CORNING

Let’s 3D.

Create *in vivo*-like models.

Accelerate Your Next Discovery

3D cell culture is exploding. Scientists are taking advantage of this evolving technology to generate innovative 3D models never before possible. Corning customers use 3D bioprinting to mimic natural tissues for drug screening. They create organoids to explore the intricacies of various diseases in hopes of delivering more effective treatments. And they produce novel spheroid cell models, along with unique methods of high throughput screening, for more effective drug screening.

Corning is ready, willing, and able to provide the in-depth knowledge, expertise, and hands-on assistance you need to create breakthrough models and deliver what's next – whatever your 3D approach. Working together, we can rock the science of 3D.

2D or 3D? It's No Longer a Question.

Why have so many scientists embraced 3D cell culture? Because cells grown in 3D more closely mimic *in vivo* behavior in tissues and organs than cells grown in a 2D culture model. 3D cell culture environments create more biologically relevant models for drug discovery which may lead to more accurate outcome predictions, higher success rates for drug compound testing, a faster path to market, and reduced development costs.

Attribute	2D	**3D**
Growth Substrate	Rigid, inert	Mimics natural tissue environment
Cell Shape Growth	Loss of cell polarity and altered shape	Maintains *in vivo*-like morphology and polarity
Architecture	Not physiological: cells partially interact	Physiological: promotes close interaction between cells, extracellular matrices (ECMs), and growth factors
Growth Factor Diffusion	Rapid	Slow: biochemical gradients regulate cell-cell communication and signaling
Gene Expression	Different patterns of gene expression	Maintenance of *in vivo*-like expression patterns

Explore 3D Cell Culture Environments

Nobody can predict the body's precise response to your next discovery. But Corning Life Sciences offers several tools to help you generate *in vivo*-like conditions for a broad range of cell types, environments, and applications. Our solutions help you create lifelike 3D models that mimic *in vivo* cell behavior for tissue development, cellular differentiation, and screening assays. These *in vitro* 3D models enable biologically meaningful experiments and more predictive results to help you accelerate research in challenging and complex areas.

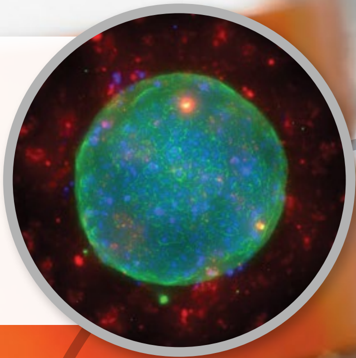
Spheroid Models

Spheroids are simple, widely used multicellular 3D models that form due to the tendency of adherent cells to aggregate. They can be generated from a broad range of cell types resulting in tumor spheroids, embryoid bodies, hepatospheres, neurospheres, and mammospheres.

3D multicellular spheroids can develop metabolic gradients that create heterogeneous cell populations with superior cell-to-cell and cell-to-ECM interactions.[1] They offer a more physiologically relevant model as compared to 2D cell culture and can successfully mimic the microenvironment of a variety of tissue types in disease states.

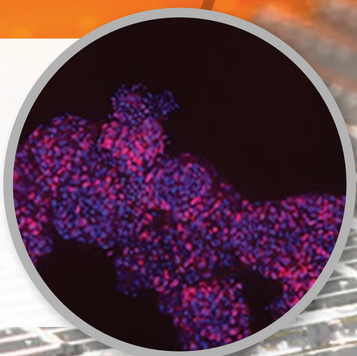
Scaffold-free Spheroid Models

A scaffold-free environment allows for the self-assembly of cells into suspension colonies without the aid of ECMs or other physical supports.



Hydrogels and ECM Scaffold Models

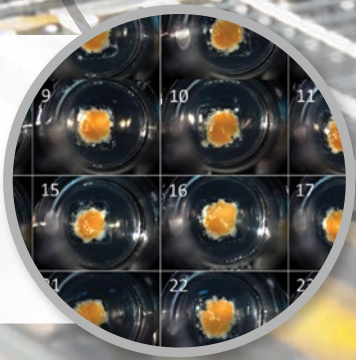
Hydrogels are a series of cross-linked polymer chains or complex protein molecules of natural (ECMs) or synthetic origin that can be used to encapsulate cells, providing an ideal 3D growth environment.



Which 3D model is right for your research?

Solid Synthetic Scaffold Models

Solid scaffolds are porous structures offered in a variety of materials and geometries that mimic 3D microenvironments for a diverse set of cell culture applications.



Corning Solutions for Scaffold-free Spheroid Models

Scaffold-free Spheroid Models

Common scaffold-free methods for generating spheroids include suspension cultures in media, hanging drop methods, or attachment-resistant cell culture surfaces such as Corning® Ultra-Low Attachment (ULA) surface, Corning spheroid microplates, or Corning Elplasia® plates and flasks. They offer a biologically inert surface that minimizes cell attachment and promotes the formation of 3D multicellular spheroids employed in cancer research, stem cell biology, and drug screening.

Ultra-Low Attachment Surface

The Corning Ultra-Low Attachment (ULA) surface is a proprietary, animal-free, covalently bonded hydrogel surface that is hydrophilic and neutrally charged. It minimizes cell attachment, protein absorption, and enzyme activation. The surface is non-cytotoxic and non-degradable. The ULA surface promotes the formation and easy harvesting of anchorage-dependent scaffold-free spheroids and is available in a variety of cell culture formats and configurations (Figure 1).

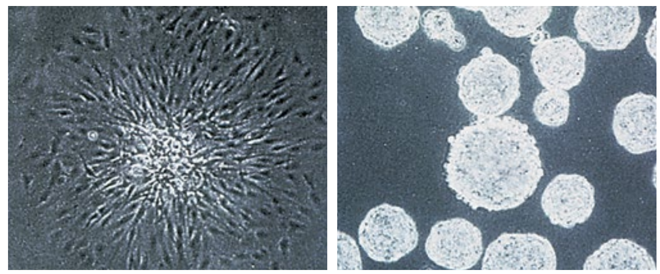


Figure 1. Tissue Culture-treated surface (left) and spheroid colonies on Ultra-Low Attachment surface (right).

Spheroid Microplates

Corning Spheroid microplates combine the ULA surface with an innovative U-shaped well geometry. This easy-to-use, highly reproducible tool is ideal for generating, culturing, and assaying individual uniformly sized 3D multicellular spheroids in the same microplate – with no need for transfer. The microplates feature opaque side walls and a proprietary gridded plate bottom to reduce well-to-well cross-talk and background fluorescence/luminescence for a variety of drug and toxicity screening applications. Corning spheroid microplates are available in 96-, 384-, and 1536-well automation-friendly formats.
They enable direct high throughput screening of spheroids in these microplates, cutting down associated scale-up steps.

Corning 3D Clear Tissue Clearing Reagent

Despite the growing relevance of 3D cell culture as a research model, imaging techniques used to characterize these models are highly limited. Due to the thickness and opacity of 3D cellular structures, most current imaging technologies cannot penetrate to the center of the tissues, resulting in only the outer 2 to 3 layers of cells being detected. This causes the dark centers often seen in images of 3D cell culture models. This is highly problematic for accurate analysis as these outer cells are most exposed to compounds, nutrients, and oxygen and do not reflect the entire cell population.

Corning 3D Clear tissue clearing reagent can be used in a tissue clearing technique to support imaging. When paired with fluorescent labeling (e.g., fluorescent protein, immunofluorescence, chemical dyes) and high content confocal microscopy, Corning 3D Clear reagent allows for complete 3D cell culture model characterization and more accurate drug screening.

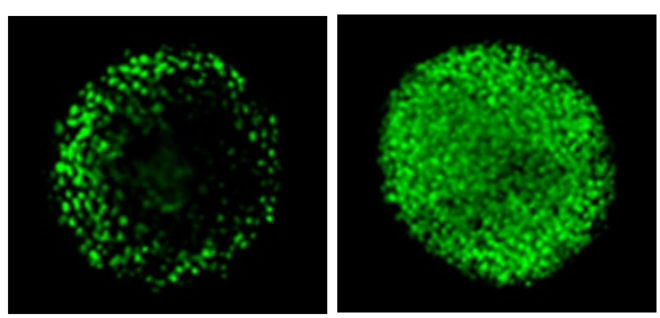


Figure 2. Imaged spheroid before (left) and after (right) tissue clearing.

Corning® Elplasia® Technology

With the effectiveness of 3D spheroids in many areas of research including anti-cancer drug screening and *in vitro* tumor studies, the need for better methods to produce replicate spheroids of uniform size in mass quantities has emerged. Corning's Elplasia technology addresses this need by enabling researchers to generate a high density of spheroids in a scaffold-free model using microcavity technology. Elplasia technology can be used for drug and high throughput screening, cancer, tumor and stem cell biology, cell therapy research, and 3D tissue engineering.

Corning Elplasia Plates

Simple Spheroid Formation and Culture
Elplasia plates use a simple "plug and play" protocol for scaffold-free, spheroid self-assembly at large volumes. Spheroids may be formed and cultured for 21 or more days in one Corning Elplasia plate.

Highly Reproducible and Consistent
Elplasia plates feature novel well geometries that promote uniform spheroid formation. Plates are offered in two surface coatings, including Corning Ultra-Low Attachment (ULA) surface on round bottom plates and plasma-treated (for self-coating) on square bottom type plates.

High Density
Produce between 79 to 15,000+ spheroids per well, depending on plate format, under the same culture conditions.

Increased Data
The high volume of spheroids generated in each well increase signals per well without increasing spheroid size. This high density format also generates increased data points, enabling image analysis of multiple spheroids vs. one spheroid per well.

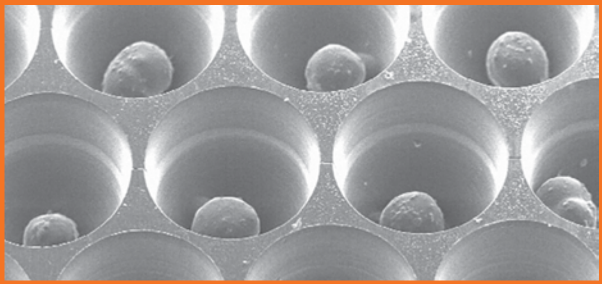
Easy Imaging
Elplasia plates feature black opaque sidewalls to reduce well-to-well "cross-talk". This also makes them well-suited for fluorescent/luminescent assays. Elplasia plates feature surfaces with optical qualities suited for image analysis. Square bottom type plates are an ideal solution for clonal selection and high magnification imaging of very small clusters.

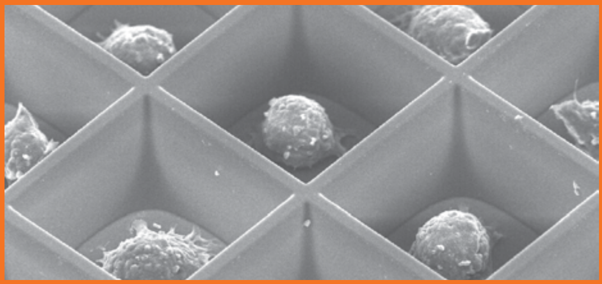
Two Unique Well Types

Corning Elplasia plates are available in multiple formats, two well geometry types, and two surface coatings. All plates are gamma irradiated and have a one-year shelf life.

Corning Elplasia round bottom plates are optimal for bulk spheroid formation, collection, and expansion. Round bottom plates feature Corning Ultra-Low Attachment surface and are available in 6-, 24-, and 96-well formats.

Corning Elplasia square bottom type plates feature a surface with optical qualities suited for image analysis, making them an ideal solution for clonal selection and high magnification imaging of very small clusters. Square bottom plates are plasma-treated for self-coating and come in 6-, 24-, 96-, and 384-well formats.





Corning® Elplasia® Technology *(continued)*

Corning Elplasia 12K Flask

The Corning Elplasia 12K flask contains 152 microcavities per cm^2 in a vessel footprint similar to that of a T-75 flask for highly reproducible bulk spheroid formation across microcavities.

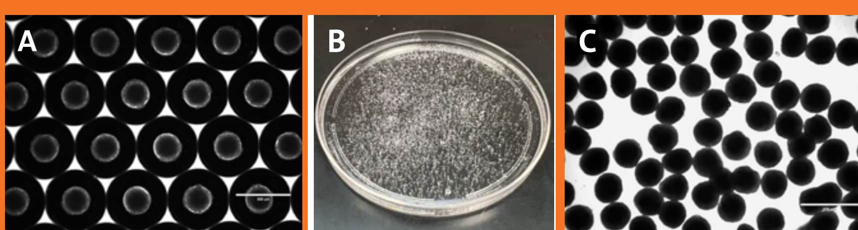
Gravity, in conjunction with the Corning ULA surface, and a rounded microcavity geometry enable formation of approximately 12,000 spheroids of similar shape and size. The ULA surface, a proprietary, animal-free, covalently bonded hydrogel, promotes the formation and easy harvesting of spheroids.

The microcavity geometry allows spheroids to remain in place during medium exchange without compromising full recovery at harvest time. The flask's internal diverter feature allows for minimal disruption of spheroids during liquid handling steps, along with a common media reservoir for equivalent culture conditions for all spheroids.

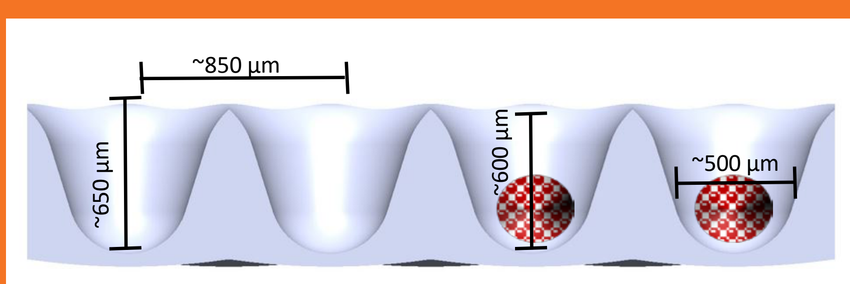
Corning Elplasia 12K Open Well Plate

The Corning Elplasia 12K plate is designed similarly to the Corning Elplasia 12K flask, with a gas-permeable polystyrene film-bottom containing 152 microcavities per cm^2 for the straightforward bulk generation of uniform spheroids in one culture condition.

The ANSI/SLAS microplate footprint design and removable lid make the Elplasia 12K plate ideal for sampling and imaging of spheroids. Media exchange ports allows for minimal spheroid disruption during liquid handling steps. The microcavity geometry allows spheroids to remain in place throughout culture without compromising accessibility/spheroid recovery at collection time.



The Corning Elplasia 12K flask and 12K open well plate were designed to enable the generation of large quantities of uniform spheroids. 14-day old MCF 7 (human breast adenocarcinoma) spheroids culture in a Corning Elplasia 12K flask (A). Spheroid collection post harvest (B). Representative image of harvested MCF 7 spheroids (C). Micrographs were taken with an EVOS® FL microscope in brightfield mode using a 2X objective.



The Corning Elplasia 12K flask and Elplasia 12K open well plate substrate contains approximately 12,000 round bottom cavities that each provide a growth area of 500 x 600 µm (diameter x depth) with gas permeable bottoms.

Scaffold-free Spheroid Model Applications

Corning spheroid models are the first choice in critical and complex research areas.

Stem Cell Research

Corning® spheroid microplates are used to create uniform embryoid bodies from induced pluripotent stem cells (iPSCs) that can be subsequently generated into high purity neural stem cells (NSCs) for the study of potential neural disease treatments (Figure 3).

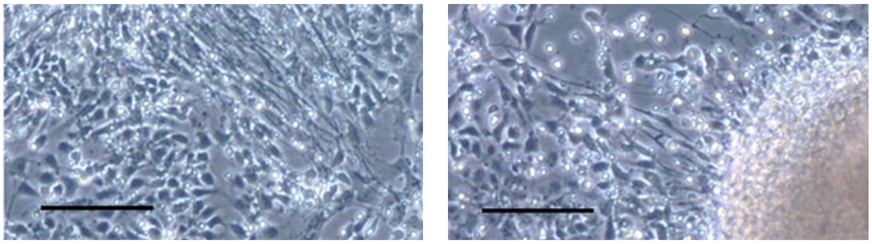


Figure 3. EB Selection in defined medium. Morphology of putative NSCs produced from iPSCs cells by various protocols. Scale bar = 100 µm.

Tumor Biology

3D tumor spheroid models generated with Corning spheroid microplates closely mimic the *in vivo* tumor microenvironment. These spheroids – grown as either single cultures or more complex co-cultures with other cell types in the tumor microenvironment – offer an opportunity to better predict the therapeutic efficacy of cancer drug models in oncology drug discovery applications (Figure 4).

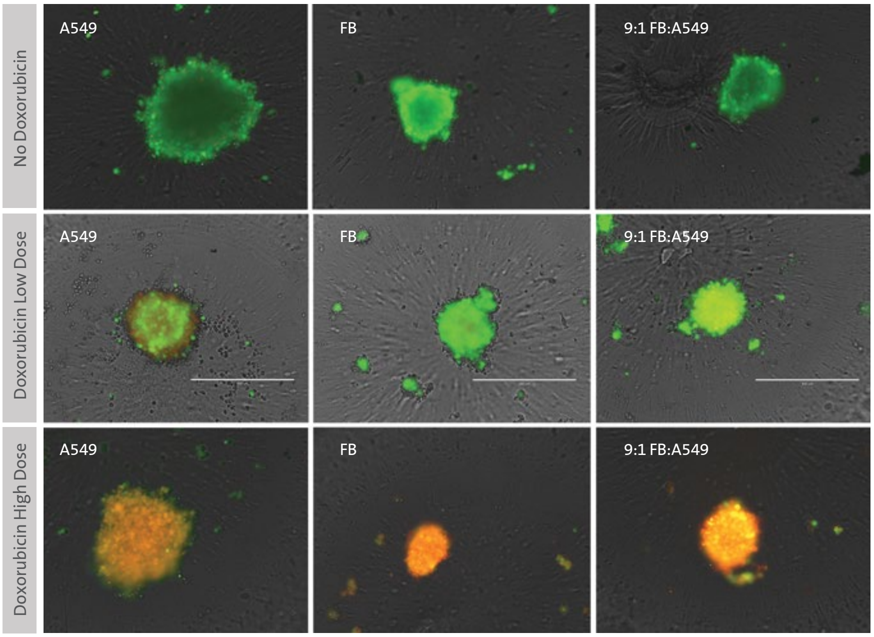
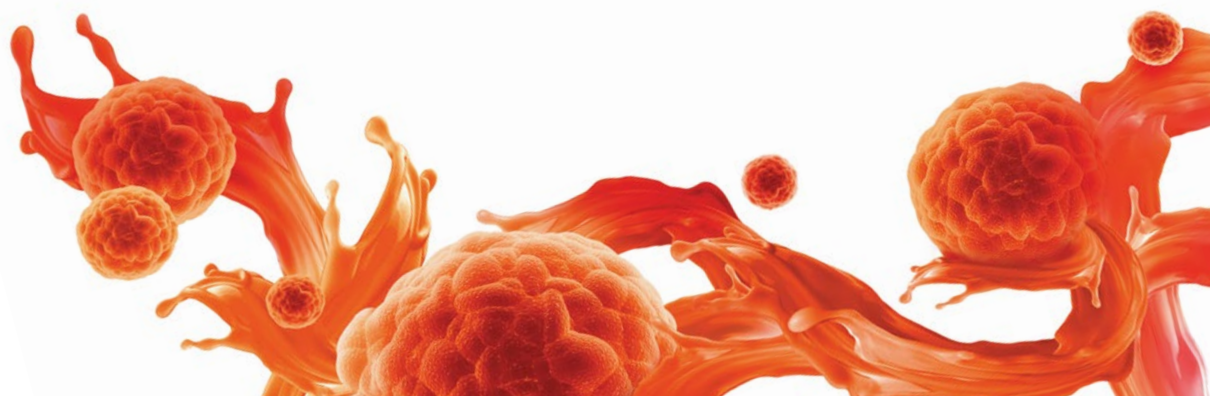


Figure 4. Live (green) and dead (red) stained 96-hour mono- and co-culture A549 and fibroblast spheroids. Spheroids were exposed to high (34.5 µM) and low (27.6 µM) doses of doxorubicin or vehicle control [no doxorubicin] for 48 hours in Corning 384-well spheroid microplates. Fibroblast (FB) monoculture displayed the most intense live staining upon low dose exposure to doxorubicin, while A549 monoculture showed increased cell death. The 9:1 ratio of FB to A549 cells also displayed a protective effect at the low dose doxorubicin exposure. All cell types displayed significant toxicity after high dose exposure. Images captured using an EVOS® fluorescent microscope at 10X objective. Scale bar = 400 µm.

Immune Oncology

Corning 384-well spheroid microplates have been successfully employed to evaluate the cytotoxic effect of CAR-T cells on tumor cell spheroids. For example, the KILR™ Cytotoxicity assay (DiscoverX Corp.) combined with KILR-transduced tumor spheroids can be formed, cultured, and assayed directly on the same spheroid microplate (Corning Application Note CLS-AN-447).



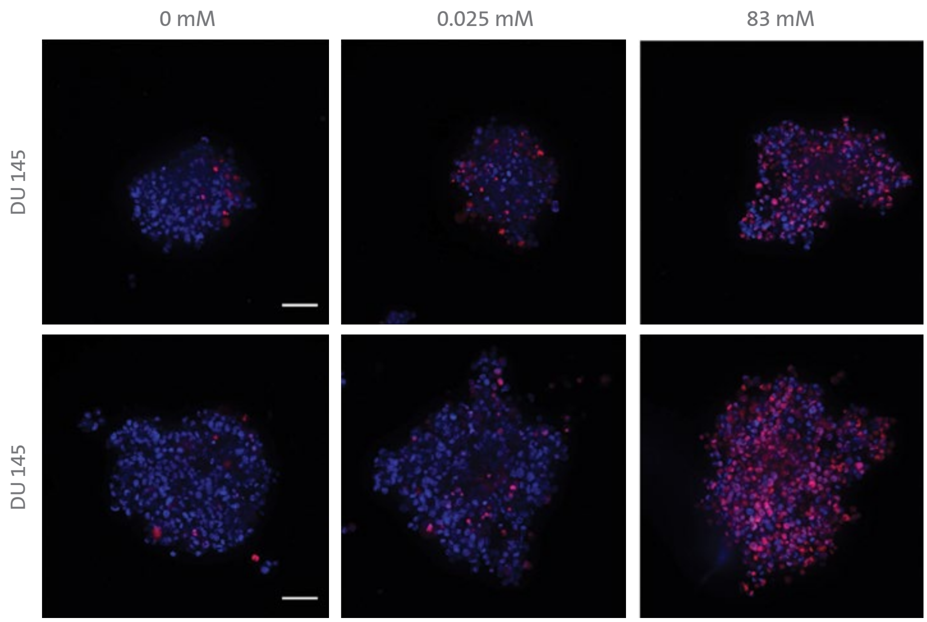


Figure 5. Confocal imaging of spheroids within Corning 1536-well spheroid microplate. Representative z-stack images of DU 145 (top) and PANC-1 (bottom) spheroids exposed to 0 mM, 0.025 mM, and 83 mM cisplatin (left, middle, right, respectively) for 24 hours. Spheroids were stained with Hoechst (blue) and PI (red) to assess cell viability. Spheroids were imaged directly in the spheroid microplate with the Thermo Scientific CellInsight™ CX7 high-content screening platform using a 10X objective. Scale bar = 100 µm.

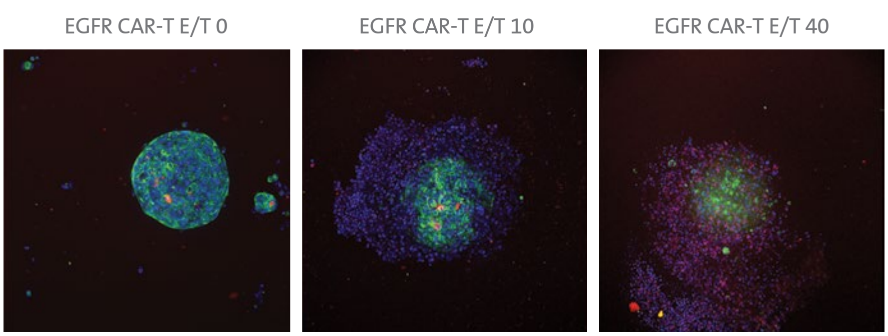


Figure 6. Representative confocal images of HCC827 KILR-transduced spheroids with CAR-T cell invasion. Twenty-four hours after CAR-T cell addition, HCC827-KILR cells were stained for cytokeratin-7 (green) and EGFR CAR-T cells were stained for CD3ζ (red). All cell nuclei were counterstained with Hoechst (blue). As effector to target ratio (E/T) is increased from 10:1 to 40:1, invasion of the CAR-T cells into the HCC827 tumor spheroid and subsequent tumor cell lysis is visible. Images obtained using a CellInsight CX7 in confocal mode using 10X objective.

Drug Discovery

As 3D models are used more and more for drug discovery, the need for high volume formats has emerged to meet the demands of high throughput systems. Corning 1536-well spheroid microplates have been successfully employed to generate uniform, single spheroids that can be assayed via imaging, fluorescence, or luminescence directly in the microplate (Figure 5, Corning Application Note CLS-AN-529).

Cell Therapy

Corning spheroid microplates have proven to be an effective high throughput tool for culturing and screening tumor spheroids with CAR-T cell assays (Figure 6, Corning Application Note CLS-AN-447). The spheroid microplate may also be combined with other Corning technologies, such as Transwell® permeable supports, to study cancer or immune cell interactions (Corning Application Note CLS-AN-425).

SCIENTIFIC RESOURCES

SCAFFOLD-FREE SPHEROID MODELS

1. Corning Spheroid Microplates (Corning User Guide CLS-AN-235)
2. Corning Spheroid Microplates Spheroid Formation (Corning Protocol CLS-AN-308)
3. Co-culturing and Assaying Spheroids in the Corning Spheroid Microplate (Corning Application Note CLS-AN-390)
4. Corning Elplasia Round Bottom Plates (Corning Guidelines for Use CLS-AN-536)
5. A Novel Three Dimensional Immune Oncology Model for High Throughput Testing of Tumoricidal Capability (Corning Application Note CLS-AN-425)
6. Corning Elplasia 12K Flask (Corning Guidelines for Use CLS-AN-713DOC)
7. Corning Elplasia 12K Open Well Plate (Corning Guidelines for Use CLS-AN-757DOC)

Visit **www.corning.com/3D** to access all Corning scientific resources.

Corning Solutions for Hydrogel and ECM Scaffold Models

Hydrogels are made up of a network of highly absorbent, cross-linked polymer chains or complex protein molecules of natural or synthetic origin. They can encapsulate and release a variety of bioactive molecules.[2] Due to their high water content, they closely resemble the tissues *in vivo* and act as excellent 3D matrices. Hydrogels can be used alone or combined with other technologies such as permeable supports. They work seamlessly with microplate formats and automated equipment used in high throughput screening applications. Hydrogels derived naturally from extracellular matrix (ECM) proteins contain endogenous elements including soluble biomolecules and growth factors that can be beneficial for supporting cell viability, cell migration, function, and differentiation. These ECMs are amenable to matrix degradation and deposition.

Corning® Matrigel® Matrix

The most widely used natural ECM is Corning Matrigel matrix, a reconstituted basement membrane extract from Engelbreth-Holm-Swarm (EHS) mouse tumors. It is rich in laminin, Collagen IV, entactin, heparin sulfate proteoglycans, and a number of growth factors, making it ideal to use in applications including cancer and stem cell research. This bioactive matrix exhibits the mechanical and chemical properties of the *in vivo* ECM. It offers a dynamic and tunable microenvironment for the cells to grow and develop.[3] The ECM components of Matrigel matrix activate cellular responses and pathways that are more physiologically relevant compared to cells grown in 2D surfaces (Corning Review Article CLS-AC-AN-245).

Corning Matrigel Matrix for Organoid Culture

This optimized ECM reduces the need for time-consuming screening, while providing the reproducibility and consistency essential for organoid research. Matrigel matrix for organoid culture has been verified to support organoid growth and differentiation including long-term expansion of mouse small intestinal organoids for more than 7 passages with typical organoid budding morphology and marker expression. It also enables growth and differentiation of polarized 3D epithelium from primary human airway epithelial cells expressing typical markers.[5] Each lot is measured for its elastic modulus, indicative of matrix stiffness that supports an organoid workflow and demonstrated to successfully grow organoids from both healthy and diseased cell origins.[5]

Collagen

Corning Collagen Type I is a natural hydrogel commonly found in stromal compartments in the dermis, tendon, and bone. It is effective as a gel and supports *in vivo*-like 3D growth and differentiation. It provides physiological interactions with receptors to modulate the expression of a variety of genes including those involved in cell invasion, cell sensitivity to anti-cancer drugs, cell proliferation, and cell migration (Corning Review Article CLS-AC-AN-245). Collagen I has also been successfully used to culture intestinal, pancreatic, mammary, and salivary organoids.[6,7,8]

Corning® Matrigel® Matrix-3D Pre-coated Plates

As 3D cell culture models migrate into a high throughput environment, there is an increased need for convenience and consistency. Corning Matrigel matrix-3D plates are ready-to-use options that enable homogenous assay formats using 3D structures, wherein organoids or spheroids can be grown and assayed directly in the plate. These plates are available in 96-well and 384-well formats for high throughput screening—to help improve workflow and increase productivity. They support formation of 3D polarized epithelial structures and cancer spheroids. Corning Matrigel matrix-3D plates enable screening of pancreatic, ovarian, prostate, and intestinal organoids with drug compound libraries.[9,10] (Corning Application Notes CLS-AC-023 and CLS-AN-607).

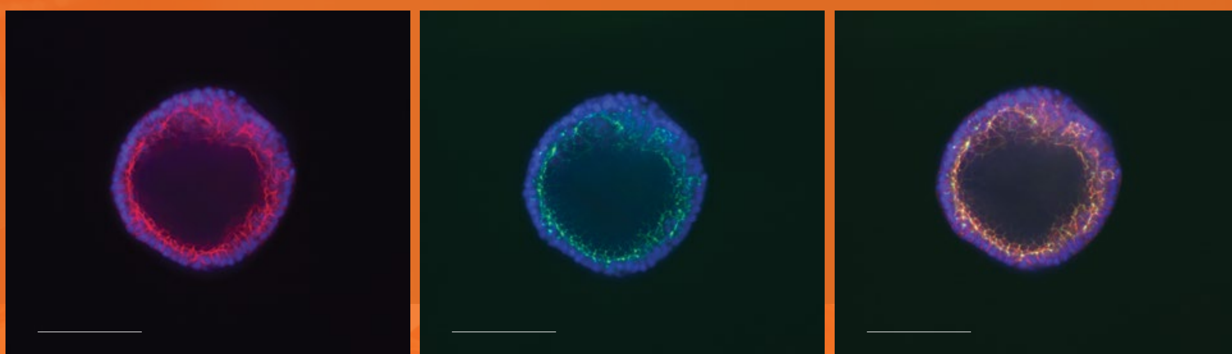


Figure 7: MDCK cyst polarity. Representative photomicrographs of fluorescently stained MDCK cysts using a 20X objective. Blue is nuclei, red is phalloidin, and green is ZO1. Right image is overlay. Scale bar = 100 µm.

Synthetic Hydrogels

In cases where bioactive compounds, such as growth factors, can potentially interfere with the specific cell behaviors or responses, synthetic hydrogels are a good choice for 3D cell culture applications. Synthetic hydrogels are biologically inert, pathogen-free, non-natural molecules that offer structural support for a variety of cell types. It is also possible to blend synthetic scaffolds with biological components to create tunable synthetic hydrogels.

Corning® Synthegel® 3D Matrix Kits

Corning Synthegel 3D Matrix Kits are defined, self-healing synthetic peptide hydrogel bio-tools for culturing many different types of cells including cancer and stem cells, in a 3D format with robustness and convenience. The Synthegel 3D matrix kits provide negligible variability and a more controlled substrate for in vivo like 3D cell culture. This platform is a critical tool that is useful for cancer research, stem cell research and drug screening in a 3D format. The Synthegel 3D matrix platform supports culture of human induced pluripotent stem cells (hiPSCs) in a 3D embedded format or in an encapsulation for suspension format, along with supporting the culture of physiological cancer spheroids.

Corning Synthemax® Vitronectin Substrate

Corning Synthemax is a vitronectin-based peptide that allows pluripotent cell expansion and propagation of various progenitor cell types, including MSCs.

Corning PureCoat™ ECM Mimetic Cultureware

Corning PureCoat ECM Mimetic cultureware contains biologically active, animal-free peptides rationally designed to mimic the cell attachment process and motifs of native ECM proteins and has 2 coating options to support the growth of stem and primary cells across a broad range of defined media formulations: Fibronectin peptide and Collagen I peptide.

Hydrogel and ECM Scaffold Model Applications

Organoids

Organoids have become an increasingly popular option for scientists in development and drug discovery. The passing of the FDA Modernization 2.0 Act, empowers researchers to use innovative non-animal methods, including the use of organoids. The ability of such methods to accurately model human physiology could transform the speed and success of bringing safe and effective treatments to market. Stem cells and/or organ progenitors from normal or diseased tissue are mixed with Corning® Matrigel® matrix to create mini-organs of the kidney, thyroid, liver, brain, lung, intestine, prostate, and pancreas. Organoids support advancements in the study of organogenesis, disease modeling, and subsequently patient-specific drug therapies. Matrigel matrix is the most published and optimum hydrogel for organoid research due to its close resemblance to an *in vivo* environment, providing necessary growth factors, proteins, and the required matrix architecture. Biological hydrogels such as Corning Collagen and Corning Matrigel matrix can be used as bio-inks to enable precise positioning and embedding of living cells during bioprinting of mini-organs.

Personalized Medicine and Drug Discovery

Organoids are derived from living cells and are cultured in a way that very closely mimics *in vivo* biological features of human tissue. These more physiologically relevant models offer a unique opportunity to test a variety of potential drugs and determine effectiveness before treating a patient.

Modeling Cancer

Tumor models using Matrigel matrix replicate the 3D structure of tumors to study formation, progression, invasion, and metastasis of cancer cells and to evaluate potential drug treatments. Cell morphology in a 3D Matrigel matrix culture can also be used to predict and/or stage cancer progression based on behaviors of cell polarization, colony formation, and proliferation. To metastasize, cancer cells must be able to cross a matrix and enter the blood vasculature. Matrigel matrix cell invasion assays can be used to assess the aggressiveness of cancer cells to demonstrate their potential of degrading the ECM proteins by proteases that can lead to metastases.

Angiogenesis

Angiogenic capability is often assessed by the ability of endothelial cells to sprout, migrate, and form vascular tubules. Matrigel matrix formulations allow *in vitro* modeling of endothelial cell behavior, including survival, apoptosis, tube formation, and invasion. These models are often used to investigate the effects of drugs or small molecules on angiogenesis *in vitro*. One option for *in vivo* evaluation of angiogenic drug compounds is the subcutaneous Matrigel matrix plug assay in mice.

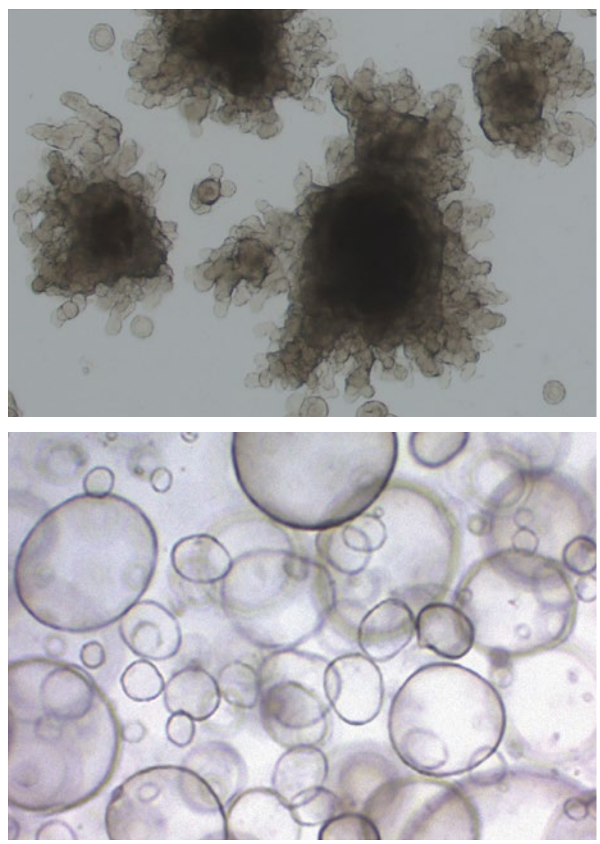
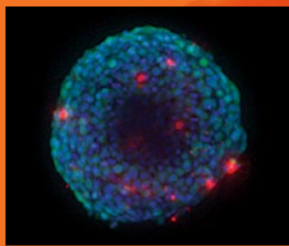
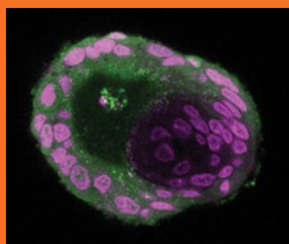


Figure 8. Top image features human rectal organoids from a cystic fibrosis cell source cultured for 10 days in Corning Matrigel matrix. *Courtesy of MDI Biological Laboratory*. Bottom image features human liver organoids in Corning Matrigel matrix (perm. L.A. Oosterhoff).





Spheroids vs. Organoids – What's the Difference?

Spheroids and organoids are both multicellular 3D structures. Although this terminology has been used interchangeably, there are distinct differences between the two. An organoid is a collection of organ-specific cell types that develops from stem cells or organ progenitors, which self-organize through cell sorting and spatially restricted lineage commitment in a manner similar to *in vivo*.[9] Multicellular tumor spheroid models were first described in the early 1970s and were obtained by culturing cancer cell lines under non-adherent conditions.[12] Tissue-derived tumor spheres and organotypic multicellular spheroids are typically obtained by tumor tissue mechanical dissociation and cutting.[11] Tumorospheres are a model of cancer stem cell expansion. Generally, there is a higher order self-assembly in organoids as opposed to spheroid cultures and the former is more dependent on an extracellular matrix for its generation.

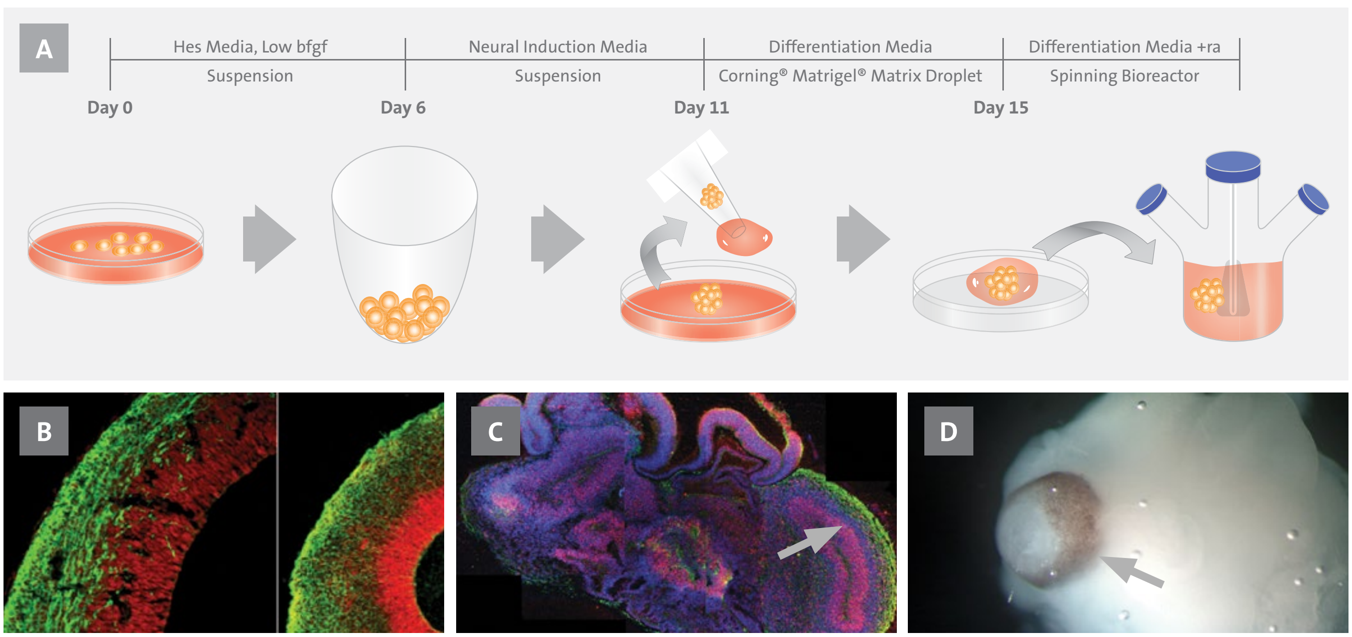
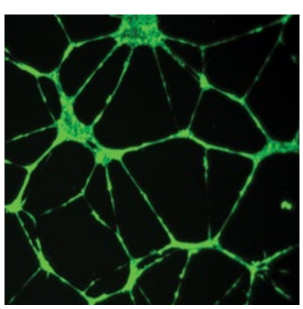


Figure 9: Description of cerebral organoid culture system. (A) Schematic of the culture system used to generate cerebral organoids. Example images of each stage are shown: bFGF, basic fibroblast growth factor; hES, human embryonic stem cell; hPSCs, human pluripotent stem cells; RA, retinoic acid. (B) A comparison between organoid and mouse brain structure demonstrates recapitulation of dorsal cortical organization. Immunohistochemistry for neurons (TUJ1, green) and radial glial stem cells (PAX6, red) in a large dorsal cortical region. (C) Sectioning and immunohistochemistry revealed complex morphology with heterogeneous regions containing neural progenitors (SOX2, red) and neurons (TUJ1, green). (D) Low-magnification bright-field images revealing fluid-filled cavities reminiscent of ventricles and retina tissue, as indicated by retinal pigmented epithelium.[12]



"Corning Matrigel matrix plug assay has become the method of choice for many studies involving *in vivo* testing for angiogenesis."

Akhtar N, Dickerson EB, Auerbach R. The sponge/Matrigel angiogenesis assay. Angiogenesis (2002) 5:75-80.

Figure 10: Corning Human Umbilical Vein Endothelial Cells (HUVEC-2) stained with Calcein AM and cultured on Corning Matrigel matrix.

Organoids *(continued)*

Explore Organoid Models

Organoids are generated from both pluripotent stem cells (PSCs) and adult stem cells (ASCs). Self-renewal and differentiation of stem cells are influenced by growth factors and extracellular matrices (ECM) that provide the required scaffold to support cell attachment and growth during organoid formation. Hydrogels such as Corning® Matrigel® matrix and Corning Collagen are popular scaffold choices to support cell expansion in organoid cultures.

Stem cells mixed with Matrigel matrix or Collagen are used to create miniorgans to advance the study of organogenesis, disease modeling, and patient therapies. For example, the combination of genome editing using CRISPR-Cas9 and organoid cultures allows researchers to evaluate DNA repair of patient-specific mutations found in certain cancers and perform genetic screens. Biological hydrogels such as Corning Collagen and Corning Matrigel matrix can be used as bioinks to enable precise positioning and embedding of living cells during printing. Organoids are also being used as physiologically relevant models for the development of new therapeutic drug candidates.

Organoid Types

Gastrointestinal
The gastrointestinal (GI) tract contains adult stem cells (Lgr+) residing at the bottom of the intestinal crypt and gastric glands. Proliferation of these cells is dependent on signaling pathways governed by cell-matrix interactions. GI organoids are an important tool to study developmental process as well as for personalized medicine.

Liver
Organoids generated from the liver have hepatic differentiation potential and can be a source for toxicology testing and serve as a model for liver diseases. Lgr5+ cells can also be clonally expanded as organoids and differentiated into functional hepatocytes both *in vitro* and upon transplantation *in vivo*. Liver organoids cultured from human and animal patients can be genetically modified *ex vivo* before transplantation or drug screening.

Neural
Neural organoids—also known as cerebral organoids—are generated from pluripotent stem cells (PSCs). Embryoid bodies derived from induced PSCs (iPSCs) are encapsulated in Corning Matrigel matrix droplets and cultured in differentiation media toward a cerebral phenotype on an agitation-based platform. These "mini-brains" can help us understand development of the human brain and help evaluate inherited and acquired brain diseases.

Organoids *(continued)*

Kidney

There are several protocols used to generate kidney organoids starting from human pluripotent stem cells (hPSCs). One method was demonstrated by Dr. Melissa H. Little's lab in 2015. There, researchers start with hPSCs seeded on cultureware coated with Corning® Matrigel® matrix through various induction stages to intermediate mesoderm. For 3D culture, these cells were then transferred to Transwell® permeable supports where they matured into kidney organoids in appropriate differentiation conditions.

Prostate

Prostate cancer is the most common cancer in men, and patient-derived biopsies can be used to generate prostate organoids. These 3D structures can be molecularly characterized and utilized to manipulate the expression of oncogenes.

SCIENTIFIC RESOURCES

HYDROGEL AND ECM SCAFFOLDS

1. Corning Cell Culture Surfaces (Corning Selection Guide CLS-C-DL-AC-006)
2. The Ultimate Guide to Corning Matrigel Matrix (Corning Guide CLS-DL-AC-016)
3. Corning Synthegel 3D Matrix Kits (Corning FAQs CLS-AN-742)
4 Corning Matrigel Matrix-3D Plate (Corning FAQ CLS-AN-571)
5. Culturing Human Intestinal Organoids with Corning Matrigel Matrix for Organoid Culture (Corning Application Note CLS-AN-569)
6. Culture of Mouse Intestinal Organoids in Corning Matrigel Matrix for Organoid Culture (Corning Application Note CLS-AN-542)
7. High Throughput Gene Expression Analysis of 3D Airway Organoids (Corning Application Note CLS-AN-534)
8. Corning Synthegel Spheroid Matrix Kit for Generating Cancer Spheroids in a Synthetic Peptide Hydrogel (Corning Application Note CLS-AN-762)

Visit **www.corning.com/3D** to access all Corning scientific resources.

Solid Synthetic Scaffold Models

Solid scaffolds can be made from a broad range of materials to mimic a given 3D microenvironment. Polymers are a common choice for generating solid scaffolds of diverse size, structure, and porosity. They can be fabricated using lithography, electrospinning, bioprinting[1] and, in the case of permeable supports, microporous membranes.

Because synthetic scaffolds are devoid of animal-derived materials, they are free of potential pathogens and other issues found with biologic products. For studies where endogenous factors are required to more realistically mimic the cellular *in vivo* environment, they can be combined with ECMs as a coating to create effective complex matrices for 3D cell culture.[2]

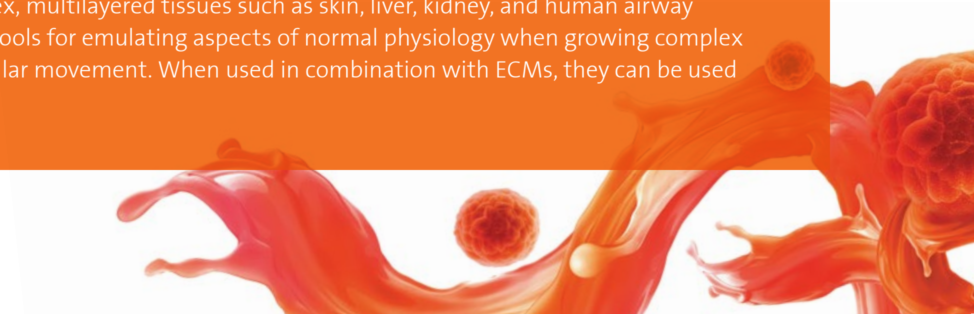
Solid Synthetic Scaffold Applications

Organotypic Tissue Models and Air-Liquid Interface (ALI) Culture

Organotypic models have been developed for a variety of tissues including skin, liver, stomach, kidney, and lung. They display a realistic micro-anatomy, mimic organ functionality, and offer insight into cell-to cell interactions, making them a valued research tool for drug discovery, regenerative medicine, toxicity testing, and disease modeling. Permeable supports are particularly well suited for growing organotypic models as their design makes it possible to bathe cells both apically and basolaterally or grow epithelial cell models at the air-liquid interface (ALI) and bathe them basolaterally. ALI culture results in more differentiated phenotypes and physiologically relevant models than traditional 2D submerged culture on solid plastic substrates[13] (Figure 11).

Corning Solutions at Work

Transwell® and Falcon® permeable supports from Corning are solid synthetic scaffold inserts with microporous membrane bottoms. They are available in multiple formats, pore sizes, and membrane types to cater to a variety of application needs. These cell culture inserts provide a dual compartment system that allows cells to carry out a variety of metabolic activities in a more *in vivo*-like manner through the exchange of media, nutrients, and other biomolecules. They have been widely used to grow complex, multilayered tissues such as skin, liver, kidney, and human airway epithelia. Permeable supports are valuable tools for emulating aspects of normal physiology when growing complex tissues, co-culturing cells, and studying cellular movement. When used in combination with ECMs, they can be used to produce cancer invasion models.



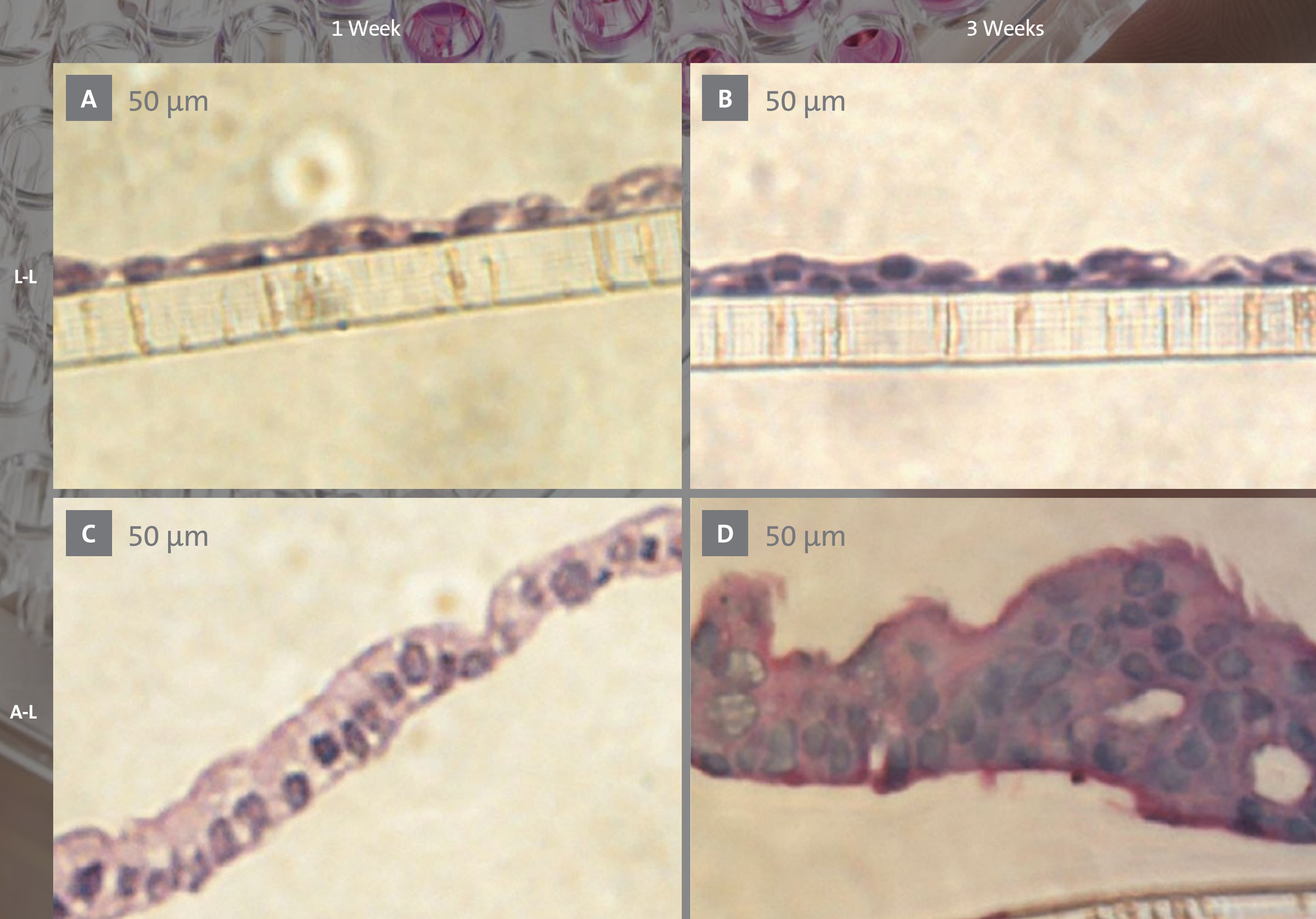


Figure 11. Haematoxylin-eosin stain of Calu-3 cells at the L-L and A-L interfaces after 1 week and 3 weeks in culture. Haematoxylin-eosin stain at the L-L interface revealed a flat cell monolayer (A, B). At the A-L interface, pseudostratified mucociliary epithelium morphology was formed (C, D).

Solid Synthetic Scaffold Applications *(continued)*

Bioprinting

Bioprinting fabricates a 3D tissue-like construct, layer-by-layer using cells, spheroids, or organoids suspended in a bioink.[14] 3D bioprinting has been used for the generation of multilayered skin, bone, liver, and cartilage tissue models in research, toxicology, and drug-screening studies. Bioprinting makes it possible to reproduce structural features seen *in vivo* and explore the cell-to-cell relationships that affect tissue functionality.[15]

Transwell® permeable supports have been successfully used as a bioprinting substrate for a variety of tissue models, including liver and kidney. The microporous surface and the compartmentalized design of the inserts provide an excellent substrate for prolonged cell culture of the bioprinted tissue in an *in vivo*-like environment that can also be subsequently used in drug discovery testing and disease modeling (Figure 12).

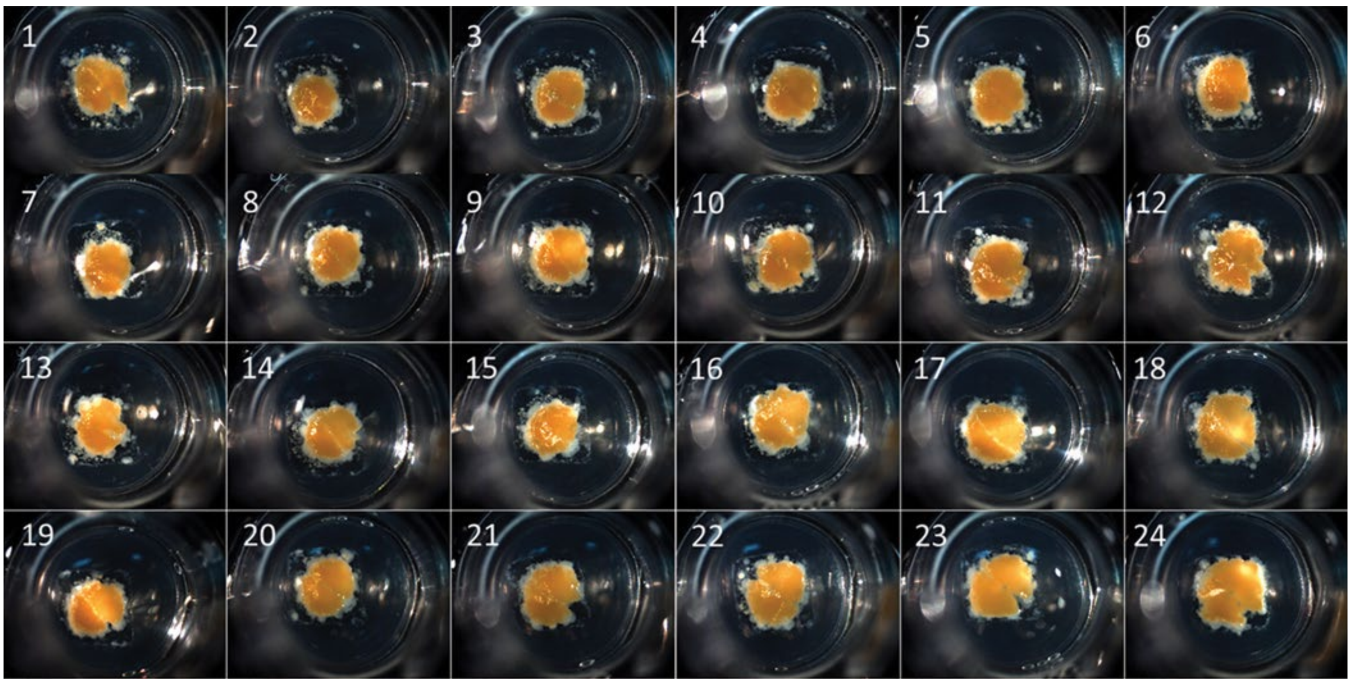


Figure 12. 3D human liver tissue bioprinted on Transwell permeable supports. Image courtesy of Organovo.

Motility Models

The movement of cells from one area to another in response to a chemical signal, is central to a variety of cell functions such as cell differentiation, wound repair, embryonic development, angiogenesis, and tumor metastasis.

Corning permeable supports offer a simple *in vitro* 3D method for studying this movement. They can be used alone in culture or coupled with ECMs, such as Corning® Matrigel® matrix or Corning spheroid microplates, to study a wide variety of cell types and movement mechanisms.

Advances in the development of a light-blocking membrane, known as Corning FluoroBlok™, have further simplified this process by eliminating the need to swab away non-migrating cells after a migration event. Fluorescently labeled cells present in the top chamber of the insert are shielded from bottom-reading fluorescence plate readers and microscopes. After labeled cells migrate through the membrane, they are easily detected by a bottom-reading fluorescence plate reader or microscope, thereby eliminating manual cell counting and additional processing steps. This non-destructive detection method enables both kinetic and endpoint migration and invasion assays. Corning offers a FluoroBlok light-blocking membrane in several insert formats designed for the study of cell movement (Figures 13 and 14).

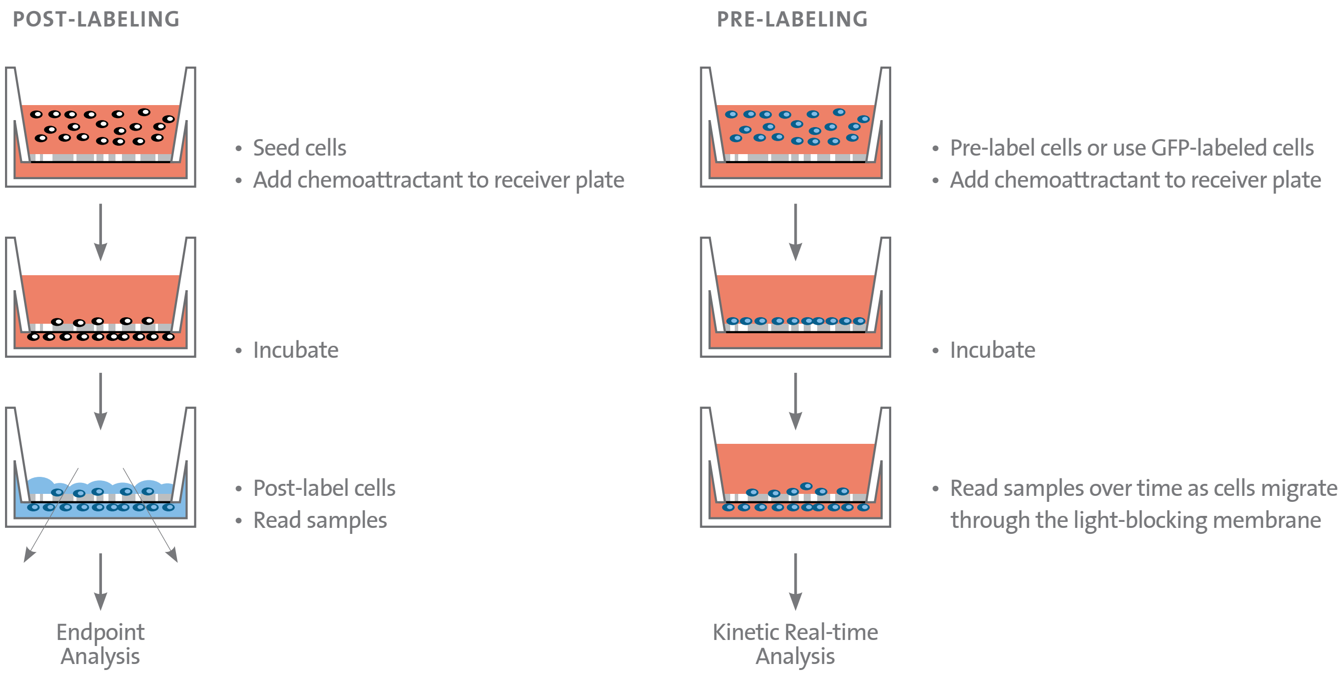


Figure 13. Corning® FluoroBlok™ Insert System

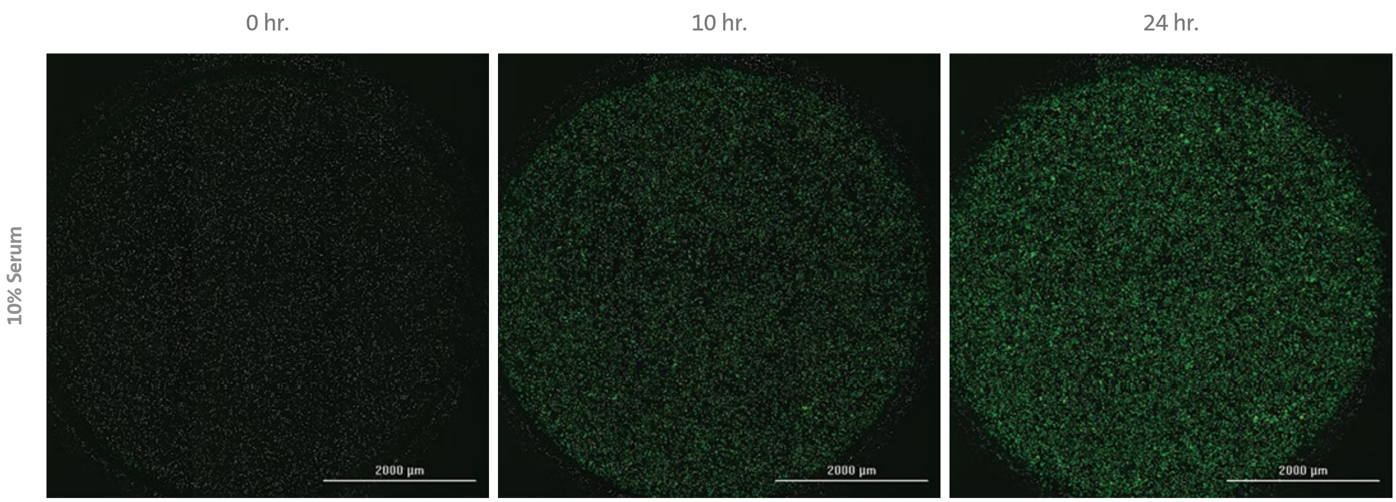
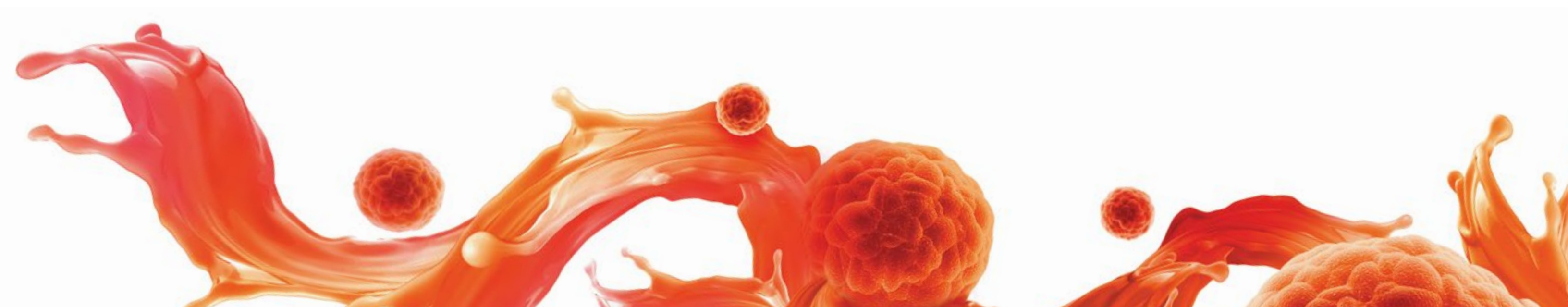


Figure 14. Kinetic images taken of Green-labeled MDA-MB-231 cells labeled prior to experiment with CellTracker™ Green CMFA dye. 10% or 0% serum was used as positive and negative chemoattractant controls, respectively. Images captured using GFP imaging filter cube and a 4X objective.



Solid Synthetic Scaffold Applications *(continued)*

Day 0

Seed MOCKII/MDR1 into HTS 96-well Transwell® inserts

Day 4

Seed LN-229 cells into Corning® Spheroid microplate

Day 5

Combine HTS 96-well Transwell inserts into a spheroid microplate and expose to drugs for 2 hours. After drug incubation, remove Transwell insert and test for monolayer integrity. Culture spheroids for 2 additional days.

Day 7

Assay spheroids with CellTiter-Glo® 3D

Figure 15. Immune Oncology Model Schematic

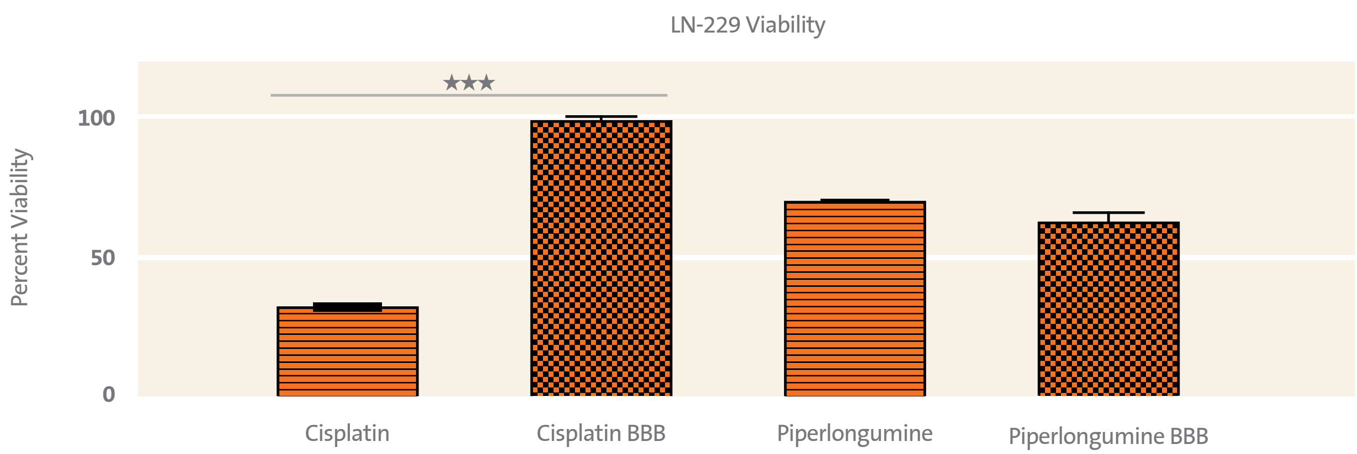


Figure 16. 3D glioma spheroid cytotoxicity assay. LN-229 cytotoxicity with, or without, blood brain barrier (BBB) surrogate. Percent viability of LN-229 spheroids 48 hours after 2-hour 250 µM drug exposure through Transwell inserts with, or without, a BBB. Viability was assessed by normalizing buffer controls to 100% viability. Data shown the average of 3 independent studies, N = 30 with 1-way ANOVA with Bonferroni's post-test. *** = $p<0.0001$.

SCIENTIFIC RESOURCES

SOLID SCAFFOLDS

1. Permeable Supports Selection Guide (Corning Guide CLS-CC-027)
2. hTERT-immortalized and Primary Keratinocytes Differentiate into Epidermal Structures in 3D Organotypic Culture (Corning Lit. Code CLS-AN-424)
3. Cell Migration, Chemotaxis and Invasion Assay Protocol (Corning Protocol CLS-AN-061)
4. Screening of Anti-metastatic Compounds by a Fluorescence-based Tumor Cell Invasion Assay (Corning Application Note CLS-DL-CC-076)

Visit **www.corning.com/3D** to access all Corning scientific resources.

Corning Tools Work Best Because They Work Together

Many Corning 3D tools can be used together to study physiological mechanisms. For example, Corning® Matrigel® matrix or Collagen, work with our permeable supports, as well as with multiwell plates and spheroid microplates for organoid and cancer biology (Corning Application Note CLS-AN-464).

One novel model that combines these 3D tools involves using Corning spheroid microplates with HTS Transwell®-96 well permeable supports for the study of a variety of complex tumoricidal events. Another unique model is high throughput brain tumor testing which traditionally requires testing for cytotoxic compounds in addition to testing for a drug's ability to pass the blood brain barrier (BBB). By combining the Corning spheroid microplates and HTS Transwell-96 well permeable supports, you can test tumor cytotoxicity and BBB permeability – all in one easy to use, 3D, high throughput assay (Figures 15 and 16, Corning Application Note CLS-AN-505).

Product Selection Chart by Application Area

With so many 3D tools available, it's not always clear where to start. We've put together this reference chart to help guide you towards the available Corning products based on your application. In some cases, you can use a proven combination of Corning products to achieve more complex, *in vivo*-like models. All Corning solutions listed are amenable to automation.

Application Areas		Cell Biology		
		Co-culture	Motility Models	Metastasis
Corning Product	**Environment Type**			
Corning® Spheroid Microplates	Scaffold-free Spheroid Models	●	●	●
Corning Elplasia® 12K Flask	Scaffold-free Spheroid Models			
Corning Elplasia 12K Open Well Plate	Scaffold-free Spheroid Models			
Corning Elplasia Plates	Scaffold-free Spheroid Models	●	●	●
Corning Ultra-Low Attachment (ULA) Surface-coated Microplates	Scaffold-free Spheroid Models	●		
Corning 3D Clear Tissue Clearing Reagent	Scaffold-free Spheroid Models	●		
Corning Matrigel® Matrix	Natural Hydrogel and ECM Scaffold Models	●	●	●
Corning Matrigel Matrix for Organoid Culture	Natural Hydrogel and ECM Scaffold Models	●		●
Corning Matrigel Matrix-3D Plate	Natural Hydrogel and ECM Scaffold Models	●		●
Corning Collagen I, High Concentration	Natural Hydrogel and ECM Scaffold Models			
Corning Synthegel® 3D Matrix Kits	Synthetic Hydrogel and ECM Scaffold Models			●
Transwell® and Falcon® Permeable Supports	Solid Synthetic Scaffold Models	●	●	●

Proven Corning Product Combinations

Corning Product	Environment Type	Co-culture	Motility Models	Metastasis
HTS Transwell-96 well Permeable Supports with Corning Spheroid Microplate	Solid Synthetic Scaffold and Scaffold-free Models	●	●	●
Corning Spheroid Microplates or Elplasia Plates with Corning Matrigel Matrix	Scaffold-free Spheroid, Natural Hydrogel, and ECM Scaffold Models	●	●	●
Transwell and Falcon Permeable Supports with Corning Matrigel Matrix	Solid Synthetic Scaffold, Natural Hydrogel, and ECM Scaffold Models	●	●	●

Cancer Research			Toxicity Screening			Regenerative Medicine	
Angiogenesis	Organoids	Tumor Biology	Bioprinting	Organoid and Organotypic Tissue Models	Immunology	Cellular/ Stem Cell Differentiation	Tissue Engineering
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A Commitment to 3D and You

Corning Life Sciences has more than 30 years of experience delivering real 3D innovations – products, platforms, protocols, applications, education, and support. Our experts work with you to overcome your most complex 3D challenges. Our solutions are designed to help you accelerate research in critical areas such as cancer biology, tissue engineering, and regenerative medicine – to bring safe, effective drugs and therapies to market in less time with greater confidence. Find out how Corning can help you create more *in vivo*-like 3D models, conduct more biologically relevant experiments, and better predict how your next discovery will behave in the real world. Visit **www.corning.com/3D.**

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