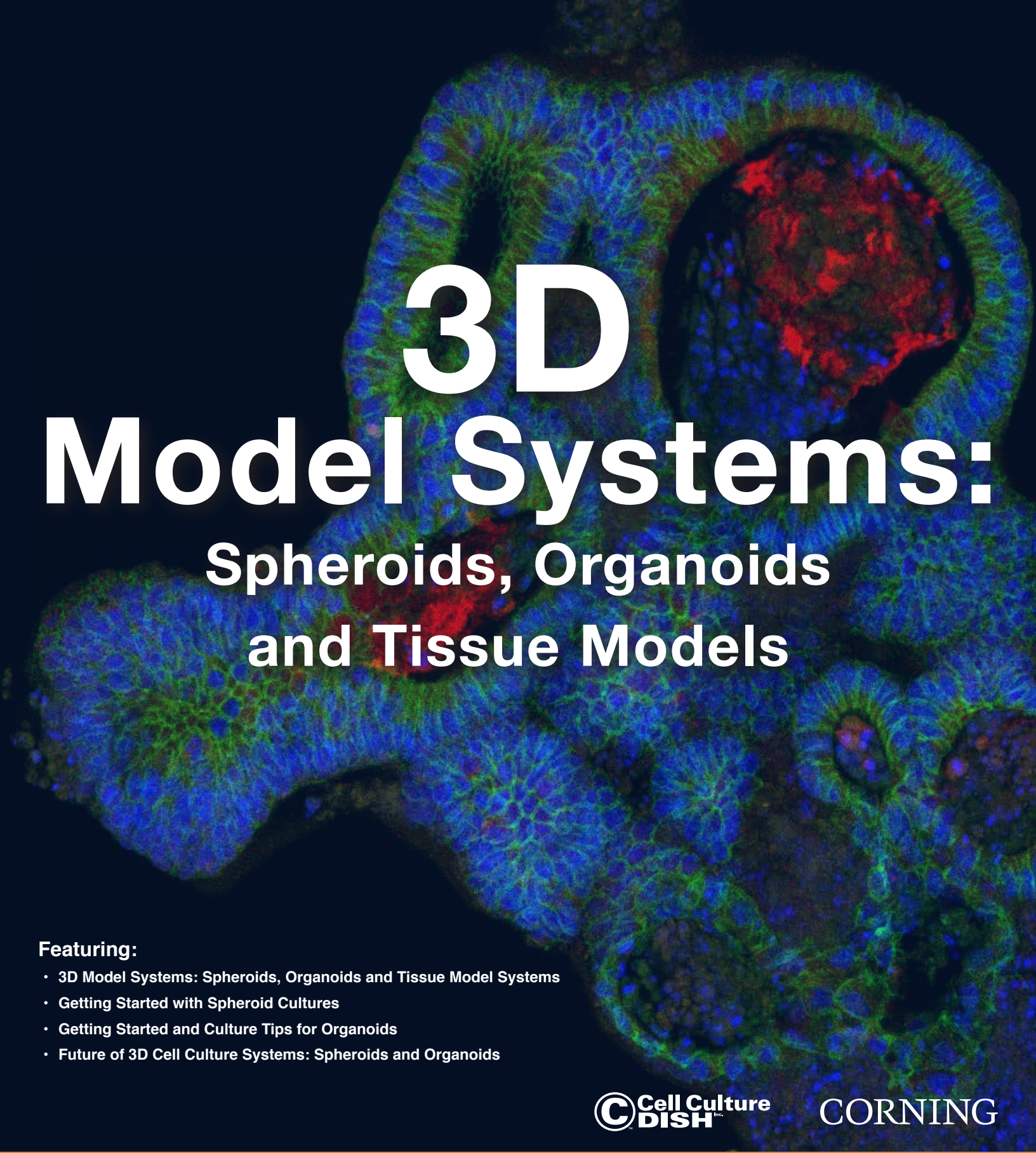

3D Model Systems:

Spheroids, Organoids and Tissue Models

Featuring:

- **3D Model Systems: Spheroids, Organoids and Tissue Model Systems**
- **Getting Started with Spheroid Cultures**
- **Getting Started and Culture Tips for Organoids**
- **Future of 3D Cell Culture Systems: Spheroids and Organoids**

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3D Cell Culture:
Spheroid, Organoid and Tissue Model Systems

Culturing cells outside of their natural environment in a laboratory under controlled conditions is essential to scientific research. Cell culture has utility in diverse areas from stem cell and cancer research, monoclonal antibody production, drug discovery, regenerative medicine, therapeutic protein production and modeling diseases. Cells used to establish *in vitro* cultures can be isolated from normal or diseased tissues, can be grown as adherent monolayers or in suspension, and can be established in two or three dimensions.

With the methodology established over a century ago, traditional two-dimensional (2D) tissue culture involves cells proliferating on flat substrates such as glass or polystyrene, resulting in monolayer cell cultures. However, this is a far departure from how cells grow *in vivo*, lacking critical cell-cell and cell-matrix interactions in their native microenvironment that drive their form, function and response to external stimuli. This has driven the development of more realistic and predictive three-dimensional (3D) cell culture models to improve the prognostic capability of *in vitro* testing systems. Culturing cells in 3D more accurately recapitulates the *in vivo* state where the cell morphology, interactions and tissue-specific architecture more closely resemble that of native tissues.

The terms spheroid and organoid are commonly used when discussing 3D cell culture systems. Spheroids and organoids in the most basic sense are both 3D structures made of many cells, so what's the difference? It can be particularly confusing since this terminology has been used interchangeably in the literature. Here, we look to demystify these two terms and discuss the distinct differences between them and their utility in scientific research as well as more advanced 3D tissue model systems like organ-on-a-chip technology.

Spheroids

The term 'spheroid' was first coined in the 1970s by Sutherland *et al*[1] when it was discovered that dissociated Chinese hamster V79 lung cells were able to form almost perfectly spherical cellular aggregates in suspension when grown in spinner flasks. Since then, spheroids have been generated from many primary cell types and cell lines in a multitude of ways from hanging drop plates, low cell attachment plates, micropatterned surfaces or rotating bioreactors to promote aggregation. Most commonly, the term spheroid is used in cancer research where tumor cells constitute the classical multicellular tumor spheroid model (MCTS), which has been invaluable to the study of solid tumor biology. The spherical geometry of the cells within the MCTS are characterized by an external proliferating region and an internal quiescent zone (caused by the gradient of nutrient and oxygen diffusion), which surrounds a necrotic core, mimicking the cellular heterogeneity observed in solid tumors. The unique cytoarchitecture of cells within the MCTS emulate *in vivo* cell morphology, proliferation, oxygenation, nutrient uptake, waste excretion and drug uptake. As such, the MCTS model is the most widely used preclinical screening tool for anticancer drug candidates[2].

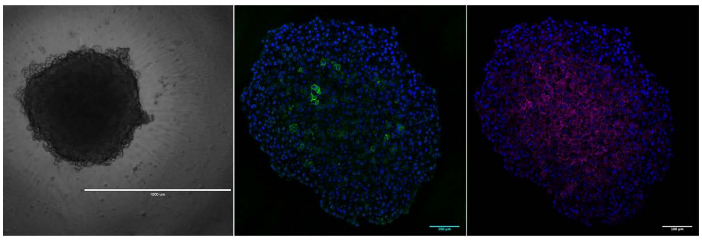

Figure 1. Brightfield and immunofluorescent (IF) photomicrographs of PANC-1 spheroids seeded at 5,000 cells for 7 days. IF images show spheroids labeled with antibodies for known hypoxia markers CA IX (green) and GLUT-1 (purple) which are preferentially located towards the center of the spheroids. Images were taken with a laser scanning confocal microscope using a 20X objective. Scale bar = 100 µm.

That said, the absence of strict terminology has resulted in confusion surrounding what should be classified as a a spheroid. For example, the term "organoid", now defined as "mini-organs", has been used in the past to describe 3D structures spontaneously formed by colon carcinoma cell line LIM1863, for 3D cultures of tumor cells embedded in basement membrane–like gel, and even the MCTS[2].

Organoids

Organoid is a general term that has been around for many years. In the past, it was defined as an aggregation of cells that contained differentiated cells with some tissue-like structures[3], which is what we now categorize as spheroids. In the recent resurgence of organoid research, organoids are are now defined as a 3D structure grown from stem cells or organ progenitors consisting of organ-specific cell types that self-organizes through cell sorting and spatially restricted lineage commitment mimicking at least one function of tissue or organ[4].

Organoids (Figure 2) can be classified into those that are tissue-derived and those that are stem cell-derived. Tissue-derived organoids typically originate from adult tissues while stem cell-derived organoids are established from pluripotent stem cells. As with their *in vivo* counterparts, organoids contain multiple cell types organized in structures that resemble the organ of interest and exhibit some of the organ function. Organoids representing different organ systems have been generated, such as the brain, liver, thymus, thyroid, lung, pancreas, intestines and heart[5]. These models have been used to study organ development, perform drug discovery/screening, model diseases, for toxicity testing and as therapeutic tools.

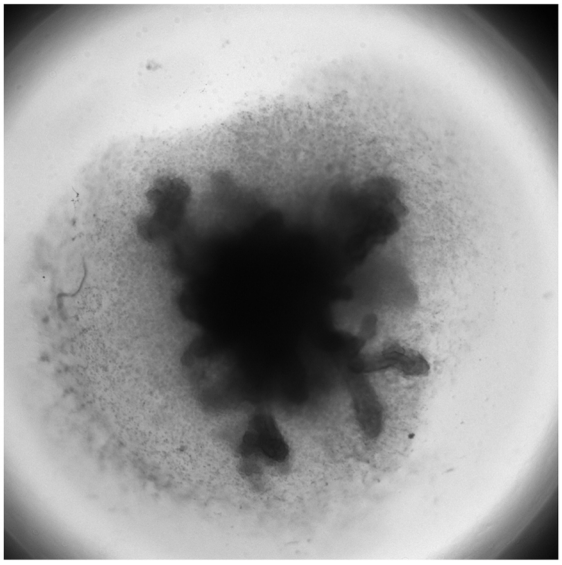

Figure 2. Human iPSC-derived 5 1/2-week-old Intestinal organoids formed using 96-well Corning® spheroid microplate. Image taken with 2X objective using Thermo Fisher CellInsight CX7.

Generally, a higher order of self-assembly is found in organoids compared to spheroid cultures, which are more simplistic, possessing little or no relevant tissue structures. The formation of organoids is more dependent on the presence of biological or synthetic matrices. Interaction between the cell and the extracellular matrix (ECM) is essential for survival, proliferation, differentiation and migration[6]. The ECM also provides a physical structure upon which cells can move and grow in 3D. Spheroid cultures are less dependent on matrices for their formation, able to form in both scaffold-free and scaffold-based conditions[7].

The reasons for pursuing 3D cell culture models is clear: it is simply a better way to grow cells to more closely resemble how they grow and interact with each other and their microenvironment in native tissues. Indeed, it is widely accepted that organoids and spheroids are superior to 2D monolayers but technical challenges and cost have hampered their adoption into high throughput screening protocols[8]. Current methodologies are bogged down by low experimental throughput, standardization issues and in some cases, poor reproducibility. Additionally, thorough validation/characterization of these models against standard 2D assays and animal models is required before they can be widely adopted for drug discovery protocols[8,9]. That said, the wealth of knowledge grows daily and advances in 3D printing and technology platforms will make it easier, faster and cheaper to put these platforms in place for large-format, high throughput protocols.

The reasons for pursuing 3D cell culture models is clear: it is simply a better way to grow cells.

3D Tissue Model Systems

While spheroid and organoid 3D cultures are a marked improvement over monolayer cultures, they still fail to mimic the full architecture of tissues, which includes vasculature and interstitial fluid flow. Emerging 'organ-on-a-chip' (OOC) technology may be able to address some of these limitations. By definition, OOCs are flexible polymer devices representing the convergence of microfluidics and tissue engineering[10]. Cells are cultured in continuously perfused micrometer-sized chambers, which creates physiologically relevant levels of of fluidic sheer force and provides nutrition, gas and waste transport to replicate *in vivo* vascularized tissues (Figure 3). The first of these was the lung-on-a-chip[11] designed by the Wyss Institute at Harvard University, which was used to study the effect of bacterial infection on lung function. Since then, tissue model systems have been designed for heart[12], kidney[13], artery[14], bone[15], cartilage[16], skin[17] and the female reproductive tract[18].

The higher degree of complexity and similarity of OOCs to native tissues make them excellent tools to elucidate organ-specific biomechanical and biochemical mechanisms and for use in drug discovery programs. This platform has the potential to decrease cost, replace animal testing and streamline drug discovery workflows to identify drug candidates that are safe and efficacious in humans. Ultimately, the goal would be a 'human-on-a-chip' system, where multiple organs are connected to assess the "whole body" response, to replace drug screening in cell culture and animal models. While the 'organ-on-a-chip' technology shows great promise, we are a long way from reaching that goal. They still lack many of the complexities observed in real tissues, therefore, more research is needed for them to become effective catalysts for drug discovery.

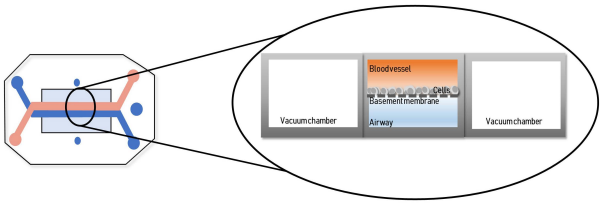

Figure 3. The most developed organ-on-a-chip is the lung-on-a-chip. The cross-section shows two hollow vacuum chambers flanking a central channel. These chambers apply vacuum force on the lung epithelial cells in the central chamber to simulate breathing. The central chamber contains lung cells on a porous membrane which separates the air and blood moving through the chamber to replicate the alveolar-capillary barrier in the lungs. Drugs can be added the blood to determine what effect the drug will have on how the cells stretch during breathing.

Footnotes

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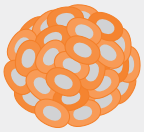
Spheroids vs. Organoids

What's the Difference?

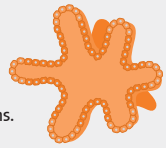
Spheroids and organoids both offer you the opportunity to create more complex three-dimensional models with the power to more accurately recreate *in vivo*-like tissue and organ conditions. Each one offers advantages and disadvantages. Which model works best for you? It largely depends on the type of application you're working on and the ultimate goal of your research. In either case, both spheroids and organoids bring incredible promise and potential to many critical areas of research.

OVERVIEW

Spheroids are a simple, inexpensive, easy way to model cells in 3D. The ability of spheroids to replicate solid tumors helps to accelerate drug discovery programs and improve our understanding of cancer biology.

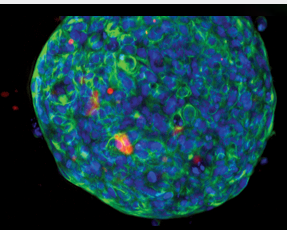


Organoids have become an increasingly popular option for scientists in disease modeling, cancer research, and drug screening because they resemble the composition and functionality of organs. These can lead to more *in vivo*-like results.



ORIGIN

Spheroids may be generated from a broad range of cell types resulting in tumor spheroids, embryoid bodies, hepatospheres, neurospheres, and mammospheres, among others. They may be made up of one cell type or multiple cell types.



Organoids may be generated from pluripotent stem cells and/or organ progenitors from normal or diseased adult tissue-specific stem cells.

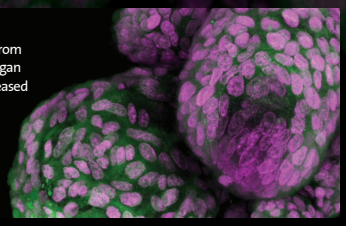
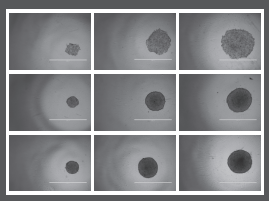


Photo Credit: MDI Laboratory, ME

MODEL ENVIRONMENT

Spheroids are typically created in a scaffold-free environment by placing cells into suspension colonies without the aid of extracellular matrices (ECMs) or other physical supports.



Organoids typically require a scaffold, such as a BME or ECM, which can be used to encapsulate cells to provide an ideal growth environment and supply biological cues to aid in self-organization.

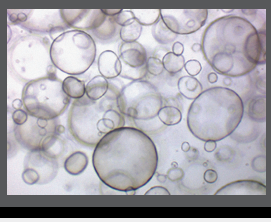
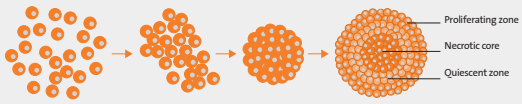


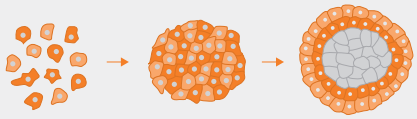
Photo Credit: L.A. Oosterhoff

POLARITY

Spheroids may or may not exhibit polarity.



Organoids exhibit polarity, cell migration, self-organizing into mini-organs.



APPLICATION AREA

Spheroid Research Areas

- Cancer biology
- Tumor modeling
- Stem cell research
- Immuno-oncology
- Liver toxicity modeling

Organoid Research Areas

- Organogenesis from stem cells
- Disease modeling including cancer
- Patient-specific therapies also known as personalized medicine
- CRISPR
- Immuno-oncology

Photo Credit: Lancaster, MA, et al., 2013

HOW TO GENERATE

Spheroids may be generated and cultured in suspension or in a low attachment culture environment such as Corning spheroid microplates or Corning® Elplasia® plates.

Organoids may be generated by mixing cells with an ECM such as Corning Matrigel® matrix and culturing in media containing specific growth factors to generate mini-organs of the kidney, thyroid, liver, brain, lung, intestine, prostate, and pancreas.

ADVANTAGES

Spheroids can develop metabolic gradients that create heterogeneous cell populations with superior cell-to-cell and cell-to-ECM interactions. They can also successfully mimic the microenvironment of a variety of diseased tissue types.

- High reproducibility
- Scalable to different plate formats
- High throughput screening (HTS) capability
- Co-culture ability

Organoids are more complex and *in-vivo*-like, resembling cell structures and microenvironments for more precise and targeted animal research and cell therapies. They are used for modeling cancer and organ development. Along with CRISPR, they allow for better genetic and drug screening disease models.

- Patient-specific
- *In vivo*-like complexity
- *In vivo*-like architecture
- HTS-enabled formats available

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Spheroid Models: Getting Started and Culture Tips

It has been well established that three-dimensional (3D) cell culture is better at replicating the physiological interactions between cells and their environment than traditional two-dimensional (2D) cell culture. In fact, many types of mammalian cells have the ability to self-aggregate into 3D aggregates called cellular spheroids when cultured in suspension or in a nonadherent environment. Common examples of spheroids include embryoid bodies, mammospheres, hepatospheres, and neurospheres. Perhaps the most widely utilized spheroid model is the multicellular spheroid model (MCTS) formed from cancer cells, which is commonly used as avascular tumor models to gain mechanistic insight into cancer invasion and metastasis and as an anti-cancer therapeutic screening tool. The heterogeneous population of proliferating and non-proliferating cells is exposed to a gradient of oxygen emulating the physio-chemical gradients found in solid tumors, therefore, making them more predictive *in vitro* models than 2D cultures. Additionally, they are also relatively easy, fast, versatile, and are inexpensive enough to enable high-throughput screening, which makes them attractive models for many applications (Figure1).

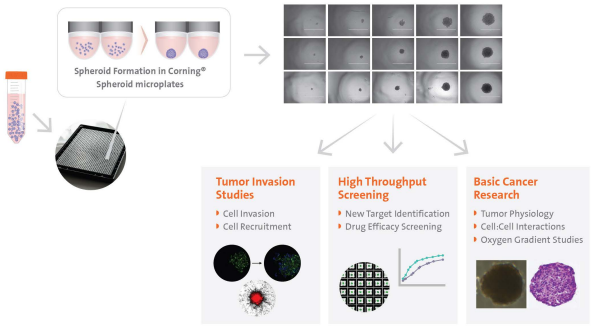

Figure 1. Potential workflow for multicellular tumor spheroids. Spheroids are easily formed in Corning® spheroid microplates and can then be used for downstream applications for basic research to high throughput screening.

Spheroid Generation

SCAFFOLD-FREE METHODOLOGIES

Scaffold-free methods are commonly used to generate spheroids where cells are cultured to prevent adhesion and promote aggregation such as hanging drop technology, rotary cultures, and bioreactors or the use of specialized non-adherent cell cultureware like the Corning® Ultra-Low Attachment microplates. Traditional methods such as the hanging drop for spheroid formation take advantage of cell sedimentation in droplets of cell culture medium to promote cell aggregation. While this method is effective, it can be time-consuming and difficult to execute reproducibly. Corning® spheroid microplates have opaque side walls, a unique clear round well-bottom design, and an Ultra-Low Attachment (ULA) surface coating that is hydrophilic, biologically inert, and non-degradable, which enables the rapid and highly reproducible formation and growth of a single spheroid per well. When these two methods were used to form human liver microtissue spheroids, the spheroids produced were comparable in size, longevity, and functional tissue characteristics (Figure 2).

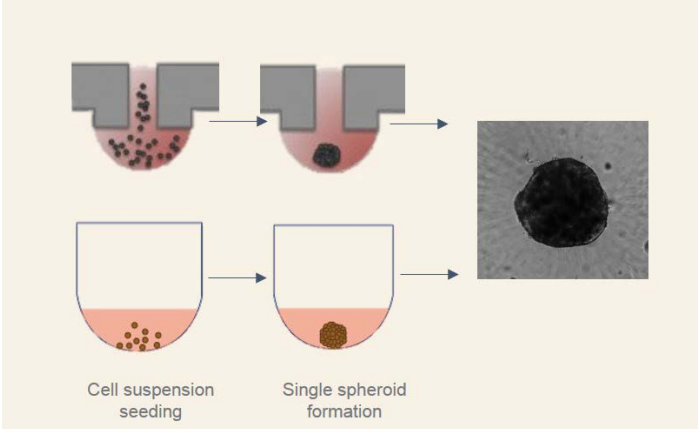

Figure 2. Spheroid formation in (A) hanging drop technique and (B) spheroid microplate with Ultra-Low Attachment surface coating.

Currently, Corning spheroid microplates are available in 96-well (Corning Cat. Nos. 4520 and 4515), 384-well (Corning Cat. Nos. 3830, 3830BC, and 4516), and 1536-well (Corning Cat. No. 4527) formats.

Below (Figure 3) are some examples of different cell types forming spheroids using different Corning® microplate formats.

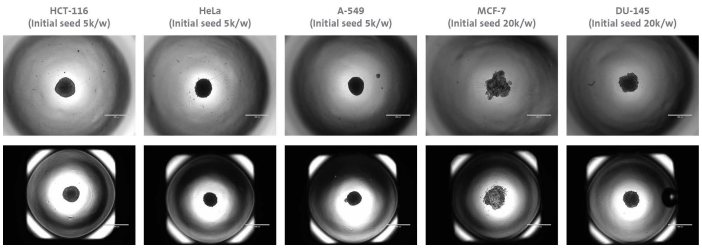

Figure 3. Multiple cell lines were shown to form single, centered spheroids in both the 96-well (top row) and 384-well (bottom row) microplate formats. Micrographs were taken after incubating samples for 72 hours (2x magnification, scale bar represents 800 µm).

HIGH THROUGHPUT SCREENING

In order to meet the demands of most drug discovery operations, a high throughput system is essential to success. The Corning® 1536-well spheroid microplate allows the formation of 1536 uniform, single spheroids that can be assayed via imaging, fluorescence, or luminescence directly in the microplate. With a maximum volume of 14 µL per well, accurate liquid handling is essential to the generation of quality data. Corning® has established an automated method for seeding, dosing, and assaying spheroids in a 1536-well microplate format using INTEGRA Biosciences liquid handling instruments.

- The VIAFILL is designed for rapid bulk liquid dispensing at volumes as low as 0.5 µL and can be fully automated with a plate stacker accessory
- The VIAFLO384 is a handheld electronic pipettor which enables transfer into 24-, 96-, 384-, and 1536-well microplates in a fast, compact, and easy-to-use manner

Helpful Tips

When using the Corning® spheroid microplates, the size of the spheroids is dependent on factors such as initial plating densities, cell-type, duration of growth phase in a spheroidal format and the desired size of spheroid at the time of assessment. A single cell suspension is required to seed into the microplates. If the cells used are particularly sticky, a 40 µm cell strainer may be required to achieve a single cell suspension. If cells are seeded manually, care should be taken to avoid touching the bottom or sides of the microwells with pipet tips to avoid damaging the ULA surface coating. The maximum working volumes for each microplate format are as follows:

96-well:	75 to 200 µL per well (maximum working volume: 300 µL)
384-well:	25 to 75 µL per well (maximum working volume: 90 µL)
1536-well:	5 to 10 µL per well (maximum working volume: 14 µL)

Many cell lines will form spheroids within a 24-hour period. However, some may require a 15-minute incubation post-seed or centrifugation at low speed (i.e. 150 x g for 5 mins) to promote cellular aggregation. The addition of media supplements such as methylcellulose or other cell types such as fibroblasts can help with cells that tend to form looser aggregates as opposed to spheroids. Optimization of the protocol is recommended for your cell type of choice.

Depending on the cell type and the duration of the spheroid culture, a media exchange step may be required. In order to leave spheroids undisturbed during media changes, sides of wells should be used to remove and add media. It is recommended that 10 to 20 µL volume be left in the well for the 96-well and 384-well formats and 5 µL for the 1536-well format so the spheroids are not left dry during the media change. Automation will be required for liquid handling of the 1536-well format.

Imaging and Staining Spheroids

Another advantage to using the ULA microplates is that spheroids may be generated, cultured, and assayed for fluorescent or luminescent signals in the same plate without the need to transfer the spheroids. The unique well geometry and opaque side walls of Corning® spheroid microplates reduces background fluorescence/ luminescence, making them suitable for automated imaging of spheroids for high content screening approaches. The plate design also includes a unique well shield to minimize well-to-well cross-talk.

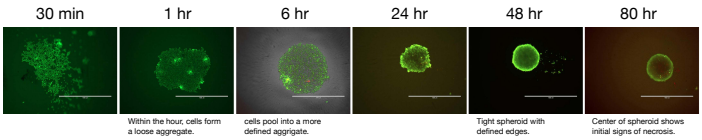

Figure 4. Formation of spheroids within the Corning® microplate using HT-29 cell line over the course of three days. The fluorescence microscopy images were obtained using LIVE/DEAD® Viability/Cytotoxicity Kit (Thermo Fisher Cat. No. L3224).

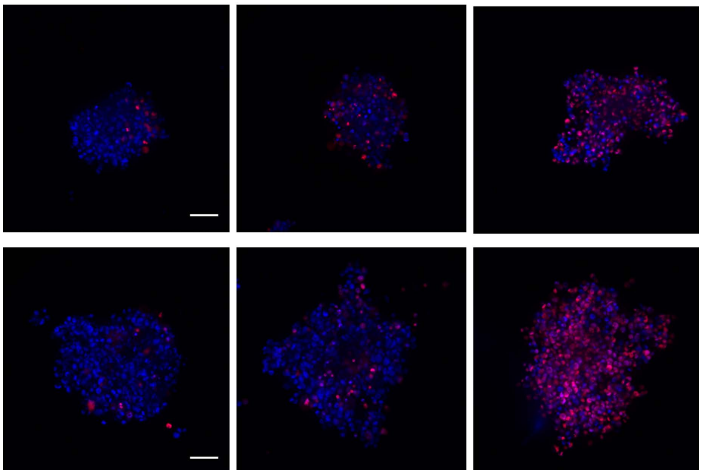

Figure 5. High-content imaging of spheroids within 1536-well spheroid microplate. Representative z-stack images of DU-145 (top) and Panc-1 (bottom) spheroids exposed to cisplatin (83 mM left, 0.025 mM middle, and 0 mM right) for 24 hours and stained with Hoechst (blue) and propidium iodide (red) to assess cell viability. Images taken with Thermo Fisher CellInsight CX7 confocal imager using a 10X objective.

Helpful Tips

The Promega CellTiter-Glo® 3D Cell Viability Assay (Promega Cat. No. G9683) for cell proliferation and cytotoxicity assay screening is recommended. Figure 6 shows results from cell proliferation and cytotoxicity assay of the human prostate cell line DU145 in response to Doxorubicin treatment.

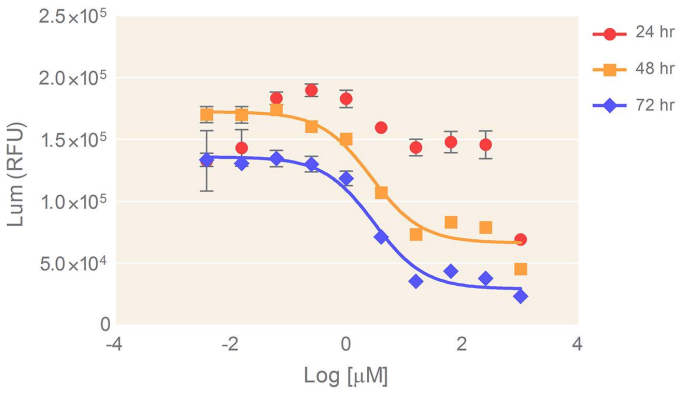

Figure 6. DU145 (human prostate cancer cell line) spheroid dose response to Doxorubicin over a 72-hour treatment in a 384-well Corning spheroid microplate.

There are a number of commercial instrumentation compatible with spheroid microplates:

Manufacturer	Instrument Name
BioTek® Instruments	Cytation® cell imaging multi-mode reader
Molecular Devices	ImageXpress® Micro XLS automated imaging system
Nexcelom	Celigo® S image cytometer
Thermo Fisher	CellInsight™ CX7 high content system
TTP Labtech	Acumen® Cellista laser scanning image cytometer

The dimensions of the Corning® microplate formats are listed below for easy instrumentation setup:

	Spheroid Microplate (Corning Cat. No.)	Well Volume (µL)	Well Depth (mm)	Well Diameter (Top/ Bottom) (mm	Plate Length (mm)	Plate Width (mm)	Plate Height (mm)	A1 Row Offset (mm)	A1 Column Offset (mm)	Well Center to Well Center Spacing (mm)	Flange or Skirt Height (mm)	Stack Height	Well Bottom Elevation (mm)	Well Bottom Thickness (mm)	Well Bottom Area (cm^2)	Distance to Bottom of Plate
96-well	4515, 4520	300	12.36	6.85/6.35	127.6	85.5	14.2	11.2	14.27	9	6.096	13.12	1.86	0.0875	N/A	1.86
384-well	4516, 3830, 3830BC	90	12.54	3.63/2.82	127.6	85.5	14.2	8.99	12.13	4.5	2.41	12.95	1.81	0.0875	N/A	1.81
1536- well	4527	14.67	6.2	1.7/1.45 (Sq)	127.6	85.5	8.0	7.87	11.01	2.25	2.16	6.70	1.81	0.1016	N/A	1.81

The physiological relevance and predictive nature of spheroids is helping to bridge the gap between *in vitro* and *in vivo* models

Oncology Models

The Corning® HTS-Transwell permeable supports (Corning Cat. No. 3387) are synthetic scaffold inserts with microporous membrane bottoms that can be fitted on the spheroid microplates. The membranes of these supports are available with small pore sizes (i.e. 0.4-8.0µm micron) that permits movement of molecules and/or the cells themselves across the membrane, which are located on opposite sides of the membrane. This format allows researchers to investigate cellular interactions and is amenable to tumor invasion studies.

CO-CULTURE MODELS

The interaction of tumor cells with other cell types in the tumor microenvironment can be one predictor of a possible therapeutic outcome. For example, evidence shows stromal cells induce chemo-resistance, protecting tumor cells from the toxic effects of anti-cancer drugs. Endothelial cells and the associated vasculature provide blood supply to the tumor allowing for its proliferation but are also responsible for carrying therapeutic compounds to the cancer cells. Therefore, the ability to model these cellular interactions *in vitro* using 3D co-culture models is very useful to study complex interactions between cancer cells and ancillary cell types to determine their impact on tumor growth, vascularization, metastasis and response to chemotherapeutic agents.

In one such study, breast cancer cells co-cultured with stromal cells with and without direct contact was studied to determine if these cellular interactions affected the response of the cancer cells to therapeutic compounds. When looking at direct cell interactions, breast cancer-associated fibroblasts (CAF), peripheral blood mononuclear cells (PBMCs) and cancer cells from the GFP-expressing breast cancer cell line BT-474 were added directly to spheroid microplates and cultured. To look at indirect interactions, the stromal cells were seeded onto the Corning® HTS Transwell-96 inserts which was seated on top of a spheroid microplate. The BT-474 cells were seeded into the spheroid microplate, separated from the stromal cells by the Transwell inserts with a 1µm pore size. The viability of the BT-474 cells was assessed before and after exposure to a chemotherapeutic agent in the control BT-474 monoculture, the direct and indirect co-cultures (Figure 7).

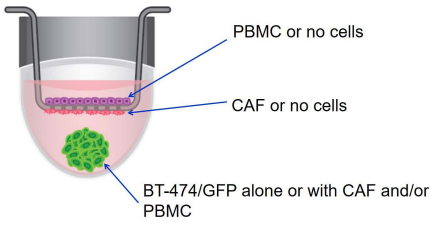

Figure 7. Corning® HTS Transwell supports fitted onto the spheroid microplate allows investigation of cellular interactions between stromal and cancer cells and its impact on response to cancer therapeutics.

TUMOR INVASION STUDIES

Traditionally, tumoricidal activity and immune evasion have been studied by utilizing 2D systems, which may not accurately reflect the complexity of a tumor in a 3D environment. The Corning® spheroid microplates to generate uniform 3D spheroids combined with Corning® HTS-Transwell inserts enable researchers to investigate immune cell homing, tumor cytotoxicity, and tumor immune evasion in an easy-to-use 3D high throughput assay (CLS-AN-425). In one study, the ability of natural killer (NK) cells to infiltrate tumor spheroids generated from A549 cells (adenocarcinoma human alveolar basal epithelial cells) was examined. Figure 8 shows the workflow for the migration and tumor cytotoxicity assays.

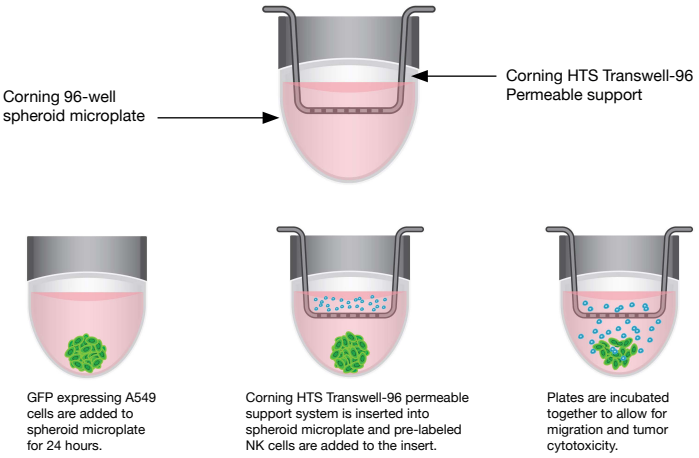

Figure 8. Corning® HTS Transwell supports fitted onto the spheroid microplate allows for migration and tumor invasion studies to better understand *in vivo* mechanisms of immune cell functions.

Undoubtedly, 3D spheroid cultures are an important addition to the research toolbox with huge translational potential, particularly for cancer and stem cell research. Their physiological relevance and the predictive nature of spheroids is helping to bridge the gap between *in vitro* and *in vivo* models, helping to drive novel discoveries and innovation.

Cell Culture DISH Inc.

CORNING

Organoid Models: Getting Started and Culture Tips

An organoid is defined as an *in vitro* 3D cellular cluster derived from primary tissue (lineage restricted adult stem cells), embryonic stem cells (ESCs), or induced pluripotent stem cells (iPSCs), capable of self-renewal, self-organization, showing similar organ functionality as the tissue of origin. Their similarity in composition and function to organs *in vivo* makes them invaluable 3D models to study organogenesis, disease modeling, and patient-specific therapies. Organoids recapitulate a large number of biological parameters including the spatial organization of heterogeneous tissue-specific cells, cell-cell interactions, cell-matrix interactions, and certain *in vivo* physiological functions generated by tissue-specific cells within the organoid[1]. Organoids bridge a gap between 2D and animal model systems by providing a stable system amenable to extended cultivation and manipulation. As such, researchers have created physiologically relevant organoid models for many organs. That said, working with organoids is not without its challenges. High quality cell culture tools are important to ensuring success when researchers are looking to incorporate organoids into cell culture workflows.

It is well known that cells in living tissues interact with other cells as well as the tissue microenvironment for survival, proliferation, differentiation and migration. The extracellular matrix (ECM) provides biological cues to support cell function as well as physical support for cells to grow and move in 3D. The presence of biological or synthetic scaffolds is important for the formation of organoids as they mimic the ECM for a more "*in vivo*–like" cell culturing system. Ultimately, the choice of scaffold will depend on the tissue of interest and research application. There are many variables to consider, such as different porosities, permeability, surface chemistries, and mechanical characteristics, which need careful consideration for successful organoid cultures.

Natural Scaffolds

Corning® Matrigel® Matrix

Corning® Matrigel® matrix is an ECM-based natural hydrogel commonly used to form 3D organoids (Figure 1). This reconstituted basement membrane is extracted from Engelbreth-Holm-Swarm (EHS) mouse tumors providing the physical support and chemical cues that resemble the *in vivo* ECM. Matrigel contains molecules important for cell function, differentiation and attachment (i.e. laminin, collagen IV, heparin sulfate proteoglycan, and nidogen/entactin). Matrigel is often used to generate cancer organoids because the components in the matrix can activate signaling pathways in cancer cells that control angiogenesis, cancer cell motility, and drug sensitivity.

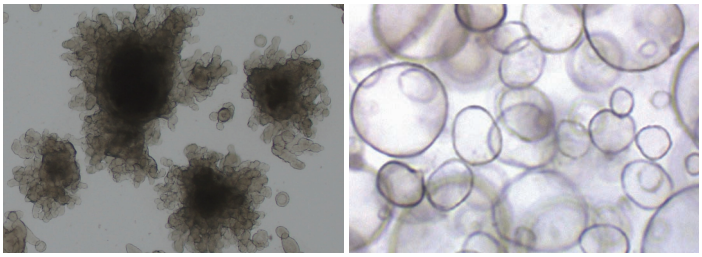

Figure 1. Left image features human rectal organoids from a cystic fibrosis cell source cultured for 10 days in Corning Matrigel matrix. Courtesy of MDI Biological Laboratory. Right image features Human liver organoids in Corning Matrigel matrix (perm. L.A. Oosterhoff).

EHS-derived matrices have been extensively published for generating viable and sustainable organoids used in drug discovery and toxicological studies. For a list of recent publications where Matrigel matrix is used for establishment of organoids, please visit www.corning.com/catalog/cls/documents/citations-references/CLS-AN-503.pdf.

An example schematic for cerebral organoids generated from human pluripotent stem cells with Matrigel matrix is shown in Figure 2.

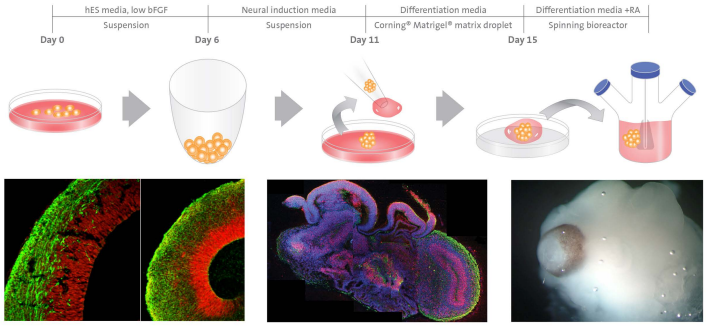

Figure 2. Cerebral organoid culture system. (A) Schematic of the culture system used to generate cerebral organoids. Example images of each stage are shown: bFGF, basic fibroblast growth factor; hES, human embryonic stem cell; hPSCs, human pluripotent stem cells; RA, retinoic acid. (B) A comparison between 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. (Lancaster, et al., 2013).

For tips and tricks on using Corning Matrigel matrix, please visit: www.corning.com/worldwide/en/products/life-sciences/resources/webforms/the-ultimate-guide-to-corning-matrigel-matrix.html.

COLLAGEN TYPE I

Corning® Collagen Type I is a natural hydrogel found in stromal compartments in the dermis, tendon, and bone often isolated from bovine skin, rat tail tendon, and human placenta. The type I molecule of collagen is a heterotrimer of 300 nm length and is composed of two alpha$_1$(I) chains and one alpha$_2$(I) chain. It supports *in vivo*-like 3D growth and differentiation and also interacts with receptors to modulate the expression of genes involved in cell invasion, sensitivity to anti-cancer drugs, cell proliferation, and cell migration. Collagen I has been successfully used to culture intestinal, pancreatic, mammary, brain and salivary organoids.

Sachs *et al*[2] found that with collagen I, murine tissue-derived intestinal epithelial organoids aligned and fused to form macroscopic hollow tubes with a single lumen and budding crypt-like domains, more closely recapitulating the native architecture of the small intestine. This is in contrast to traditional Matrigel matrix derived intestinal organoids which form as microscopic single budding cysts. It is postulated that these collagen I-derived tubes may be better for investigating drug effects and development of the intestinal epithelium, where a larger overall size and/or a bigger villus compartment would be advantageous. This also highlights how the choice of scaffold can greatly affect the 3D structure of the resulting organoid.

For answers to frequently asked questions when using Collagen I, please visit: www.corning.com/catalog/cls/documents/faqs/faq_DL_AC_004_Collagen_Products.pdf.

Hydrogels such as collagen I and Matrigel matrix can be used to engineer organoids with precise geometries using novel 3D bioprinting. When mixed with cells, biological inks (bio-inks) can create organoids that reproduce the structure of native tissue by allowing control over the spatial positioning of cells in 3D. Corning 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 are suitable culture of the bio-printed tissue that can be subse-quently used in drug discovery testing and disease modeling (Figure 3).

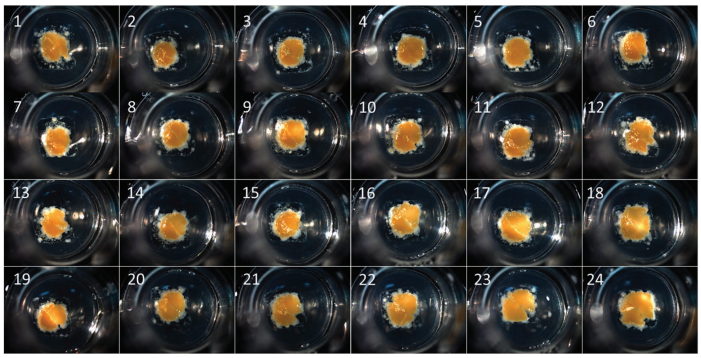

Figure 3. 3D human liver tissue bioprinted on Corning Transwell permeable supports. Image courtesy of Organovo.

Synthetic Scaffolds

In cases where bioactive compounds, such as growth factors, can potentially interfere with the specific cell behaviors or responses, synthetic hydrogels are an excellent alternative for 3D cell culture applications. Matrigel matrix is animal-derived and generally not well defined with batch-to-batch variability, which can make controlling the ECM environment and downstream applications difficult. To circumvent this, essential components identified in the native ECMs can be incorporated into synthetic polymer scaffolds to produce custom, defined and tunable scaffolds tailored specifically for the organoid system of choice. Synthetic hydrogels are composed of biologically inert, pathogen-free molecules that provide structural support for a variety of cell types. They are simple to process and manufacture making them amenable to large-scale, high-throughput workflows.

CORNING® PURAMATRIX™ PEPTIDE HYDROGEL

PuraMatrix™ is a synthetic peptide mixture capable of self-assembly into a 3D hydrogel with nanometer-scale fibrous structure. When combined with bioactive molecules (i.e. growth factors, ECM components, etc), it creates a defined 3D environment for cells and has been shown to promote differentiation of hepatocytes and neural cells. PuraMatrix™ (RADA-16) also has utility for 3D encapsulation applications, tumor cell migration and invasion, angiogenesis assays and *in vivo* analyses of tissue regeneration. The transparent nature of this hydrogel makes it easy to visualize samples using standard staining and microscopy techniques. This peptide mixture was found to be superior to collagen I as a scaffold for ovarian cancer cell lines to adhere and migrate in a 3D environment[3].

For more information on PuraMatrix™ please visit: www.corning.com/media/worldwide/global/documents/faq_DL_028_Corning_PuraMatrix_Peptide_Hydrogel.pdf.

Organoids representing many different organs allow researchers to better understand developmental processes as well as to establish more accurate disease models. Human diseases that are poorly recapitulated in animal models can be studied with patient-derived organoids. The hope is that organoids could be used as a tissue source for transplantation into patients; however, many obstacles still need to be addressed along the way, such as proper maturation and the lack of vascularization. Stem cell-derived organoids may only recapitulate the first few months of development, but not the stages beyond, therefore, potentially lacking in cell types of interest for re-searchers. In this case, tissue

organoids generated from isolated adult stem/progenitor cells or resected fragments of organ tissues (i.e. intestinal crypts, liver, or pancreas ducts) may be more suitable. Currently, the size of the organoids is constrained by the maximum distance of diffusion for nutrients and gas exchange. Larger organoids are desired for certain applications because their increased complexity improves their biological relevance. Several strategies being implemented include co-culturing organoids with endothelial cells to develop vasculature and using microfluidics to generate "organ-on-a-chip" systems (Figure 4) to improve nutrient delivery to the cells allowing larger, more complex organoids to be developed.

While this area of 3D models is relatively new, organoids have emerged as a powerful tool to complement existing animal and cell line models offering unprecedented insight into human development and disease. This opens up innovative approaches to medical research, drug discovery, toxicology testing and for advancing precision and regenerative medicine bearing great promise for the future.

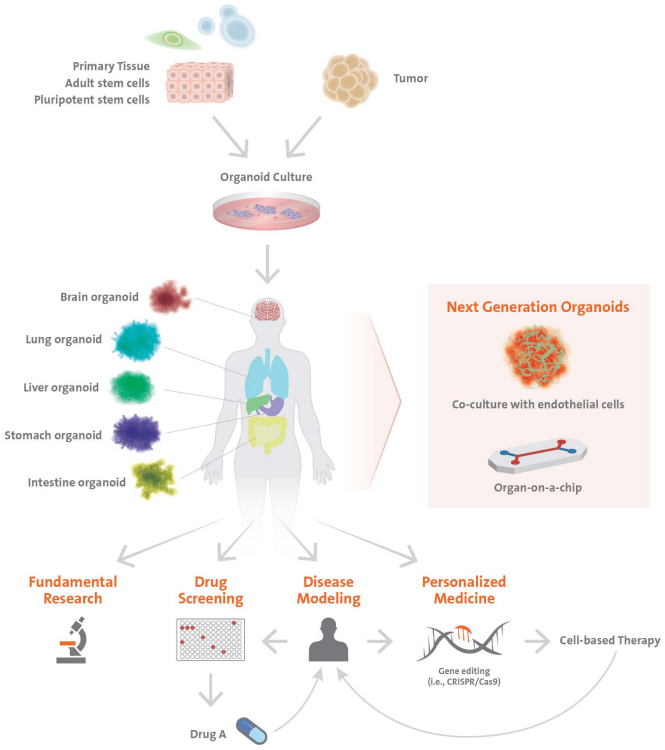

Figure 4. Schematic of organoid culture workflow. Organoids can be established from stem cells and tumor/cancer cells representative of many organs in the body including the brain, intestine, stomach, liver and lung. They can be used for many applications including basic research, identification of drug targets and personalized medicine. Gene editing technology opens the door to cell-based therapies where mutations can be corrected in patient-derived organoids and then re-infused into the patient as treatment. Next generation organoid cultures include co-culture with endothelial cells to improve vascularization and organ-on-a-chip where perfusion dynamics improve nutrient and oxygen exchange.

Footnotes

1. Yin, X., Mead, B. E., Safaee, H., Langer, R., Karp, J. M., & Levy, O. (2016). Engineering Stem Cell Organoids. Cell stem cell, 18(1), 25–38. doi:10.1016/j.stem.2015.12.005
2. Sachs, N., Tsukamoto Y., Kujala P., Peters, P.J., Clevers, H. (2017). Intestinal epithelial organoids fuse to form self-organizing tubes in floating collagen gels. Development, 144(6): 1107–1112. doi: 10.1242/dev.143933
3. Yang, Z., & Zhao, X. (2011). A 3D model of ovarian cancer cell lines on peptide nanofiber scaffold to explore the cell-scaffold interaction and chemotherapeutic resistance of an-ticancer drugs. International journal of nanomedicine, 6, 303–310. doi:10.2147/IJN.S15279

A Look Towards the Future of 3D Cell Culture

A panel discussion

Panel Members:

- **Elizabeth Abraham**, Ph.D., Senior Product Manager, Corning Life Sciences
- **Audrey Bergeron**, Applications Scientist, Corning Life Sciences
- **Debbie King**, Scientific Technical Writer, Cell Culture Dish
- **Brandy Sargent**, Editor in-Chief, Cell Culture Dish and Downstream Column (Moderator)
- **Hilary Sherman**, Senior Scientist, Corning Life Sciences

Introduction and Overview

Debbie King

Researchers have used 2D cell culture since the early 1900s, but we know that growing cells on planar surfaces have some drawbacks. Cells grown *in vitro* in 2D space don't behave like cells found *in vivo*. They lack critical cell-cell and cell-matrix interactions that drive their form, function and response to external stimuli. This limits their prognostic capabilities. More recently, 3D cell culture techniques have become popular because the cell morphology, interactions and tissue-specific architecture more closely resembles that of *in vivo* tissues. Spheroids, organoids and more complex 3D tissue systems, such as 'organ-on-a-chip' are examples of 3D cultures used by researchers to model native tissues.

Spheroids are simple, widely used 3D models that form based on the tendency of adherent cells to aggregate and can be generated from many different cell types. The multicellular tumor spheroid model is widely used in cancer research.

Organoids are more complex 3D aggregates, more like miniaturized and simplified versions of an organ. They can be tissue or stem cell-derived with the ability to self-organize spatially and demonstrate organ-specific functionality.

More complex yet, are technologies like organ-on-a-chip, which is a multi-channel 3D microfluidic cell culture system that mimics whole organ function with artificial vasculature. Cells are cultured in continuously perfused micrometer-sized chambers that recreate physiologically relevant levels of fluidic sheer force to allow for gas, nutrient and waste transport to the cell just as is observed *in vivo* vascularized tissues.

How are spheroids impacting cancer research and what do you see as future applications for the technology?

Audrey Bergeron

Spheroids can be an improved model for cancer in the lab compared to standard 2D cell culture. When cancer cells are cultured as spheroids, they are able to maintain the shape, polarity, genotype, and heterogeneity observed *in vivo* (1). This allows researchers to create models that are much more reflective of what's going on in the body. For a simple example, if you think about drug penetration into a 2D monolayer of cells it's completely different from drug penetration into a solid tumor. In a 2D monolayer each cell is exposed to the same concentration of drug whereas in a spheroid, like a solid tumor, there are gradients of drug exposure.

More and more we're seeing researchers move away from cancer cell lines and move more toward specialized cancer models such as patient derived models. The hope here is to find the appropriate therapies for each individual patient.

(1) Antoni, D., Burckel, H., Josset, E., & Noel, G. (2015). Three-dimensional cell culture: a breakthrough *in vivo*. International journal of molecular sciences, 16(3), 5517–5527. doi:10.3390/ijms16035517.

What tools and technologies are needed to fully realize the potential of spheroid culture models?

Debbie King

One of the key parameters for success with spheroid culture is controlling the size of the spheroids. It can be very difficult to get consistent, reproducible results if the starting spheroid culture is not uniform in size and shape. Cell culture tools available on the market now, such as ultra-low attachment plates, facilitate the formation of uniformly sized spheroids for many research applications from low to high throughput modalities.

Hilary Sherman

There always needs to be a little bit of a balance between throughput and complexity in terms of creating models for research. That's why there are so many options available for 3D research. Low attachment products such as Corning® spheroid microplate and Eplasia® plates are great for creating high throughput 3D models, but can lack some complexity. Organ-on-a-chip and hydrogel models add biological complexity to the model, but are typically not as high throughput.

What is the most interesting achievement so far in using organoids?

Elizabeth Abraham

Personalized medicine. Due to their unique ability of unlimited self-renewal, organoids are different from spheroids. Organoids can be made from patient-derived stem cells in a selective medium containing Corning® Matrigel matrix. These organoids can then be exposed to varying drugs to identify the best treatment to fight that particular cancer; thus personalizing medicine to treat disease. Taking this idea even further is the ability to repair genes in cells that can form organoids, then using those organoids to understand treatment regimens. Organoids thus serve as a converging platform for gene editing, 3D imaging and bio-engineering. Thus the therapeutic potential of organoids in modeling human disease and testing drug candidates is in my opinion the most interesting achievement thus far.

Audrey Bergeron

There has been a recent report of researchers at the Cincinnati Children's Hospital Medical Center (2) developing the world's first connected tri-organoid system, the human hepato-biliary-pancreatic (HBP) organoid. This is a remarkable achievement since it moves the field from individual organoid research to connected organoid systems, which more physiologically mimic the interplay between human tissues. There have also been challenges to date with current liver organoid approaches failing to adequately recapitulate bile duct connectivity, which is important for liver development and function. The authors describe optimized methods to create the multi-organ model from differentiated human pluripotent stem cells via the formation of early-stage anterior and posterior gut spheroids which fuse together and develop into hepatic, biliary and pancreatic structures. This is an exciting new basis for more dynamic and integrated *in vitro* systems-based organoid models to study organogenesis, for use in research and diagnostic applications and for potent applications in precision medicine and transplantation studies.

(2) Hiroyuki Koike, Kentaro Iwasawa, Rie Ouchi, Mari Maezawa, Kirsten Giesbrecht, Norikazu Saiki, Autumn Ferguson, Masaki Kimura, Wendy L. Thompson, James M. Wells, Aaron M. Zorn, Takanori Takebe. Modelling human hepato-biliary-pancreatic organogenesis from the foregut–midgut boundary. Nature, 2019; DOI: 10.1038/s41586-019-1598-0.

Debbie King

The generation of cerebral organoids is one of the most interesting achievements. These "mini-brains" derived from pluripotent stem cells can self-organize into functioning neural structures. Neural cells are notoriously difficult to culture *in vitro* and obtaining sufficient cells for experiments can be challenging. Cerebral organoids offer a way to study neural tissues, replicating aspects of human brain development and disease that was once impossible to observe in the laboratory. Scientists have used them to make discoveries about neurological disorders like schizophrenia and autism. These organoids have been useful models to examine fetal brain microcephaly caused by Zika virus infection.

What technologies played have helped scientists overcome the biggest challenges to using organoids?

Elizabeth Abraham

3D extracellular matrix, like Matrigel® matrix, provide the appropriate scaffold to be able to generate and grow organoids. This matrix can also be modulated by matrix metalloproteases secreted by cells within the organoid to grow and differentiate.

The discovery of Wnt signaling is also important as the Wnt pathway is the heart of the organoid technology.

Lastly, 3D imaging, the ability to see inside 3D structures such as organoids has also enabled scientists learn how to use organoids in disease modeling.

Audrey Bergeron

One of the biggest challenges is the lack of vasculature in organoid systems, which hinders *in vivo*-like expansion and limits organoid size. Technologies have been developed (and are continuing to be developed) which improve long-term culture conditions and the delivery of nutrients and gaseous exchange to the developing organoid. These include spinner flasks and bioreactors to increase "flow" in the culture system and microfluidics-based platforms for efficient nutrient diffusion, oxygenation and waste metabolite disposal (a key example is cerebral organoids).

It is also interesting to see the evolution of permeable membranes such as the Transwell® permeable supports and other semi-permeable membrane materials being integrated into perfusion systems and 3D bioprinting techniques to improve nourishment to the organoid during maturation. These technologies have helped to increase the life- span and utility of organoids to months.

Another challenge in high throughput pharmacological and toxicity screening applications has been the formation of reproducible, single organoids per well. The Ultra-Low Attachment (ULA) surface cultureware or microplates coupled with established biological hydrogels such as Corning Matrigel matrix have provided a platform to generate uniformly sized organoids compatible with HTS applications. Concurrent advancements in high content screening platforms has also helped to elucidate the 3D complexity of organoids in terms of multi-parameter imaging and quantitative analysis.

What advancements do you see with organoids in the next five years?

Elizabeth Abraham

Organoids fulfilling the need of "organ-donors" that can be used in patients awaiting transplantation and using organoids as a diagnostic tool to detect and treat cancers.

Audrey Bergeron

More complex, vascularized multi-organoid systems will continue to be developed to advance precision and regenerative medicine closer towards transplantable organs. I also think that protocols and models will continue to be optimized to generate data and improve clinical predictively of organoid models in pharmacological and toxicity testing - this could potentially mitigate the need for animal models during drug development.

Debbie King

Researchers are also looking to combine genome-editing technologies like CRISPR-Cas9 in particular with patient cell-derived organoids. For monogenic diseases, it opens up the possibility of performing gene correction through gene editing prior to autologous transplantation as a curative solution. It's already been shown that the defective CTFR gene in cystic fibrosis patient-derived organoids can be corrected using CRISPR/Cas9 homologous recombination (3).

(3) Schwank G, Koo BK, Sasselli V, Dekkers JF, Heo I, Demircan T, Sasaki N, Boymans S, Cuppen E, van der Ent CK, Nieuwenhuis EE, Beekman JM, Clevers H. Functional repair of CFTR by CRISPR/Cas9 in intestinal stem cell organoids of cystic fibrosis patients. Cell Stem Cell. 2013 Dec 5;13(6):653-8. doi: 10.1016/j.stem.2013.11.002.

What technologies will enable those achievements?

Hilary Sherman

I think more defined reagents such as ECM's and media will help to make organoid culture easier and more consistent. Also, better bioprinters with higher resolution will aid in generating more complex 3D structures.

Debbie King

Automation platforms will allow for precise control of culture conditions and enable high-throughput screening in drug discovery workflows. Also, high-content imaging technology will be key to capturing morphological and gene expression data to study organoids. Live cell imaging within organoids will allow us to visualize, for example, early events in human development in real time. Overall, the field would also benefit from standardization in protocols, reagents such as the type of culture media/ECM to use and the best cell sources so that comparisons between labs can be made to help advance research forward.

What areas of research do you think will be most impacted by 3D culture systems?

Audrey Bergeron

Cancer research, to better model cancer *in vitro* to better understand cancer biology and for personalized medicine.

Hilary Sherman

Regenerative medicine, a branch of therapy that involves engineering biological tissue or organ to establish normal function, this includes the hope to someday be able to 3D print organs.

Debbie King

3D culture systems will continue to have a large impact on developmental biology. To study human development, this has largely been limited to observational studies on pre-implantation embryos or from progenitor cells isolated from fetal tissues, which are then cultured *in vitro.* The advent of organoid models derived from iPSCs opens up the ability to study human embryonic development in a way we couldn't do before. As well, organ-specific progenitors generated from iPSCs provide a wealth of insight into the morphogenesis of different organ systems.