Exploring What’s Possible With Stem Cell Therapies

Overcome the Pitfalls, Realize the Promise With Cell Culture Innovations

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Over the past several years, stem cell research has experienced a significant upswing, evidenced by not only activity in the lab and clinic but also investments industrywide.

The biotech boom is on track through 2022 and beyond, spurred in large part by in vitro stem cell research driving new precision approaches in tissue engineering, cancer immunotherapies, neurological and endocrine diseases, and much more.

But amid the surge of activity and promise surrounding applications in mesenchymal stem cells, induced pluripotent stem cells and emerging acellular therapies, such as extracellular vesicles, challenges remain in making these advanced therapies production-ready for clinical trials and, ultimately, market entry.

From difficulties in efficiently optimizing cellular expansion and downstream processing to the ongoing need to prioritize cell quality in tandem with cell quantity, these barriers have precluded many bench projects from achieving the milestones necessary for commercial success.

As one scientist told Nature in fall 2021, it can seem like stem cell researchers are around-the-clock babysitters — as if keeping cultures alive and thriving is a full-time effort.[1]

But new technologies, culturing platforms and materials have made the costs, responsibilities and pressures less cumbersome for lab scientists looking to realize the promise of stem cell therapies while overcoming the many pitfalls.

In this playbook, we've asked Corning Life Sciences experts to explore the changing landscape of stem cell research and cell therapy production and how emerging solutions have facilitated a new era of more effective and efficient workflows.

Corning® CellSTACK® Culture Chambers are a scalable culture vessel system that may be used to culture larger quantities of adherent cells, with a growth surface area ranging from 636 cm2 to 25,440 cm^2.

[1] Bender, E. Stem-cell start-ups seek to crack the mass-production problem. Nature. 2021;597(7878):S20-S21. doi:10.1038/d41586-021-02627-y

Stem Cell Applications and Therapies: An Overview

Healthy bodies can regenerate human tissue, but many diseases and injuries can stop that natural process from occurring. However, if this happens, stem cell therapies can help the body fight cancer and repair and regenerate human tissue. But because different cell types behave uniquely depending on the setting, scientists' approach to these therapies will vary widely.

Very often, that approach is customized to match the unique characteristics of the cell type, such as pluripotent cells (which have a wide range of differentiation possibilities) or multipotent or unipotent adult stem cells (which don't have the same differentiation potential of pluripotent stem cells but may be more readily available). Some technologies, such as induced pluripotent stem cells, give scientists the best of both worlds by reprogramming adult stem cells to act like embryonic stem cells.

"You have this continuum with pluripotent cells on one end and more differentiated cells on the other end that are already well-defined and can turn into just a few cell types," said Tom Bongiorno, Ph.D., field application scientist at Corning Life Sciences. "There's a delicate balance where you want to match the right cell type to your therapeutic application, but you might not want fully differentiated cells because they won't replicate as quickly."

That continuum packs a lot of promise into stem cells and their applications in modern medicine, but it also creates a great deal of complexity. After all, these are living organisms. The cells themselves are the therapy, and you could need billions of them for one dose.

High volumes require a scale-up strategy using either 2D adherent culturing or, a recent innovation, 3D techniques. Picking such a system means sifting through many choices, from cultureware and substrates to scale-up platforms optimized for the cells' unique characteristics.

Along with these scale-up choices are operational concerns, such as labor or equipment costs, as well as time to market.

But without a lot of industry guidance on how to efficiently culture these complex cells to get more of them without affecting quality, labs are often left to navigate these decisions on their own. Here, our experts walk through some considerations and best practices.

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Factors That Can Influence Cell Quality

Cell quality is the holy grail of stem cell therapy. You may have billions of these cells, but if quality gets lost along the way, then all that work, time and money could go to waste.

"In cell therapy, cells are part of the actual product, so the fact that cells are healthy and performing as they should is integral," said Hilary Sherman, senior applications scientist at Corning Life Sciences. "If the cell isn't behaving as it should, the therapy could be less effective, or maybe even dangerous if you have a situation where cells are differentiating into something they shouldn't."

Substrates

Several factors throughout the scale-up seed train can influence cell quality. One is the substrate, where anchorage-dependent cells attach and expand. Some substrates are better for certain applications than others, after factoring in different considerations such as synthetic or biological materials. Teams that carefully and cautiously choose their surfaces will be best set up for a successful and timely scale-up.

"Substrate choices can depend on your ultimate needs," Sherman said. "You might not want the added variability of a biological component, or maybe you do because you're trying to replicate something that's happening in the body within an in vitro environment."

Substrates can also affect cell behavior. For example, one study found that when mesenchymal stem cells were cultured on different substrates, the cells differentiated into different cell types based on the material's stiffness.[2]

It can come down to a cell's sensitivities, according to Alejandro Montoya, senior product line manager at Corning Life Sciences.

"We've seen that adult mesenchymal cells are comparatively easier to grow because they have less sensitivity to not having an extracellular matrix (ECM), while more complex cells, like induced pluripotent or embryonic stem cells, may need the signaling and structure of an ECM, such as Corning® Matrigel® matrix or a more defined biological relevant substrate such as Corning rLaminin-521," Montoya said. "So much can be optimized according to these unique cell types."

[2] Engler, A.J.; Sen, S.; Sweeney, H.L.; and Discher, D.E. Matrix Elasticity Directs Stem Cell Lineage Specification. Cell. 2006;126(4):677-689. doi:10.1016/j.cell.2006.06.044

Vessel Type

Another factor to optimize is the vessel itself. Typically, most labs start with a small-scale option, such as a T-flask, before moving up to options that provide more surface area without taking up a ton of space, such as multilayer flasks and stacked solutions. The Corning® HYPERFlask®, for example, expands the attachment area from a standard flask's 175 square centimeters to 1,720 square centimeters while providing the gas exchange, footprint and operator responsibilities of a single flask.[3]

Closed-system tubing manifolds can also expand stacked systems, such as Corning HYPERStack® or CellSTACK cell culture vessels, for more scale as needed. For maximum output, microcarrier options also provide an adherent surface within high-volume bioreactors. Each of these diverse systems emphasizes the opportunities in using more efficient vessels to generate more cells without sacrificing quality and cost.

"You want to develop a very efficient seed train that can yield as many cells as you can while replicating them as few times as you can," Bongiorno said. "That's because one of the big challenges with some stem cells, such as mesenchymal stem cells, is that after you isolate them, they can only replicate a limited number of times in vitro."

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[3] A Flask for Any Task. Accessed Jan. 6, 2022.
https://www.corning.com/catalog/cls/documents/infographics/CLS-CC-114.pdf

Allogenic Cell Therapeutics: Successfully Creating Master Cell Banks, Working Cell Banks, And Drug Product.

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Microenvironment

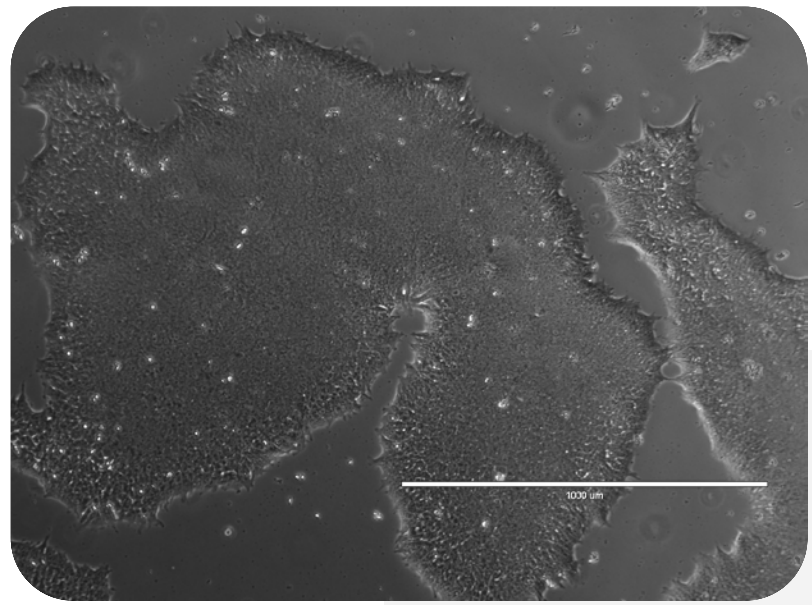
New techniques for stem cell culture, such as generating spheroids, organoids, extracellular vesicles (EVs) and inroads into organ regeneration are underscoring the need for more advanced microenvironments that provide the molecular signaling and scaffolding needed to expand stem cells within 3D, more vivo-like conditions.

When building the right microenvironment, one high-stakes consideration is not just the vessels, microplates and substrates that home these 3D structures, such as spheroids, but also the hydrogels, extracellular matrices (ECMs), and specialized surface treatments and coatings that keep them intact.

"Many hydrogels have some degree of lot-to-lot variability, so using specialized products that are screened and made for stem cells is critical," Bongiorno said. "It may be a minor change, but it can have a big impact on stem cell quality."

Such products may include those that are hESC-qualified or optimized exclusively for organoid cultures.

"Corning Matrigel matrix versions are optimized and qualified for these specific applications, minimizing the lot-to-lot variability," Montoya said. "Matrigel matrix has been an integral part of feeder-free stem cell culture and just as important if not more in the origin, pioneering and continuous development of organoid research."

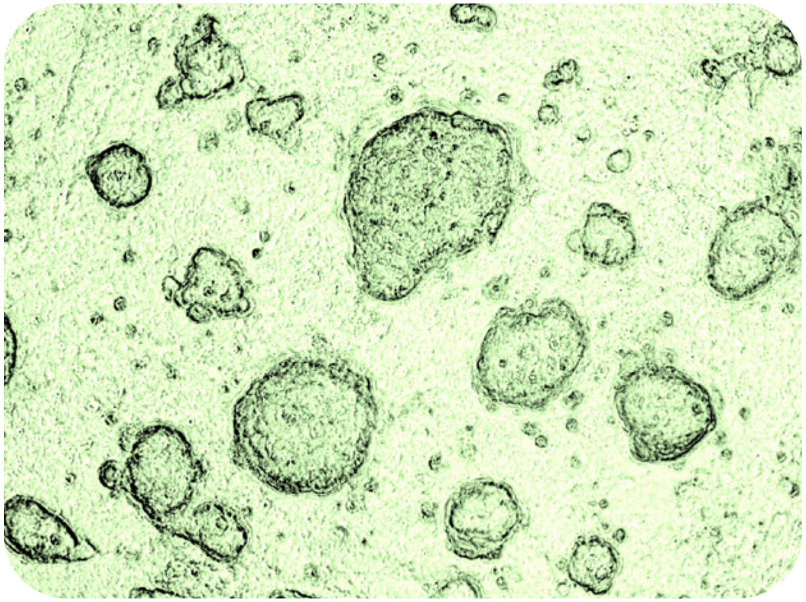


Human iPSCs cultured on hESC qualified Corning® Matrigel® matrix.

Beyond the vessels and the specialized media and reagents, it is critical that the correct stimuli are activated at the right time with the right intensity. These stimuli can be achieved with appropriate geometry and fluid dynamics, whether that means static, dynamic or perfusion media conditions. With emerging fields such as EV production and acellular therapies — and the opportunities in creating EVs in MSCs and other stem cells4 — it is imperative that as we transition from 2D cultures to 3D models, we accurately represent what is happening in whole tissues and organs. The end goal for 3D models is to recapitulate nature. Continuing to build knowledge of and applying these critical stimuli will be essential to drive quality cultures for the production of desired acellular products, such as EVs, to achieve high quantity without sacrificing quality.

Microenvironments can also be governed by simple principles, such as geometry and surface chemistry. Microwells or cavities that have hydrophilic, nonstick coatings can facilitate the formation of 3D cell cultures without scaffolding or hydrogels. By having no localized points of attachment, adherent cells in a single-cell suspension will aggregate and self-assemble naturally in spheroids. The geometry of the cell culture substrate or surface will govern size and potentially shape of the resulting spheroids as well. Simplicity can be the key for developing 3D culture models that have no impediments to the culture environment that a scaffold or hydrogel could impose, permitting optimal exchange of nutrients, gases and other critical needs.

[4] Teng, F., and Fussenegger, M. Shedding Light on Extracellular Vesicle Biogenesis and Bioengineering. Advanced Science. 2020;8(1):2003505. doi:10.1002/advs.202003505



Extracellular vesicles have shown great promise in early-stage clinical trials, expanding momentum and interest in the transition from 2D to 3D cell culturing.

Listen to this podcast to learn more

Breaking Through Expansion Barriers With Scale-Up Strategies

Navigating the many factors of stem cell expansion calls for a scale-up strategy that addresses these cells' diverse and complex needs — for example, the need to simultaneously harvest billions of attachment-dependent cells while preempting issues with quality, senescence or variability. Here are a few factors to consider:

Seed Train Compatibility

Keeping the various parts of the seed train consistent and compatible is essential for an efficient scale-up strategy. For example, a surface treatment you might use for a smaller vessel like a single-layer flask would ideally stay consistent as production expands. This upstream effort can save downstream time and costs.

"You do need to carefully consider how different cell types will respond to different variables, including surface treatment as you're moving from one platform to the other," Bongiorno said. "Sometimes that can require a bit of testing at small scale to make sure that the cells are compatible with the surface you eventually want to use later on."

Mitigating Contamination and Toxicity Risk

As yield expands across the seed train, beware the threat of contamination and toxicity risk — both from external contaminants as well as internal substances such as cryoprotectants.

To mitigate these risks and their associated effects, including cost and time, scientists are increasingly turning to closed-system platforms, such as Corning® HYPERStack® vessels that have tubing and connectors pre-attached to ensure a sterilefluid path. Such systems preempt opportunities for contamination by limiting how often or long platforms are exposed to the environment.

With regard to cryoprotectants, such as DMSO, advanced closed-system washing platforms, such as the Corning® X-WASH® system, are another important part of the efficient scale-up workflow.[5]

Learn how to reduce DMSO when working with cryopreserved mesenchymal stem cells with Corning's free application note.

Get the Note

[5] X-WASH System. Corning.com. Published 2022. Accessed Jan. 6, 2022. https://www.corning.com/worldwide/en/products/life-sciences/products/bioprocess/x-series-cell-separation-platform/x-wash.html

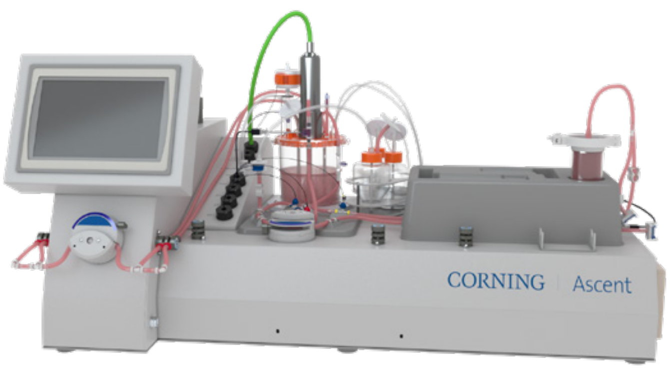
Automation

As scale increases, automation can significantly speed things along so that labs — even smaller startups — can do more with fewer resources. Automated manipulators with stack compatibility as well as adherent-supportive platforms, such as the Corning® Ascent™ FBR system[6], are among those options.

"When you have a lot of vessels, you need a lot of different operators to work with them," Bongiorno said. "And that brings in some challenges of time, but also operator-to-operator variability. Bringing more automated solutions into your workflow allows you to accelerate the scale-up strategy and reduce labor costs and variability, while also getting much better repeatability."

Learn more about how to accelerate adherent scale up to produce high cell yields more efficiently.

Watch the Video



[6] AscentTM FBR System. Corning.com. Published 2022. Accessed Jan. 6, 2022.
https://www.corning.com/worldwide/en/products/life-sciences/products/bioprocess/ascent-fbr-system.html

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Work With a Trusted Scientific Partner

In the complex world of cell and gene therapy, having a seasoned partner to guide you through uncharted production territory is critical to success. Technical teams from scientific suppliers, like Corning Life Sciences, can help you:

- Improve manufacturing efficiency by manifolding multiple stack vessels in compact designs.
- Streamline liquid manipulations through customized closed systems.
- Increase stem cell yields for clinical applications by optimizing culture conditions.

Making the Most of Stem Cell Innovations

Stem cells offer much promise, and yet researchers are only scratching the surface of their potential. With applications across therapeutic approaches and disease types, the need to expand research cultures to production-ready scale — cost-effectively and quickly — is an ongoing challenge for life science companies.

As your team explores the path from the research bench to upstream bioproduction for your program, consider scale-up strategies that account for the complexity of these cell types. Even small choices, such as the substrate or vessel, can have an important impact. More broadly, look for consistency and compatibility in your scale-up approach, as well as opportunities to mitigate contamination risk and automate where appropriate.

Learn how you can outfit your stem cell research program for production-ready success.

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Corning Life Sciences is a global, leading manufacturer of lab tools for growing cells, bioprocess manufacturing, liquid handling, benchtop equipment, among other solutions for life sciences. Corning strives to improve efficiencies and develop innovations that enable scientists and therapy manufacturers to harness the power of cells to create breakthrough discoveries in areas like gene, stem cell, immune therapies, as well as vaccine development. Visit www.corning.com/lifesciences to learn more.

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