled environment.

A truly closed system with no environmental exposure eliminates the risk of contaminants, but the nature of certain process steps in cell therapy production may not currently be achievable in a closed system manner. As such, a “functionally closed system” allows users to perform unit operations in a realistic and logical but safe manner. In a functionally closed system, any open events, such as media exchange, are done in a controlled space such as a biological safety cabinet, and materials are handled in a manner to avoid contamination from the environment, operators, or other reagents.

Closed Systems for Cell Therapy

In the paradigm of cell therapies where “the process is the product”, an efficient process is also a safe one. Consistent production schemes avoid batch failure and prevent costly investigations as well as recertification of the working space and extensive environmental testing. The implementation of closed systems can help developers achieve efficiency, and there are many technologies available to create a system compatible with a cell therapy process.

Why is it important?
A closed system, by design, provides physical barriers to protect the operator when working with infectious agents and conversely, to protect the work from the risk of microbial contamination or cross-contamination from other patient samples. Particularly for large-scale manufacturing, closed systems mitigate the risk and cost associated with the failure or loss of batches as a result of contamination but convey the additional benefit of allowing flexibility in the manufacturing space. Enclosing a process eliminates the outside environment, which lessens the need for mechanisms that control that environment, such as dedicated clean rooms and biological safety cabinets (BSCs). Removing the requirement of cleanrooms permits a reduced facility footprint and better utilization of space, which all result in cost savings.

Closed system technologies can also be incorporated with automation platforms, that not only improve product consistency by reducing operator variability, but also improve manufacturing efficiency and can reduce the vein-to-vein time critical in cell therapies.

How to Achieve a Closed System?
The implementation of closed systems provides common benefits in process efficiency and safety, but there is no ”one-size-fits-all” in the design of a closed system process; a successful process will take into consideration the diversity of cellular products as well as facility layouts and capabilities; as such, processes are highly customized and will require development. The design of any closed system starts with a detailed analysis of the current process to identify key unit operations that are suitable for closure. For example, if there is a step in the manufacturing process that requires introducing materials to the product, sterile tube welding or aseptic connectors can be used to replace spike port needles or open pouring in a BSC.

Another consideration in closed system design is the balance between risk and cost. Within a process, certain steps may bear lower contamination risk than others, or mechanisms to close that step may not yet be practical, either due to budgetary or facility constraints. While the ultimate goal is to have a fully integrated and complete closed system, designing such a process can be

time consuming and financially costly. Therefore, an assessment can determine the risk level for unit operations where, if the contamination risk is low, closing that specific process step may not be necessary. As such, system closure can be achieved in a stepwise manner that prioritizes high-risk unit operations.

Once system closure has been designed and built out, it is necessary to validate it to ensure both its readiness and ability to maintain an aseptic environment. There are many resources available to help validate a closed system, but it can be valuable to leverage the expertise of your vendor(s). There are three criteria that define the readiness of a closed system: bioburden level, cleanliness level, and degree of environmental segregation or integrity[2]. Satisfying these criteria can be done with direct and indirect measures. For example, a direct way to test the integrity of the closed system is to undergo bioburden testing, which is accomplished by running bacterial growth media like TSB (tryptic soy broth) through the closed system and then culturing the media to detect any microbial growth. Indirect measures such as verification of sanitization records (correct time, temperature and concentration) also help ensure the integrity of a closed system[2]. Additionally, vendors of closed system components have quality systems in place consisting of appropriate validation studies and compliance documentation that can provide further evidence that the systems and materials utilized are fit for use.

A final piece in the implementation of a closed system is the personnel within a facility. Adequate staff training in the transition to closed system operating technologies should ensure that operators are comfortable with the new equipment and procedures.

When to Consider a Closed System?

Within the life cycle of a cell therapy product, successes in research and development become discussions of process development, and concerns shift to scale-up or possible outsourcing to a contract manufacturer. This transition presents an opportunity to initiate closed system design and is a common point for Corning team members to interact with both developers and their chosen manufacturing partners. The natural consideration of scale in a process development discussion is also a key aspect of closed system design. Your chosen components and operations should be scalable across the phases of manufacturing, and flexible enough to allow a process to run in diverse types of manufacturing spaces.

Summary

As demand for cell therapy products increases, viable cell therapy manufacturing processes must be scalable, consistent, and cost-effective. Implementation of a closed system in cell therapy manufacturing requires an initial investment but provides both short and long-term benefits. From batch to batch, closed systems lead to more robust and consistent manufacturing that helps manufacturers avoid the significant costs incurred by batch failures. In a larger context, closed systems enable manufacturers to work in more flexible environments that reduce capital and operational costs. Ultimately, closed system processes in cell therapy manufacturing provide a means for improving patient access to life-changing therapies.

References

1. Johnson J, Probst S, Palberg T, Gil P, Kennedy M, Rogalewicz J, Green K, Chalk S. Challenging the Cleanroom Paradigm for Biopharmaceutical Manufacturing of Bulk Drug Substances. BioPharm International. Aug. 01, 2011.
2. Estape D, Walker AL, Orichowskyj ST, Vega H, J. Pratt DJ. Proof of Closure: Life Cycle of Closed Systems. Pharmaceutical Engineering. Special Reports September/October 2017.

Cell Culture DISH Inc.

CORNING