WHITEPAPER

3D Culture and Assay Systems

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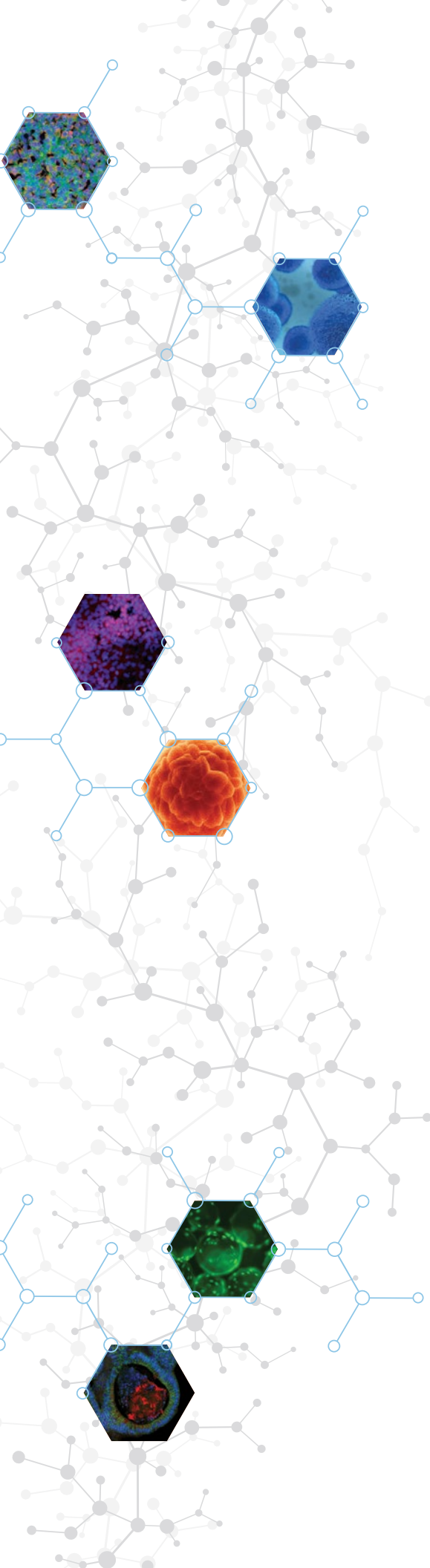

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For decades, mammalian cell culture meant growing monolayers of cells in a dish. While these 2D cell culture models have provided remarkable insight into the function and dysfunction of a stunning array of cell types, the limitations of 2D cell culture have become obvious. In living organisms, cells interact with a 3D environment that includes other cells and cell types, the extracellular matrix (ECM), and a complex cellular microenvironment. These 3D interactions are essential to understanding the function of healthy organs and the dysfunction of disease states. Our past reliance on 2D cell culture and animal models hindered our ability to understand complex human disorders and find effective treatments.

In response, researchers have developed an impressive array of 3D cell culture models that build upon advances in stem cell biology. These 3D cell culture models fall into three main categories: spheroids, organoids, and bioprinted tissues. The protocols for these models vary based on the category. All of these models recreate aspects of *in vivo* tissues in an *in vitro* culture but have significant differences that make them suitable for different applications.

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Spheroids

Spheroids are a simple, inexpensive way to model cells in 3D. They form due to adherent cells' tendency to stick together and don't require an ECM or other scaffolding. A wide range of cell types can be used to generate spheroids, including tumor tissue, embryoid bodies, hepatocytes, nervous tissue, and mammary glands. Spheroids may be formed from one or multiple cell types and may or may not exhibit polarity. While spheroids can't self-organize or regenerate like organoids, they're an easy-to-use 3D model with the power to mimic *in vivo* tissue and organ conditions.

Most notably, tumor spheroids can closely mimic the *in vivo* tumor microenvironment, with cells in the center of the spheroid having less access to oxygen and nutrients than cells on the surface of the tumor spheroid. This has helped researchers develop a better understanding of cancer biology, more accurately predict drug efficacy in cancer research, and study the cytotoxic effects of CAR-T cells.

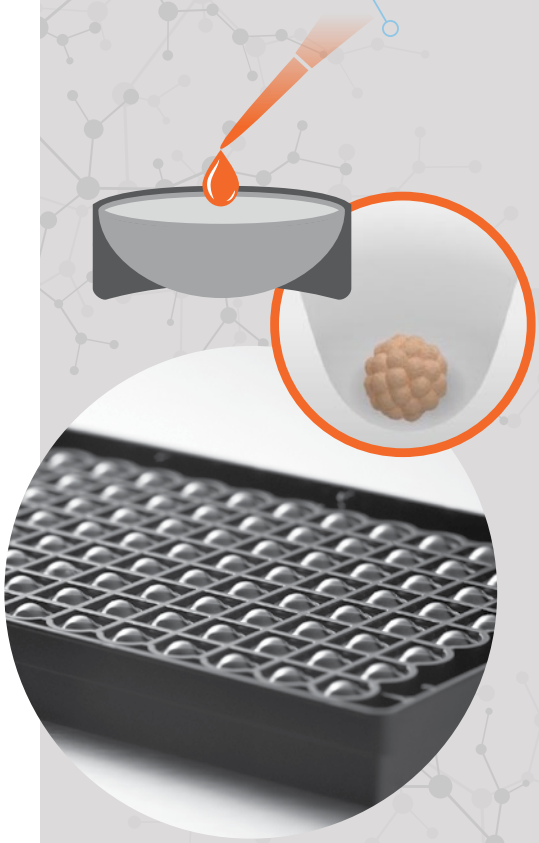
Spheroids can also be used in stem cell research to develop embryoid bodies from induced pluripotent stem cells (iPSCs), which can then be turned into many cell types such as high-purity neural stem cells useful in studying neural diseases and related treatments.

Growing Spheroids

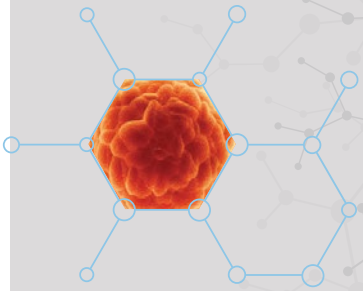
Spheroids are typically created in a scaffold-free environment although newer technologies that use scaffolds/ECMs to generate spheroids are now available. Methods for growing spheroids include employing the hanging drop method, using an attachment-resistant cell culture surface, and/or placing cells in suspension cultures without the aid of physical supports or using a hydrogel that promotes cell aggregation to generate spheroids.

The hanging drop method involves depositing drops of a single-cell suspension onto the underside of a tissue culture lid, then inverting the lid over a hydration chamber. Aggregates typically form within 24 hours and are then transferred to a shaker flask so that spheroids may form in suspension culture for about 48 hours.

Employing an attachment-resistant cell culture surface can simplify the process, improve consistency, and facilitate scale-up. The Corning® Ultra-Low Attachment (ULA) surface is an animal-free, covalently bound hydrogel that effectively inhibits cellular attachment. The ULA surface promotes the formation and easy harvesting of scaffold-free spheroids. A wide variety of vessels are available with the ULA surface, including traditional culture dishes, T-flasks, and microplates.



Spheroid Microplates



Spheroid microplates promote the growth of one spheroid per well in a scaffold-free environment, and are available in 96-, 384-, and 1536-well formats. The plates are designed for optical clarity and are compatible with brightfield and fluorescent microscopy. The opaque side walls reduce well-to-well cross-talk and background fluorescence and luminescence. The ability to image directly in the microplate eliminates the need to transfer delicate spheroids, reducing damage and ultimately improving results. Corning® Elplasia® plates use microcavity technology to further simplify the process. Each U-shaped microcavity is designed to hold a single spheroid as it's generated, cultured, and analyzed. This allows hundreds to thousands of spheroids to be cultured in each well of a microplate for up to 21 days or more. The uniform conditions and easy-to-use "plug and play" protocol promotes producing spheroids of similar shape and size, which improves consistency.

Elplasia open well plates are available in 6-, 24-, 96-, and 384-well formats, with ULA-coated round wells or plasma-treated square wells for self-coating. The square well plates are ideal for clonal selection and high-magnification imaging of tiny clusters. To maximize production, the Elplasia 12K flask enables approximately 12,000 spheroids to form in a vessel footprint similar to that of a T-75 flask. The Elplasia 12K flask's internal diverter feature allows for minimal disruption of spheroids during liquid handling steps, without compromising full recovery at harvest time.

This technology is compatible with many tumor, normal, and primary cell types often used for 3D culture, and it's been successfully employed for drug screening, cancer biology, stem cell biology, cell therapy research, and 3D tissue engineering.

Corning Disposable Spinner Flasks can be used to culture a large number of spheroids in suspension. Advantages include improved nutrient access and gas exchange from all sides of each spheroid. In addition, it's possible to grow larger 3D structures because the size constraints of a culture plate are removed.

Synthetic hydrogels can provide even more flexibility. Innovative Corning Synthegel® 3D matrix is a defined, self-healing synthetic hydrogel that supports the culture of human induced pluripotent stem cells (hiPSCs) in a 3D embedded format or in an encapsulation for suspension format. The Synthegel Spheroid matrix kit provides high gel strength to support high content 3D self-assembled spheroid cultures on a traditional culture surface. The Synthegel hiPSC Suspension matrix kit supports large-scale manufacturing of physiological 3D hiPSC spheroids in suspension culture. These kits allow for reproducible results and work with standard methodologies.

Corning Elplasia Plates

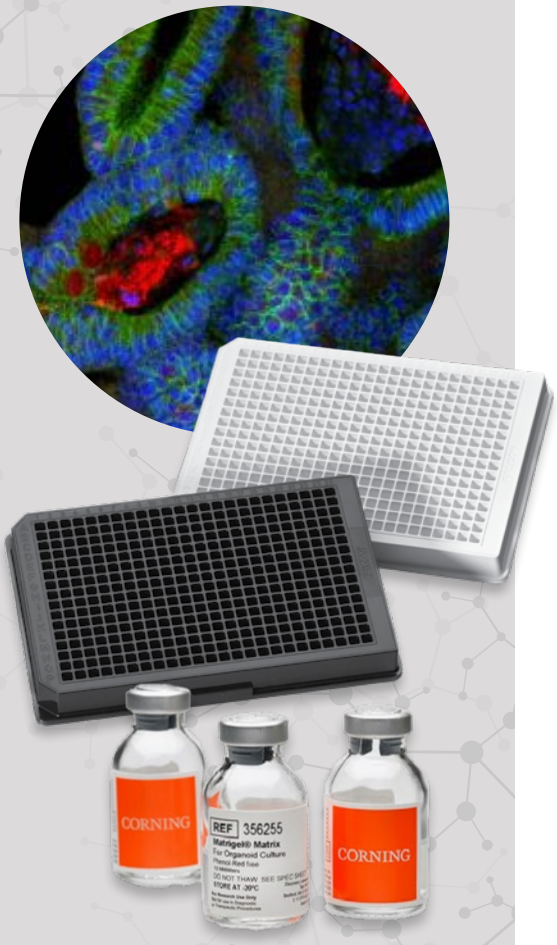
Corning Elplasia 12K Flask

Corning Synthegel® 3D Matrix

Organoids

An organoid is a complex mixture of organ-specific cells derived from stem cells or progenitor cells. When provided with the appropriate culture conditions and an ECM for scaffolding, the cells grow and self-assemble into a tiny 3D version of the appropriate organ. Researchers have succeeded in generating organoids for the brain, breast, colon, liver, pancreas, stomach, esophagus, small intestine, ovary, uterus, fallopian tubes, prostate, retina, and more. By more faithfully replicating organs' structure and physiology, organoids overcome many limitations of conventional 2D cultures and even live-animal disease models. They're a popular choice for disease modeling, cancer research, and drug screening.

Organoids can be propagated, expanded, and frozen. When combined with the ability to collect or generate stem cells from individual patients and the power of CRISPR-Cas9 genome editing technology, the possibilities for organoids are immense. For example, lung organoids are used to study infectious respiratory diseases, including COVID-19, and lung disorders such as cystic fibrosis and asthma. Brain organoids are being used to improve glioblastoma treatment and better understand neurodegenerative diseases including Alzheimer's disease and Parkinson's disease. Patient-derived pancreatic tumor organoids are being used to test potential cancer therapies.



Matrigel matrix-3D plates and Corning Matrigel matrix for organoid culture

Growing Organoids

Organoids may be generated from pluripotent stem cells (PSCs) or tissue-specific adult stem cells (ASCs). Depending on the process, organoids may be produced in as little as 6 days or may require several weeks of culture. Unlike spheroids, organoids typically require a scaffold to form. This is often an ECM that can be used to encapsulate cells and supply biochemical cues and structural support that aid in self-organization. Some spheroid applications also benefit from the addition of an ECM.

Corning® Matrigel® matrix is a natural hydrogel that has been used extensively to culture 3D organoid models due to its ability to mimic an *in vivo* environment and provide natural growth factors, proteins, and the required matrix architecture. To simplify the process, the formulation of Matrigel matrix for organoid culture has been optimized to support organoid growth and differentiation. To facilitate high throughput applications, pre-coated Matrigel matrix-3D plates are available in ready-to-use 96- and 384-well formats. Organoids can be grown and assayed directly in the plate. Other natural hydrogels used for organoid culture are Collagen and Laminin.

Methods used for organoid models include the dome method and bioreactor, permeable support, and low-adhesion microplate culturing. For the dome method, cells are suspended within an ECM such as Matrigel matrix, and the mixture is dispensed as droplets in a microplate. Once the matrix has gelled, the self-contained domes are covered in a growth medium. This method is often used to generate organoids from ASCs.

Bioreactor culturing is often used to expand iPSC-derived organoids for high throughput applications because using a spinner flask or bioreactor improves the availability of oxygen and nutrients and accelerates cell proliferation.

A variety of methods have been developed to support organoid culture in suspension. One approach is to add ECM to the growth media in a very dilute form. Using 5% ECM causes small pieces of ECM to polymerize in the culture media. Organoids can attach to these small pieces to grow in suspension culture. Another option is to generate individual droplets of ECM seeded with organoid cells. This is achieved by mixing organoid cells with unpolymerized ECM on ice, then injecting small volumes directly into warm media that promotes ECM polymerization.

Tissue Models

Permeable support culturing uses porous scaffolds such as Transwell® or Falcon® permeable supports, which are designed to be inserted into a 100 mm dish or 6-, 12-, 24-, or 96-well microplates. These porous scaffolds allow cells to be bathed apically and basolaterally to provide the optimal structure and conditions for cell differentiation. For example, one side of the culture can be exposed to a liquid medium, while the other side of the culture is exposed to air. This is ideal for studying respiratory tract epithelial cells, which interact with both liquid and air. These complex 3D models are used to study respiratory infections including COVID-19. Transwell and Falcon permeable supports are available in various pore sizes to help create a cell culture environment that mimics the desired *in vivo* environment.

Low-adhesion microplate culturing can employ the ULA surface to create 3D structures that can then be embedded in an ECM to generate organoids. This low-attachment mechanism, combined with the unique round bottom shape of the spheroid and Elplasia microplates, allows scientists to culture one organoid per well or cavity of the microplate.

Other applications may benefit from Corning BioCoat® cultureware, which provides highly consistent and biologically functional precoated surfaces. BioCoat cultureware is available in a wide range of vessel and microplate formats. Available coatings include Collagen I, Collagen IV, Fibronectin, Gelatin, Laminin, Matrigel Matrix, Poly-D-lysine, and Poly-L-lysine.

Bioprinted Tissues

Spheroids and organoids have many advantages, but they're tiny. The lack of a circulatory system limits the transfer of oxygen and nutrients. Furthermore, researchers can't control the overall shape of an organoid and the distribution of cell types, and cell culture conditions that differ significantly from physiological conditions for particular cell types may skew these factors. To create even more physiologically relevant models, some scientists have turned to 3D tissue engineering and bioprinting.

Bioprinting, such as with the Corning® Matribot® bioprinter, is used to create 3D constructs using cells, spheroids, or organoids that are mixed with hydrogels such as Matrigel matrix or Collagen. These temperature-sensitive hydrogels are stored at low temperatures and polymerize as temperatures increase. Therefore, the Matribot bioprinter printhead uses cooling syringe technology to dispense bioink layer by layer. It can also be used to dispense hydrogels that work at ambient temperature, such as alginate-based hydrogels.

Bioprinters are incredibly versatile. The Matribot bioprinter can precisely distribute a single layer of an ECM into a Petri dish or microplate wells as small as a 384-well. It can also dispense 3D droplets or droplet arrays for organoid applications. These applications save time and improve consistency. The Matribot bioprinter also allows cells to be bioprinted in layered 3D geometries to better emulate *in vivo* environments in ways that are advancing biomedical research.

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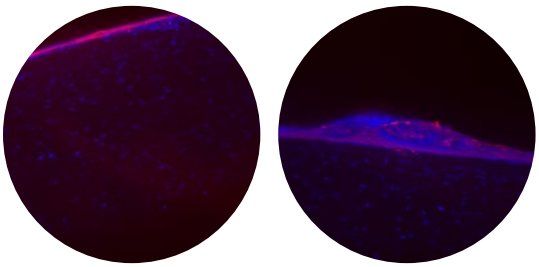
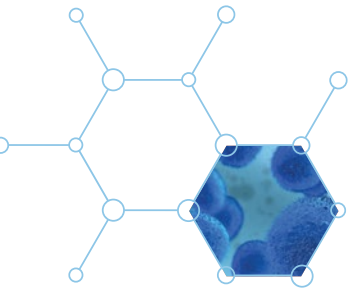


Cross-section view of multilayered 3D printed skin stained with Hoechst (blue) and Cytokeratin (red). Scale bar is 100 µm.

Corning Matribot Bioprinter



One example is skin, which is made of fibroblasts topped by keratinocytes. The cells' orientation is essential to their function, so simply coculturing the two cell types in a dish isn't enough to understand the tissue. Skin organoids can be generated, but they're limited in size. Bioprinting can create a layered 3D structure composed of multiple cell types that more closely mimics skin anatomy. In fact, the Matribot bioprinter can bioprint a full-thickness skin model directly into a Transwell® permeable support so that cells can be ultimately exposed to air and still receive nutrients from below just like *in vivo*.

By using the appropriate cells and conditions, 3D bioprinting has also been used to generate bone, liver, stomach, kidney, lung, and cartilage tissue. These models typically display a realistic microanatomy, mimic organ function, and offer insight into cell-to-cell interactions. These properties are useful in research, toxicology, and drug-screening studies. The field continues to advance as techniques are refined and developed. For example, sacrificial inks have been developed to create scaffolding for vascularization in 3D-printed tissues. This will allow bioprinted models to more closely mimic organ structure and size.

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The Path Forward

In vitro 3D cell culture models have become increasingly sophisticated and varied while the methods for generating, culturing, and analyzing them have become increasingly standardized and controlled. New technologies have facilitated miniaturization so individual spheroids or organoids can be tracked in a single microcavity of a single well of a 1536-well microplate. Additional technologies further facilitate automation and high throughput studies that are now commonly used for drug discovery and toxicity screening.

The potential to generate any tissue in the body — at least in a miniature organoid — is now combined with the relative ease of generating stem cells from any person and the power of CRISPR-Cas9 genome editing technology. This is leading to extraordinary progress in research for complex human disorders that had been especially difficult to study in 2D cell culture and animal models. Advances are also being made in the increasingly complex art of bioprinting cells and scaffolds so that they produce lifelike tissues that might one day be commonly used to replace damaged human tissues.

Previous generations couldn't have imagined the immense potential we now have at our fingertips to understand cellular life in all of its complexity and to prevent or even reverse illness. Around the world, researchers are working to make those possibilities a reality.

Curious about 3D cell culture? Learn more about 3D models and tools.

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