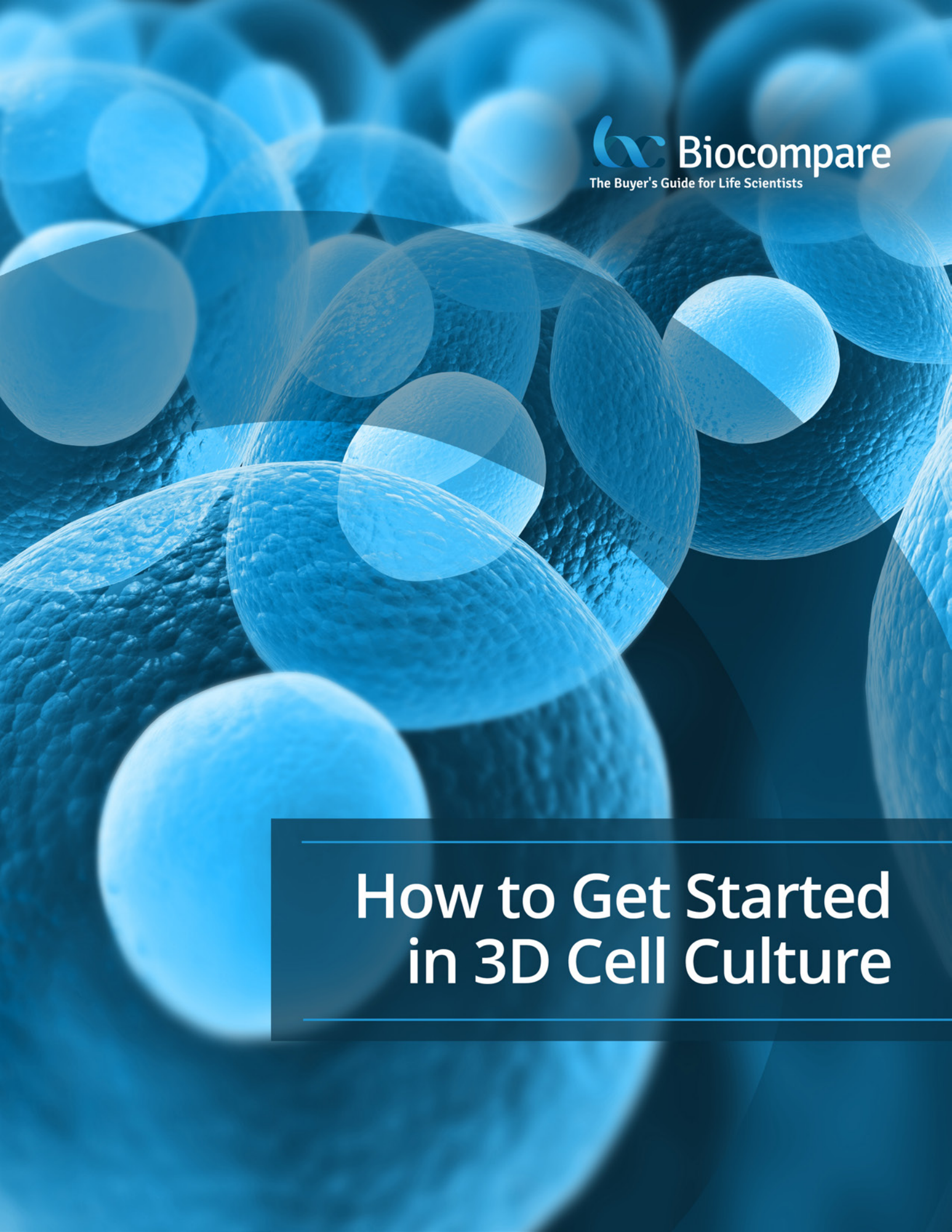

How to Get Started in 3D Cell Culture

Contents

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Choosing a 3D Cell Culture System

Guidance on how to determine which model would be best for your research.

Increasingly, labs are turning to 3D models to take advantage of these cultures' ability to mimic the physiological environment of *in vivo* tissues. With several types of 3D cell culture model systems available today, making the leap from 2D to 3D requires consideration: Would model systems, such as spheroids, organoids, or bioprinted tissues, be appropriate for your research? "Choosing the right model will help to elicit the data needed, without spending more time on optimization than necessary, or performing studies that don't provide the desired information," says Hilary Sherman, Senior Applications Scientist at Corning Life Sciences. Here are some guidelines and advice for choosing a 3D cell culture model system.

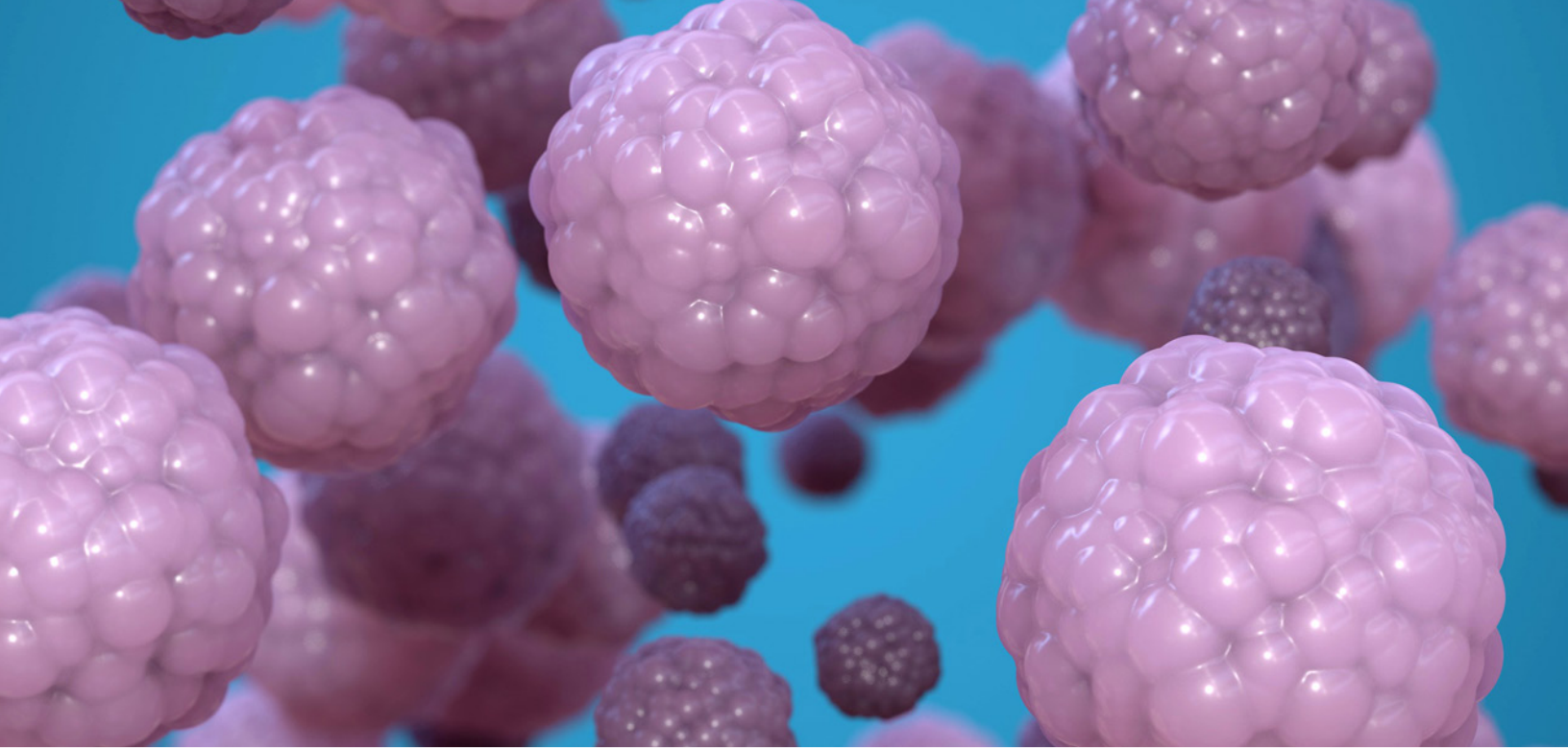
Literature before lab

Before choosing a model system, spare your future self from troubleshooting frustration by researching the literature. Learn what worked—and didn't—for other researchers who might be using the same cell type, model system, pharmacological compounds, or who might have similar research goals. "Taking the time to ensure the right model is being used is going to reduce extra work needed later to troubleshoot or repeat experiments," says Sherman.

Choosing a 3D culture model

There are several considerations to take into account before choosing a model system, such as the nature of the research question, the cell type(s), cost and available resources, and whether additional training is required. Scientifically, one of the most important considerations is whether the model allows you the freedom to conduct your planned experiments without introducing potential difficulties that can stem from unnecessary complexity. "Finding the right model for a scientist's specific needs is an important first step," says Sherman. "Ultimately, it really comes down to what information is the researcher trying to attain." Yet for many researchers, it isn't necessarily obvious which model system would be the best for their new project. Many 3D cell culture systems fall into the categories of spheroids, organoids or bioprinted tissues.

Spheroids. A spheroid is a cluster of cells (from a range of one or more cell types) that stick to one



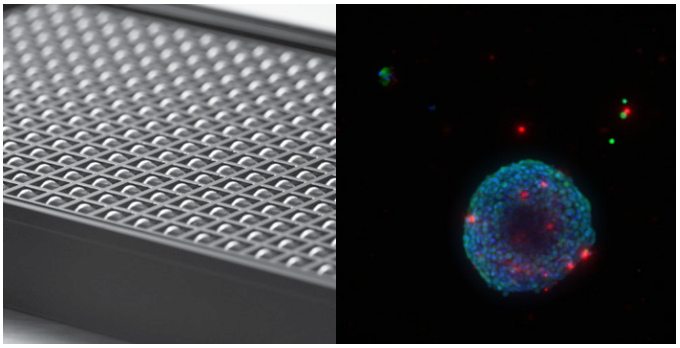
another without requiring extracellular scaffolding material and are a convenient model for solid tumors. Spheroids are considered less complex than organoids, form relatively easily in low-attachment, coated multi-well plates such as Corning spheroid microplates, and are compatible with high-throughput screening applications. Tumor spheroids, for example, are used to screen anti-cancer drug candidates and to study the cytotoxic effects of CAR-T cells. Other types of spheroids include embryoid bodies, neurospheres, hepatospheres, and mammospheres.

Organoids. An organoid is a complex mixture of organ-specific cells derived from stem or progenitor cells. Given extracellular matrix material for scaffolding, such as Corning Matrigel matrix, the cells grow and self-assemble into a tiny version of their tissue of origin. With their greater complexity, organoids offer more possibilities for representing disease models and intricate research questions. Their ability to replicate more faithfully the structure and physiology of organs makes organoids important in research, medical applications, and drug screening. But consider the level of complexity you really need before jumping in. "Organoids often can provide more information than spheroid models, but the experiments are more complex and costly," says Sherman. "The complexity of an organoid model might be required to address the question the researcher is asking, but sometimes a simple spheroid model might provide enough information."

Bioprinted tissues. 3D bioprinting refers to the construction of models or tissues by carefully extruding bioink—extracellular matrix containing cells—using a 3D bioprinter such as the Corning Matribot bioprinter, which gives the researcher greater precision in the placement of cells. This allows researchers to seed distinct layers composed of different cell types, such as in skin tissue models, and to specify precise locations or orientations of cells within the bioprinted tissue. This can be vital for mimicking the structure of complex tissues.

There is no limit to the number of cell types one can print into tissue models, but it's best not to use more than you really need. "Adding more complexity does not always add value to the model," notes

Sherman. "Sometimes it just adds unnecessary complication and cost." Assessing tissue health and the effectiveness of any experiments require assays that are easier to interpret without extraneous complexities. For example, in bioprinted skin models, "permeability assays or histology are often performed to assess the quality of the model," says Sherman. Other assays might be used, for example, to assess a drug's effectiveness in other organoid models, such as when screening potential therapeutic compounds in a 3D culture model for cancer.



Tools like the Corning spheroid microplate are a "plug and play" option that allow you to generate and analyze 3D spheroids in the same microplate. A unique well geometry and ultra-low attachment (ULA) surface enable uniform and reproducible spheroid formation.

Choose complexity when warranted

When taking into account the above considerations, it's important to balance them realistically given your research aims and available resources. Above all, remember to scale the complexity of the model system appropriately, but only as needed. "Sometimes the answer to a particular question can be determined with a really inexpensive or simple model," says Sherman. "Other times the question itself is complex, so a more complicated or expensive model might be required to get useful information." For example, if you are testing severmost appropriate 3D model system. On the other hand, spheroids could be a better option if you are screening new drug candidates for any anti-cancer effects.

With the increasing adoption of 3D bioprinted tissue models, Sherman hopes their use will extend to benefit patients via precision medicine. "Currently, most bioprinting applications are for basic research and drug testing," she says. "But I think many, including myself, dream of bigger aspirations, like someday being able to print tissues and organs for those in need."

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Lessons Learned in the Lab

Weill Cornell Medical College Researchers' Perspectives with 3D Cell Culture

As 3D cell culture is increasingly adopted in labs around the world, more scientists are making the leap from 2D to 3D. This is typically due to the fact that 3D models more closely resemble *in vivo* conditions, for example, in terms of tissue architecture, physiology, and cellular interactions. Like many scientific techniques, cell culture benefits from consistency and routine. Yet over time, experience teaches us important lessons that could not be learned by simply reading up on a topic or lab technique.

Oftentimes, the knowledge gained from experience proves to be the most beneficial. There can be a lot of value delivered when seasoned experts generously share their own learned wisdom. Biocompare interviewed three researchers who use 3D cell culture techniques to create patient-derived tumor organoids at the Englander Institute for Precision Medicine at Weill Cornell Medical College in New York City. This type of 3D culture is difficult to establish and maintain, requiring knowledge and experience to cultivate successfully. When scientists first learned how to culture cells in three dimensions, what did they wish they had known then? Which important reminders do they wish someone had emphasized to their 3D novice selves from the beginning? Here is some of their hard-earned wisdom and generously-given advice for researchers who are just getting started in 3D cell cultures.

What advice would you give regarding the choice of a 3D culture system, and setting up versus maintaining 3D cultures?

Enrique Podaza, Postdoctoral Associate: The selection of which 3D culture strategy to use depends on the goal of the experiment. A crucial determinant is the selection of the extracellular matrix. Be careful in your selection since your experimental readouts could be conditioned by it.

Jenna Moyer, Lab Supervisor for ex vivo Models: Some tissue types prefer denser 3D matrices than others, so try out a few percentages of your matrix of choice and keep track of the final matrix protein concentration used. You may also have to test various media formulations before you find the specific media that a new culture responds to.

The most difficult part of 3D culturing is establishing new cultures from very small primary tissues. Sometimes the cell viability and count for these are so low that we plate the cells into a very small 40 µL Corning® Matrigel® matrix. Then, we very gradually expand the cultures to full 6-well plates with millions of cells.

How do the day-to-day cell culture tasks differ for 2D versus 3D cultures?

Florencia Madorsky Rowdo, Postdoctoral Associate: The tasks that are more similar [between 2D and 3D] are media changes, cryopreservation of the cells, and checking the cultures under the microscope.

Jenna: Feeding organoid cultures is similar to and almost as easy as with 2D, but each well must be checked to make sure the 3D matrix droplets haven't lifted off the plate before aspirating the old media. Splitting is somewhat different, as the cells must be harvested from the matrix before washing and replating back into new matrix.

Enrique: The splitting and biobanking of 3D culture cells isn't more difficult, but it's for sure more time-consuming. For 2D cell lines this process is pretty straightforward, but for 3D cultures it may take longer, especially if you are working with several lines at the same time.

Looking back now, is there anything you wish you'd known when you were first beginning to use 3D cultures?

Jenna: Often organoid cultures grow better when they are more densely packed. When splitting, be more gentle than you might be with an established 2D line. Because the culture may do better when split as clusters of cells, pay close attention to the time the culture remains in Accutase or TryplE.

Enrique: If you are starting a new lab or thinking about starting new lines with 3D cultures, consider that you will have a fixed expenditure just for the maintenance of the cultures. For example, we work with patient-derived tumor organoids, and the media composition is crucial to maintain the tumor heterogeneity. This means that a lot of growth factors and supplements are required, also increasing the cost of the cultures.

Florencia: Once you establish your cultures, think carefully about the types of experiments that you plan to do, as 3D cultures may have some limitations regarding scalability. For example, if you are planning to do an experiment that requires millions of cells, it may be very time-consuming and expensive to get enough cells. Imaging 3D cultures may be challenging in some cases, even though there are new technologies that are optimized for doing it.

What general advice would you offer researchers who are familiar with techniques for 2D but new to 3D cell culture?

Jenna: Be patient when establishing new primary 3D organoid cultures. Organoid cultures at early passages may not yet have the high growth rate expected for many fully established cultures. The more difficult-to-grow cultures may have a splitting ratio as low as 1:3 every 3 weeks.

Florencia: In general, 3D cultures tend to grow slower than 2D cultures, so you have to be patient and very gentle with splitting. It is very important to optimize the cell culture conditions, including what type of media to use and when it is convenient to split the cells.

In addition, 3D cultures present several advantages over 2D cultures, as they mimic tissue architecture, cell-cell and cell-matrix interactions in a more accurate way.

Enrique: The selection of which 3D culture strategy to use depends on the goal of the experiment. 3D culture systems constitute valuable tools for biomedical research since they enable cell-cell interactions that ultimately modulate cellular behavior.

Organoid vs. Spheroid: What's the Difference?

How they're made and what they do are among the distinctions.

The terms "spheroid" and "organoid" are kind of like jam or jelly.

Sure, they mean similar things, and they're often used interchangeably — most of the time, you'll get by just fine using either. But there are distinct differences between the two in how they're made and what they do.

If you're just looking to make a sandwich, either jam or jelly will do the job. But if you want to get started in **complex 3D cell culture**, you need to know the difference — and pick the right one.

Organoid vs. Spheroid: Learning the Basics of Cell Clusters

Spheroids and organoids are 3D structures composed of multiple cells. Each can be useful in 3D cell research — but in different ways because they're made differently.

- **Organoids** are complex clusters of organ-specific cells, such as those from the stomach, liver, or bladder. They're made of stem cells or progenitor cells and self-assemble when given a scaffolding extracellular environment, such as **Corning® Matrigel® matrix** or collagen. When that happens, they grow into microscopic versions of parent organs viable for 3D study.
- **Spheroids** are simple clusters of broad-ranging cells, such as from tumor tissue, embryoid bodies, hepatocytes, nervous tissue, or mammary glands. They don't require a scaffolding to form 3D cultures; they do so by simply sticking to each other. However, they can't self-organize or regenerate, and thus aren't as advanced as organoids.

Organoid vs. Spheroid: Scientific Applications

Organoids and spheroids can each produce *in vivo*-like iterations from *in vitro* cultures, but they have unique applications, and different lab scenarios might call for different multicellular structures.

Organoid Applications

Organoid technology has been used to great success in personalized medicine — in disease modeling as well as optimizing drug discovery and regenerative medicine. The applications of organoids in CRISPR research could similarly help scientists better study organ development within the context of gene editing.

Specific to cancer research, 3D organoids can provide insight into the mutational signatures of selected cancers because they can mimic the pathophysiology of human tumors.

Organoids can also function as a self-assembling miniature manifestation of a parent organ, which can be of particular benefit to researchers. For example, **neural organoids** bring us closer to understanding diseases in the brain, while researchers have studied **intestinal organoids** to better understand cystic fibrosis.

Spheroid Applications

Perhaps most notably, **tumor spheroids** can help scientists understand the *in vivo* microenvironments of tumors, which can help researchers predict drug efficacy in cancer research. The earliest iterations of spheroids were **developed in the 1970s** to study the impact of radiotherapy on human tumor cells.

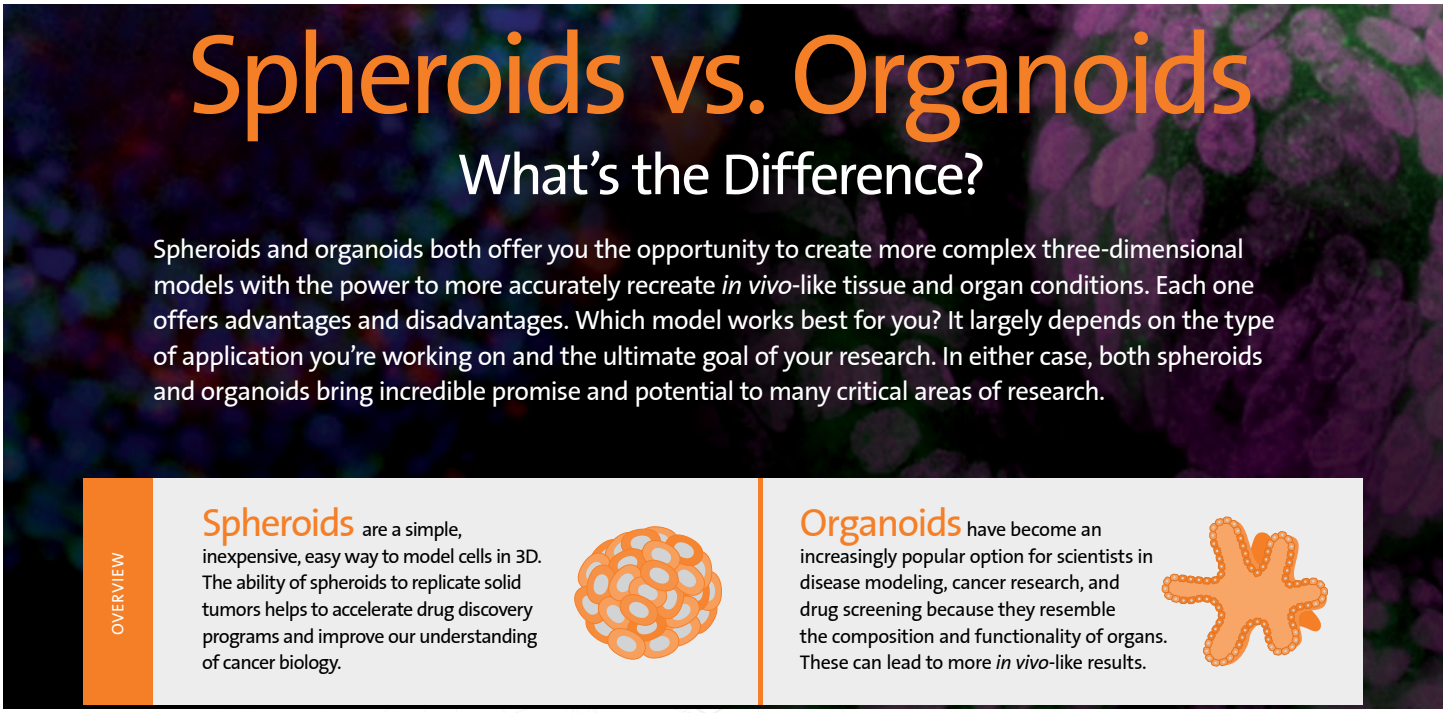
Spheroids can also be used in stem cell research to develop embryoid bodies from induced pluripotent stem cells, which can then be turned into high-purity neural stem cells useful in studying neural diseases and their related treatments.

Scientists have also used tumor spheroids to study the cytotoxic effects of CAR-T cells — such as with the KILR® Cytotoxicity assay developed by DiscoverX. When CAR-T cells are grown in KILR-transduced tumor spheroids, scientists can form, culture, and assay on the same **spheroid microplate.**

Making 3D Models Work for You

Whichever structure suits your needs, spheroids and organoids can unlock greater insights for cell research in ways 2D studies simply can't. As research continues, the field is primed for even greater success — which means more opportunities to tap into the growing power of 3D research.

Free Infographic Poster: *Learn more about the origin, model environments, applications, and advantages of spheroids and organoids. Request your poster here.*



A 3D Cell Culture Primer: Freezing, Culturing, and Measuring Organoids

Best practices that can help you dive into 3D cell culturing right away.

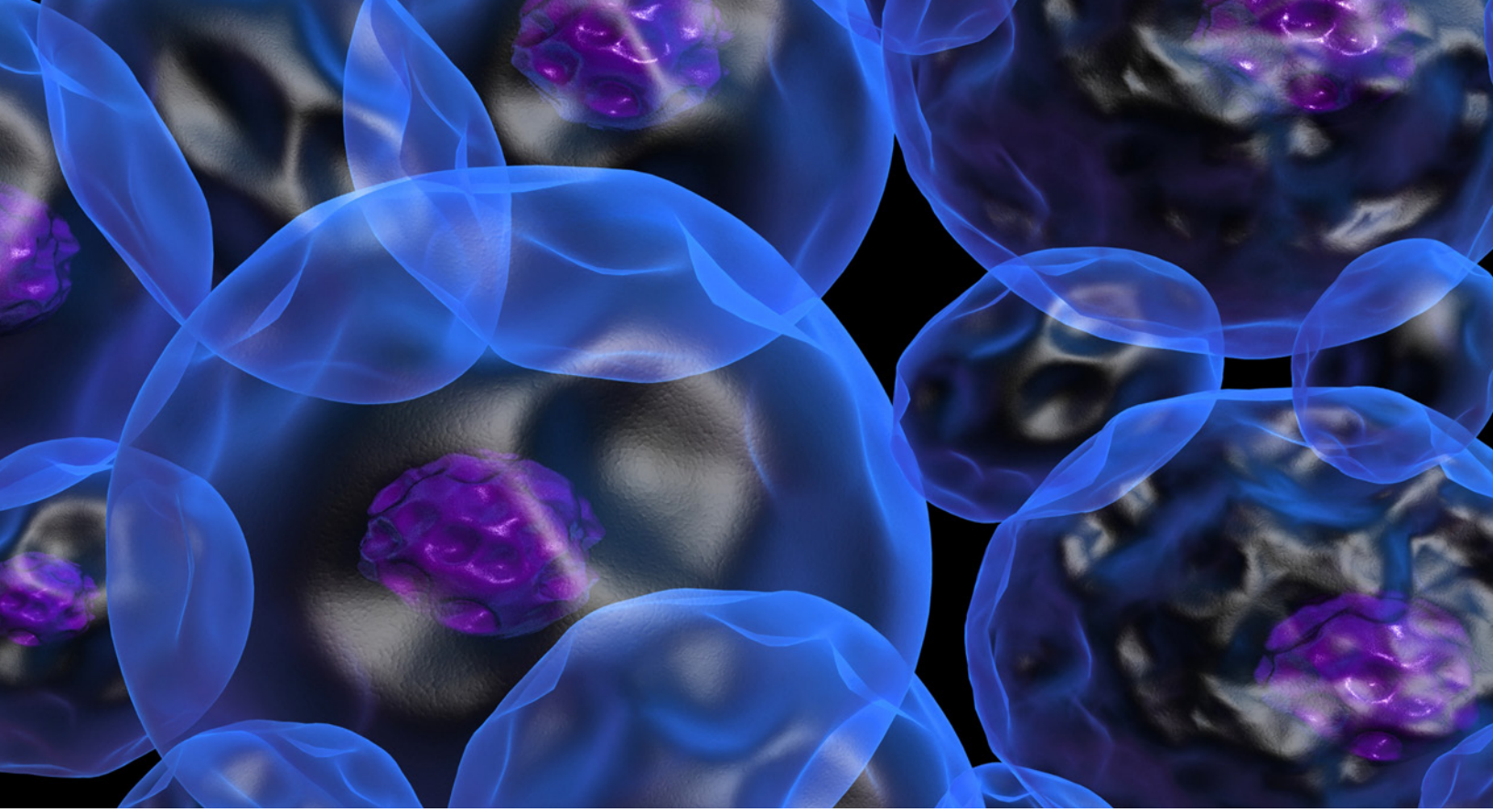
Working with 3D cell culture — **organoids**, especially — can be fascinating and enlightening. Organoids are a vital tool in disease modeling and **drug discovery** because they better mimic the composition and functionality of organs, resulting in miniature *in vitro* versions of *in vivo* material.

Organoid applications have virtually limitless potential, but they're much more complicated than 2D cultures or 3D spheroids. Don't let that complexity intimidate you from diving into this emerging research field, though. We know a few tips, tricks, and best practices about freezing, culturing, and measuring organoids that can help you dive into 3D cell culturing right away.

Freezing Organoids

Proper freezing can mean the difference between cells that will last for the long haul and those that die right away. According to **Nature Protocols**, a good methodology follows these 10 essential steps.

1. Cool a freezing container to 4°C. Have at least one or two confluent wells ready for each cryovial, depending on whether you're using 6-well or 48-well plates.
2. Pipette the media up and down using a 1,000-µL pipette and between 500 µL to 1,000 µL of basal medium to break up the basement matrix, which contains has the organoids.
3. Move the suspension with the organoids to a 15-mL centrifuge tube.
4. Add cold basal media to the organoids and repeatedly resuspend to remove the basement matrix.
5. Set the centrifuge to between 100 × g and 200 × g at 8°C. Run it for five minutes.
6. Aspirate all but about 2 mL of the supernatant. Avoid breaking the organoids into single cells.



7. Add more cold basal medium to the organoids.
8. Set the centrifuge to between 200 × g to 250 × g at 8°C. Run it for five minutes.
9. Aspirate all of the supernatant and resuspend it in 500 µL of cold freezing medium per one well or two wells, depending on whether you are using 6-well or 48-well plates.
10. Transfer the suspension to 500-µL cryovials and set in the cooling container. Immediately transfer the container to -80°C; keep it there for at least 24 hours before transferring organoids to liquid nitrogen for long-term freezing.

Please note: The freezing medium contains cryoprotective agent (CPA) like DMSO which is toxic to cells at room temperature. For cell viability when freezing, cells need to avoid excessive exposure to CPA at room temperature during harvesting.

You're not freezing single cells — you're freezing organoids, which grow bigger and more complex every day.

"The size of the organoids when frozen can have a big impact on their recovery when you ultimately thaw them out," said Hilary Sherman, a senior applications scientist at Corning Life Sciences. "Typically, smaller organoids survive the freezing-thawing process much better."

If carefully preserved, cultures can last for a long time — even up to a decade, Sherman says — giving experiments the benefit of long-term utility as new information arises.

"There are many reasons why a researcher would want to store their organoids for that long," Sherman said. "If you're making a library, you might have a potential therapy that you want to test against a patient's sample from many years ago. Or it could be that you're picking up work that someone else started before their postdoc and moved on."

Culturing Organoids

There's more than one way to create an organoid — you can form them in droplet domes, use bioreactors to scale them up for high-throughput use, or from permeable supports and low-adhesion microplates. The method you choose will depend on what you're looking to learn from the experiment.

- **Matrix domes** place single cells or tissues in a self-contained **extracellular matrix** dome so that the organoid can assemble. Researchers might choose this method if they don't have a large amount of the organoid material or if they're concerned about long scan times from thick ECM cultures.
- **Organoids** made from pluripotent stem cells in an extracellular matrix can be added to bioreactors culture and scale. They're ideal for high-throughput uses, where you need to scale up a large amount of organoids or culture for extended periods of time .
- **Permeable supports and low-adhesion microplates** mechanically support the organoid with **air-liquid interface** materials or ultra-low attachment, which prevent cell binding. They're ideal for researchers looking to further differentiate cells or conduct endpoint assays. **Precoated plates** are yet another format option for culturing organoids that can save even more time.

Depending on your process, you can grow an organoid in as little as six days or upwards of several weeks. But Sherman recommends maintaining size similarity whenever possible during passaging.

"When you're breaking up organoids, you're breaking them into all different-sized pieces," she said. "The more consistent you can keep them, size-wise, the more you'll be able to ensure they grow at the same rate for future applications. One way to help reduce that variability is to allow gravity to settle the heavier pieces of organoids to the bottom of the tube so that the smaller organoids remain at the top. That way, you can collect the population that you want for more uniform harvesting."

Measuring Organoids

There's a lot at stake when it comes to measuring organoids. You need to get it right.

"Size is one of several factors that are measured to determine an organoid establishment, so it's very important," said Kyung-A (Katie) Song, Ph.D., a scientific support specialist at Corning Life Sciences. "In addition, organoids can only be grown to a limited size *in vitro*, due to the lack of a circulatory system and limitation in the transfer of oxygen and nutrition."

Measurements are hard to do manually, given organoids' irregular shape and the challenge of keeping them sterile. Processing programs can help researchers take accurate microscope measurements without breaking the bank.

"As long as you have a basic microscope camera, you can take images of your organoid cultures and process them with free software, such as ImageJ," Sherman said.

But, she notes, such services aren't ideal for large volumes of work.

"If you're instead trying to do high-throughput research," she said, "you might need to invest in more expensive equipment."

Watch our ***Organoids Master Class Series: Introduction To & Getting Started With Organoids*** webinar for more best practices and exclusive tips on setting up your organoid culture program.

Corning® Spheroid Microplates

Spheroid formation protocol

In vitro 3D cell culture models are widely recognized as more physiologically relevant systems com- pared to 2D formats. To recapitulate features of native tumor microenvironments, cancer cells can be cultured in Corning spheroid microplates, which combine the Corning Ultra-Low Attachment surface with innovative well geometry to provide an ideal tool for generating, culturing, and assaying 3D multicellular spheroids in the same plate, without the need for a transfer step.

This protocol describes a basic method for generating and culturing tumor spheroids in a 96-well spheroid microplate format. This basic protocol for culture and assay can be adapted for all spheroid microplates. Table 2 provides suggested volumes for miniaturization of the 96-well culture volumes to both 384- and 1536-well formats. Since plating volumes and seeding densities may vary with cell type and downstream application, assay specific optimization of conditions is recommended.

Methods and Materials

HT-29 human colon cancer cells (ATCC® Cat. No. HTB-38TM) cultured in McCoy's 5a medium (Corning Cat. No. 10-050-CV), A549 human lung cancer cells (ATCC® Cat. No. CCL-185TM) cultured in F-12K (Kaighn's Mod.) medium (Corning Cat. No. 10-025-CV), and MCF7 human breast cancer cells (ATCC® Cat. No. HTB-22TM) and DU 145 human prostate cancer cells (ATCC® Cat. No. HTB-81TM) both cultured in Dulbecco's Modification of Eagle's Medium (DMEM) (Corning Cat. No. 10-013-CM) were used for these studies. All growth media contained 10% Fetal Bovine Serum (FBS) (Corning Cat. No. 35-010-CV). Cell cultures were maintained according to ATCC recommendations and harvested using standard cell culture methods.

Initial plating densities for spheroid formation depend on factors such as cell type, duration of growth phase in a spheroidal format, and the desired size of spheroid at the time of assessment. To best eval- uate spheroid formation and growth, cells were plated at densities of 40, 200, 1,000, 5,000, and 10,000 cells in 100 µL of growth media per well using 96-well spheroid microplates (Corning Cat. No. 4520). Spheroid cultures were analyzed at 0, 24, 48, and 72 hours using CellTiter-Glo® 3D cell viability assay (Promega Cat. No. G9683). The same seeding methods were used for all four cell lines.

1. Harvest cells ensuring a healthy, single cell suspension.

   Note: Cells can be passed through a 40 µm cell strainer (Corning Cat. No. 352340) or a 5 mL round bottom polystyrene test tube with cell strainer snap cap (Corning Cat. No. 352235) to achieve a single cell suspension.

Table 1. Seeding Density Preparation		
Seeding Density (cells/well)	Cell Concentration (cells/mL)	Total Cells (in 5 mL)
0	0	0
40	400	2.000
200	2.000	10.000
1,000	10,000	50,000
5,000	50,000	250,000
10,000	100,000	500,000

2. Prepare 5 mL dilutions for each seeding density (Table 1) in order to seed eight wells per seeding density for each time point (four 96-well spheroid microplates).

3. Dispense 100 µL of cell suspension to eight wells per concentration. For control wells, dispense 100 µL of growth medium without cells.

   Note: Spheroid microplates can be seeded manually* using a multichannel pipettor (Corning Cat. No. 4083 or 4087), or automated** using a liquid dispenser instrument such as the Multidrop™ Combi reagent dispenser (Thermo Fisher Cat. No. 5840300). Manual liquid addition is adequate for both 96- and 384-well spheroid microplates. Automated liquid handling is recommended for a 1536-well spheroid microplate.

   * During manual seeding, ensure pipet tips do not scratch the bottom or sides of the wells to avoid damaging the Corning® Ultra-Low Attachment surface coating.

   ** If using an automated liquid dispenser, increase the dilution volume to take into account priming the instrument and the length of tubing (10 mL).

4. Place spheroid microplates in a humidified incubator set to 37°C and 5% CO_2.

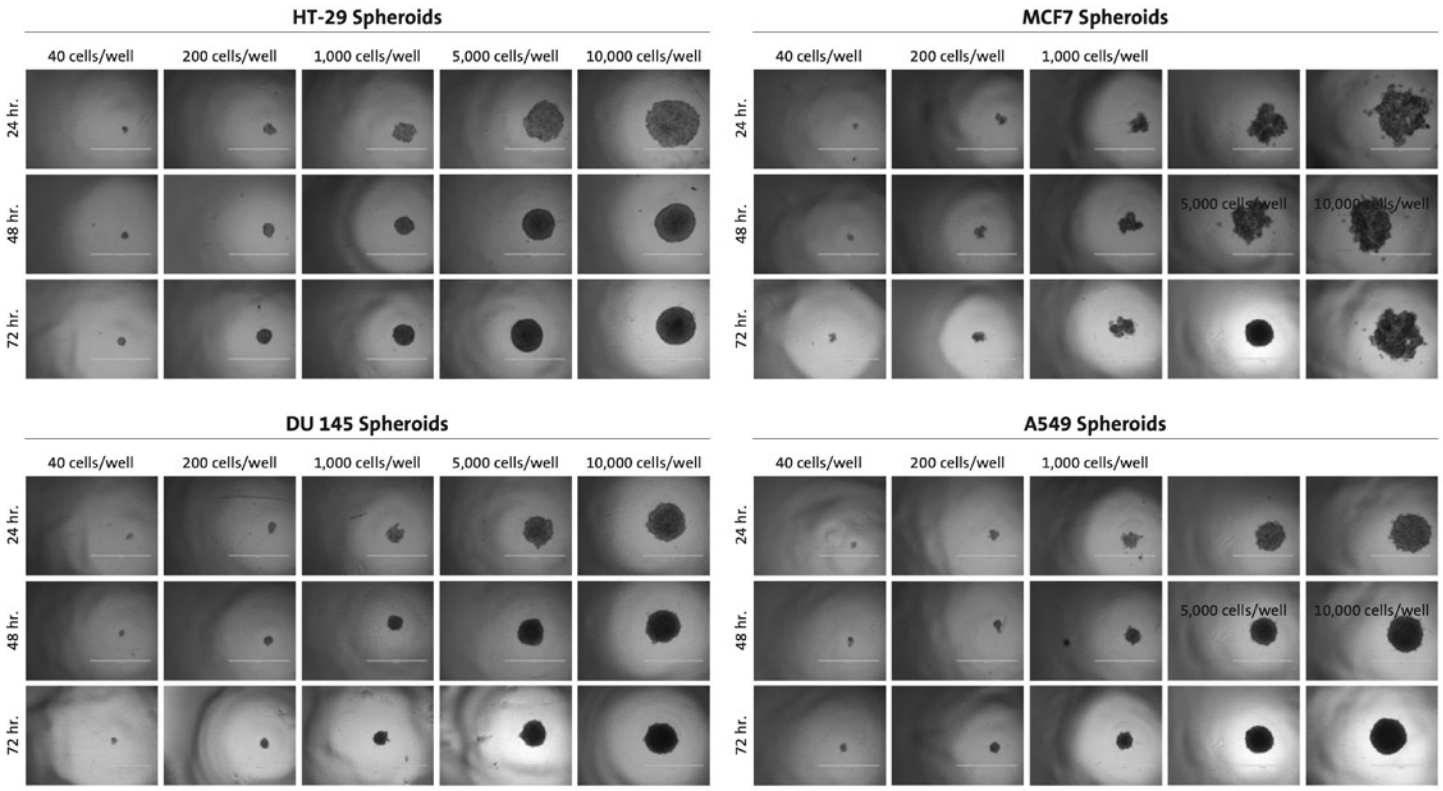
5. Evaluate spheroid formation and growth at 0, 24, 48, and 72 hour time points visually and performing a cell viability assay.

Visualization

Spheroid formation and growth was assessed via microscopic examination using an inverted microscope and by photographing spheroids for all five seeding densities at each time point.

Visual Observations:

- At the 24-hour time point, cells formed loosely aggregated multicellular spheroids.
- After 48 hours of incubation, all but the MCF7 cells formed tight, defined spheroids.
- At 72 hours, HT-29 and A549 spheroids showed a slight increase in size when seeded at the lower densities (<5,000 cells/well), but DU 145 and MCF7 spheroids did not appear to increase in size after 48 hours.



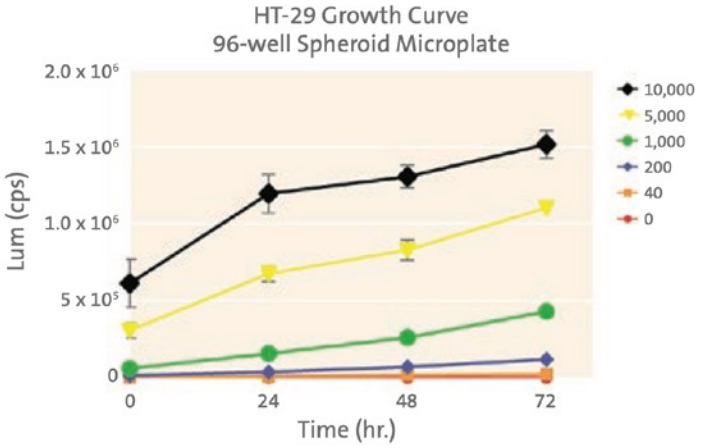
Cell Viability Assay

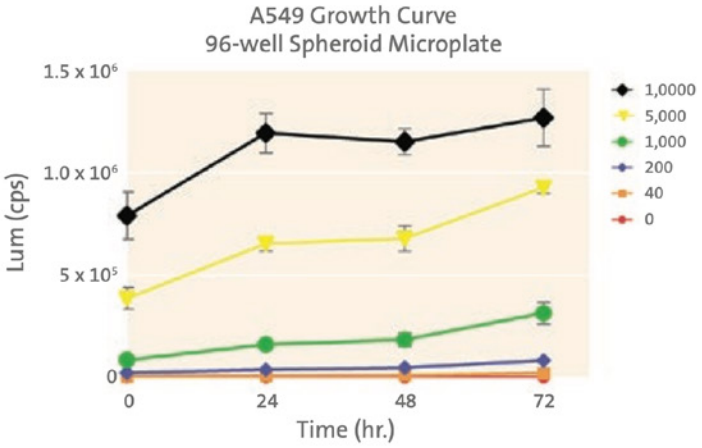
The CellTiter-Glo® 3D Cell Viability Assay protocol was followed. Briefly, the CellTiter-Glo® 3D reagent was thawed; the reagent and assay plates were allowed to reach room temperature for 30 minutes. The CellTiter-Glo® 3D reagent was added directly into wells in a 1:1 dilution (Table 2). The solutions were well mixed by shaking the plate for 5 minutes using an orbital plate shaker and then incubated at room temperature for a total of 30 minutes. After incubation, luminescent signal was read using an EnVision™ Multilabel Plate Reader (PerkinElmer Cat. No. 2104-0010).

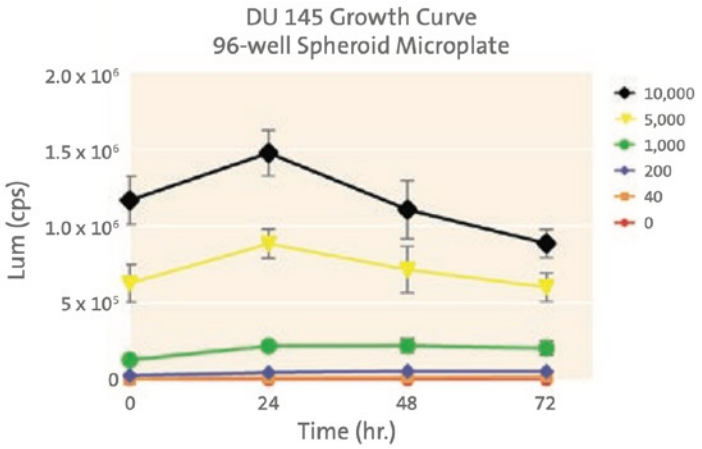
Table 2. Suggested Assay Volumes

	Spheroid Microplate Size		
	96-well	384-well	1536-well
Well volume (µL)	300	90	14
Working volume (µL)	75 - 300	20 - 80	12
Single cell suspension (µL)	100	50	5
CellTiter-Glo 3D (µL)	100	40*	5

*Reduce culture media to 40 µL before adding CellTiter-Glo 3D reagent at 1:1 ratio with culture media.







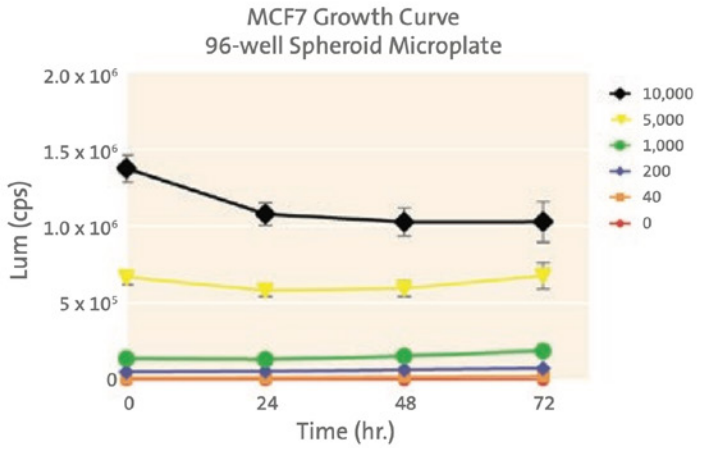


Table 3. Multicellular Spheroid Generated Using Spheroid Microplate

Cell line	ATCC® Cat. No.	Tumor type	Medium	Spheroid Morphology
BT-474	HTB-20™	Human breast/duct carcinoma	RPMI/10% FBS	T
A549	CCL-185™	Human lung carcinoma	F-12K/10% FBS	T
HEK-293	CRL-1573™	Human embryonic kidney	DMEM/10% FBS	T
5/9m alpha3-18	CRL-10154™	Hamster (CHO-K1 derived), M-CSF production	DMEM/10% FBS	A
DU 145	HTB-81™	Human prostate carcinoma	DMEM/10% FBS	T
IMR-32	CCL-127™	Human brain neuroblastoma	DMEM/10% FBS	A
Detroit 562	CCL-138™	Human pharynx, SCC	DMEM/10% FBS	T
MCF7	HTB-22™	Human breast, adenocarcinoma	DMEM/10% FBS	A
PANC-1	CRL-1469™	Human pancreatic carcinoma	DMEM/10% FBS	T
Hep G2	HB-8065™	Human hepatocellular carcinoma	DMEM/10% FBS	A

Table 3. Multicellular Spheroid Generated Using Spheroid Microplate (cont'd)				
Cell line	ATCC® Cat. No.	Tumor type	Medium	Spheroid Morphology
U-2 OS	HTB-96™	Human, bone osteosarcoma	McCoy's 5a/10% FBS	T
HCT 116	CCL-247™	Human colon carcinoma	McCoy's 5a/10% FBS	T
HT-29	HTB-38™	Human colon adenocarcinoma	McCoy's 5a/10% FBS	T
PC-3	CRL-1435™	Human prostate adenocarcinoma (grade IV)	F-12K/10% FBS	L
MDA-MB-231	HTB-26™	Human breast adenocarcinoma	L-15/10% FBS	A

T= tight spheroid formation
A = cell aggregate formation
L = loose cell aggregate formation

Note: For the 0-hour time point (cell in suspension), plates were shaken for 2 minutes and incubated for a total of 10 minutes prior to reading signal.

Cell Viability Assay:

- Exhibited continuous growth of HT-29 and A549 spheroids.
- Growth inhibition of MCF7 and DU 145 spheroids observed.
- Large spheroids (5,000 and 10,000 initial seeding densities) did not appear to have the linearity of growth exhibited with the smaller sized spheroids.

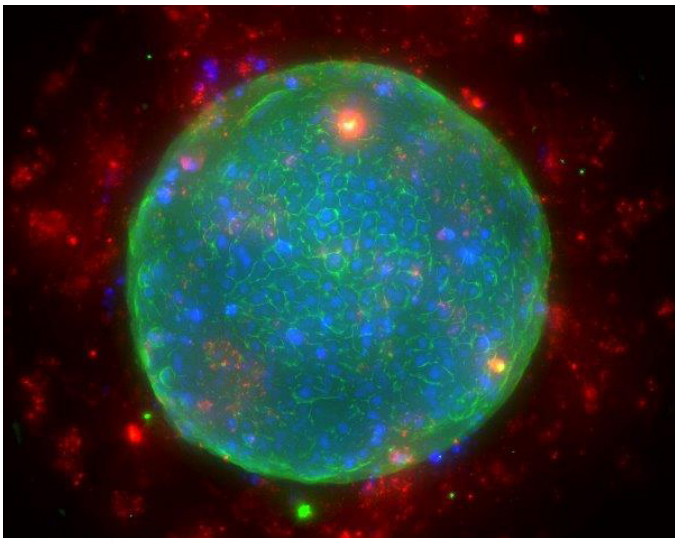
For more specific information on claims, visit the Certificates page at **www.corning.com/lifesciences**.

Related Resources

Spheroid Processing and Embedding for Histology: Guidelines for Use

3D Imaging of Optically Cleared Spheroids in Corning® Spheroid Microplates: Application Note

Corning Spheroid Microplates: User Guide



3D Bioprinting for Future Drug Discovery and Toxicology Research

Helping replace animal studies and avoiding errors in translating the results across to human physiology are among the benefits.

Bioprinting, especially 3D printing, is opening doorways to *in vitro* drug discovery and toxicology research. Increasing the utility of *in vitro* studies, 3D printing can help replace animal studies and avoid errors in translating the results across to human physiology.

Tools like the **Corning Matribot® Bioprinter** expand research horizons and bring consistency through automation to high-throughput approaches such as organoid and spheroid culture. In addition, being able to use the patient's own cells in printing can also make it easier to obtain meaningful therapeutic results in personalized medicine.

3D Tissue as *In Vitro* Mimics

3D bioprinting can involve creating layer-by-layer models that mimic tissues and organs. This is done by seeding living cells within a supportive extracellular matrix ink (bioink) and then printing up a tissue replica.

By using software and precision printing tools researchers can layer or print cells in a close approximation to the ways cells orient within natural tissues. The result is an organ or tissue model that better represents the *in vivo* environment. This is important for studying cell-cell interactions that are essential to tissue and organ function.

Sacrificial inks are also available to create scaffolding for vascularized 3D bioprints that mimic organ structure even more closely.

Software Tools Print for Cellular Orientation

As Corning Applications Scientist Hilary Sherman notes, "With 3D printing, you can actually direct the three-dimensional structure. You can form layers of tissues where the cells are in a specific location or specific orientation, rather than randomly growing in culture." Bioprinters such as the Matribot bioprinter often come complete with STL files, which

are easy to use in setting up a new tissue print. More are available online, making 3D printing an incredibly accessible and versatile investment.

Sherman goes on to give the example of skin, which is made up of fibroblasts topped by keratinocytes. "Those are different, distinct cell types, and their orientation matters if we want to create a good model," she says.

Instead of *in vitro* cultures composed entirely of a single cell type, such as fibroblasts, toxicology research and cosmetic screening can be carried out on layered prints that mimic skin anatomy.

"Simply having a co-culture in a dish is really not enough," says Sherman. "We want to create a structure in which the cells are organized in a way that's more similar to how they're organized in the body in order to see the true impact."

"At the most basic research level, 3D bioprinting gives a better understanding of how multiple cell subtypes interact together," explains Sherman. "In the example of skin, if you want to study the impact of a lotion or something that gets placed on top of the skin, it wouldn't make sense to test your compound with fibroblasts, because that's not what's actually exposed to the drug. It makes more sense to expose it to the keratinocytes which are on the top layer, but with them interacting with the fibroblasts."

Extrusion Versatility for Organoid Consistency

Being able to accurately dispense bioinks helps with consistency. The Corning Matribot bioprinter, for example, is the only benchtop 3D printer that can dispense a cell matrix product, **Corning Matrigel® matrix**, with accuracy, as its printhead is temperature controlled.

Not only does this help with cell orientation and precision within the bioprint, but it also means that **accurately dispensing cells or organoids** in temperature sensitive hydrogels is possible. This is important for toxicology research since data from studies will be highly consistent.

Frontiers in Medical Technology notes that creating heterogeneous tissues for drug screening in personalized medicine studies is highly important for validity. Being able to recreate 3D bioprints accurately helps to bridge between *in vitro* and *in vivo* by providing study materials that have biological relevance to living tissue.

The temperature control is key. "Doing it by hand is really challenging," explains Sherman, "because instead of having a temperature-controlled head that holds your syringe, you need to pre-chill all your pipette tips and then use ice baths and work very quickly. It's just more challenging."

Automation and Scaling Up for High Throughput

Drug discovery and toxicology studies rely on scale and repeatability for meaningful data. The ability to automate dispensing can lead to consistent designs suitable for high-throughput applications, such as multiwell plate assays and automated plate readers. Choosing a 3D bioprinter such as the Matribot bioprinter, which can

dispense temperature-sensitive viscous solutions with high levels of accuracy into a multitude of vessels, supports scaling up.

Personalized Medicine: Bioprinting for Hard-to-Source Cells

Drug discovery studies often rely on using hard-to-source cells, such as those obtained from a particular patient's cancer biopsy. **Archives in Toxicology** notes that 3D bioprinting techniques are often ideal for screening chemotherapy drugs to identify the best option.

Sherman describes how this approach helps patients with cystic fibrosis:

"The most notable cell model for most organoid researchers would be cystic fibrosis because those were the first ever organoids used to determine treatment for a patient," she explains. "Since cystic fibrosis can be caused from a variety of different mutations and not any one in particular, it's really hard to treat because everybody's needs are different. Patient biopsies have been screened to determine what is the best drug or combination of drugs to treat a specific individual's cystic fibrosis."

Related Resources

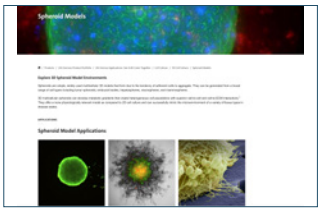
Bench Tip Video: Bioprinting Applications, Tools, and Tips for Success

Infographic: Automate to Accelerate—Meet the Matribot Bioprinter

Article: How to Achieve a More Consistent Organoid Culture

Resources

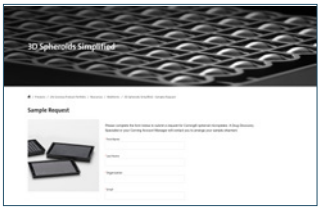
Organoid Culture Models



Spheroid Models

Tissue Models

3D Cell Culture Models E-Book Bundle



3D Spheroids Simplified

Spheroids vs. Organoids Infographic