3D Biomatrix™
Three-Dimensional Cell Culture

Using **Perfecta3D™** Hanging Drop Plates to Assess Chemosensitivity

White Paper

In this white paper

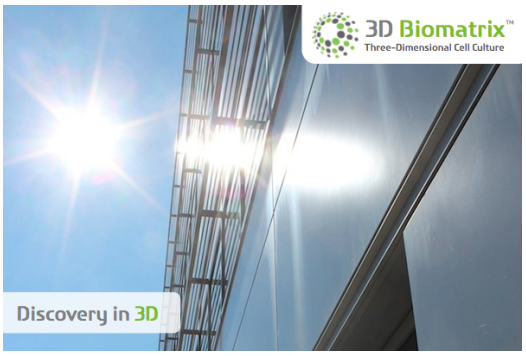
3D spheroid culture to revolutionize the next generation drug development offensives

Introduction

Three-dimensional (3D) cell culture is motivated by the strong and growing need to carry out experiments in cellular models that better mimic physiological tissues. Conventional two-dimensional (2D) cell cultures often fail to capture the cellular functions and responses that are present in tissue. As a result, drug assays and biological research findings based on conventional 2D cell cultures tend to be skewed and offer limited predictive capability. [1]

To obtain more physiologically relevant data, researchers have developed various 3D cell culture techniques to replicate the *in vivo* characteristics of physiological tissues. Some of these methods involve the use of extracellular matrix, hydrogels, and scaffolds. Other methods require the use of bioreactors and surface engineered substrates. [2]

3D spheroid culture offers simplicity, reproducibility, and similarity to physiological tissues



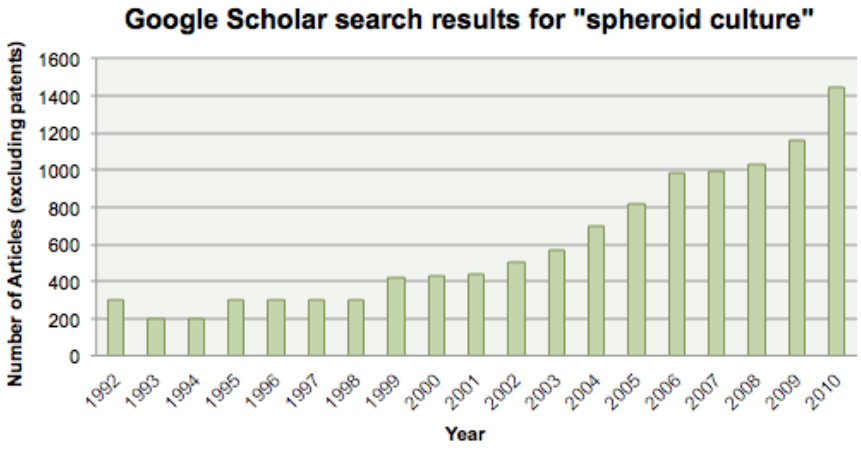
3D spheroid culture is gaining momentum

Among various methods, spheroid formation is one of the most well characterized models for 3D culture and screening due to its simplicity, reproducibility, and similarity to physiological tissues. [3, 4]

3D spheroid culture has been around for many decades. One of the earliest accounts of spheroid culture of mammalian cells was Holtfreter's work on spherical aggregates of embryonic cells in 1944. Moscona was another pioneer in the 1950s who studied the capacity of embryonic and malignant cells for reaggregation, proliferation, differentiation, and invasion.

These earlier works sparked a sustained interest in using spheroids as a model system to study the interaction of tumor cells with their microenvironment, as well as their responsiveness to radiotherapies and chemotherapies. These progresses were thoroughly summarized by Robert M. Sutherland in his article published in Science in 1988. [5]

Recently, there has been a surge of new methods developed to measure and correlate 3D spheroid's resemblance to in vivo systems, for example, using genomic and proteomic profiles. We are also seeing the emergence of new tools for generating spheroids and creating more sophisticated 3D cell culture systems. As a result of these recent advancements, we are now witnessing an explosion of research interests in 3D spheroid culture, which we believe will revolutionize the next generation drug development offensives.



Its has been difficult to scale up spheroid culture for screening and testing

Conventional spheroid culture methods suffer from variability in spheroid properties

Spheroid Culture Methods and Systems

The most well known spheroid formation method involves cultivation of suspended cells in hanging drops on the underside of a Petri dish lid. This process requires inverting of the lid following placement of the drops. As a result, the drops are susceptible to perturbation, resulting in falling, excessive spreading, and merging with neighboring drops. Although inexpensive, this method is tedious, does not permit mass production, and is not compatible with automated instruments for high-throughput screening. In addition, it is difficult to perform media exchange without damaging the spheroids. Therefore, this method usually requires another labor-intensive step to transfer the spheroids manually to a multi-well culture plate for longer-term cell culture experiments.

Other conventional spheroid formation methods include culture of cells on non-adherent surfaces, spinner flask cultures, and rotary cell culture systems.[6] These methods similarly suffer from an array of problems, such as variability in spheroids properties, low throughput, and need for expensive equipment.

Recently, new materials and devices have been designed and developed to improve culture, manipulation, and analysis of spheroids. For example, microfabrication technologies have been used to build microstructures to form spheroids of controlled sizes and shapes. New coating materials have been developed to induce and control spheroid formation on flat surfaces. Researchers have also exploited ultrasound, magnetic field, and electric field to form spheroids. [6]

Although some of these new techniques offer better control of spheroid sizes and simplify handling procedures, all of them still suffer from problems such as long-term culture and incompatibility with existing liquid handling robots for performing high- throughput screening (HTS).

Overall, it has been difficult to scale up spheroid culture in a high-throughput manner for screening and testing.

Spheroid Formation Methods and Culture Systems

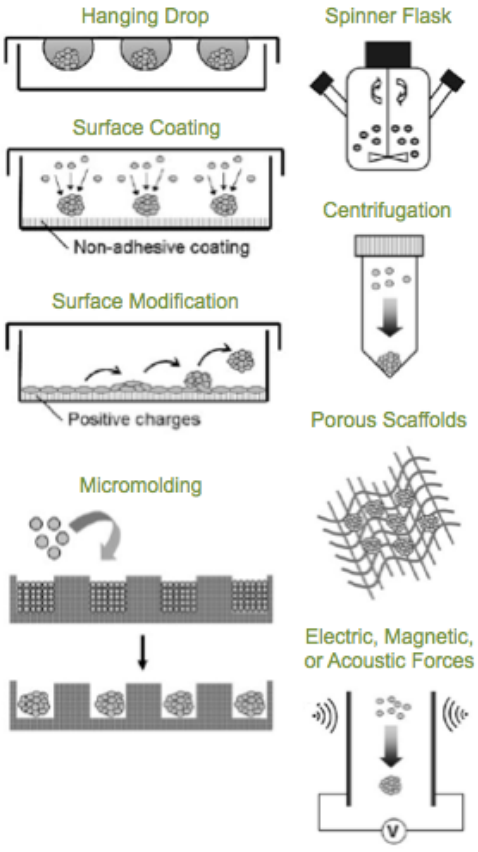


Figure adapted from Lin and Chang [6]

3D structure and environment are critical to cells

Perfecta3D™ Hanging Drop Plates

The development of anti-cancer therapeutics relies on screening libraries of compounds using in vitro tumor models to identify drug candidates that would enter animal studies and clinical trials. Unfortunately, typical *in vitro* models do not faithfully recapitulate the physiological environments in vivo because they utilize conventional 2D culture plates and petri-dishes.

Realizing that 3D structure and environment are critical to cells, researchers are beginning to adopt 3D cell culture systems to better mimic physiological tissues. However, scaling up and maintaining reproducibility of 3D cell cultures have been difficult due to lack of standardized tools and methods that are simple to use and compatible with existing equipment. These complications have hindered the adoption of 3D cell culture into routine use in research and pharmaceutical development.

One of the most exciting prospects among recent advances in 3D cell culture is standardized mass production of 3D spheroids for high-throughput screening applications. This is a promising path in which 3D culture systems can be incorporated into the mainstream drug development processes.

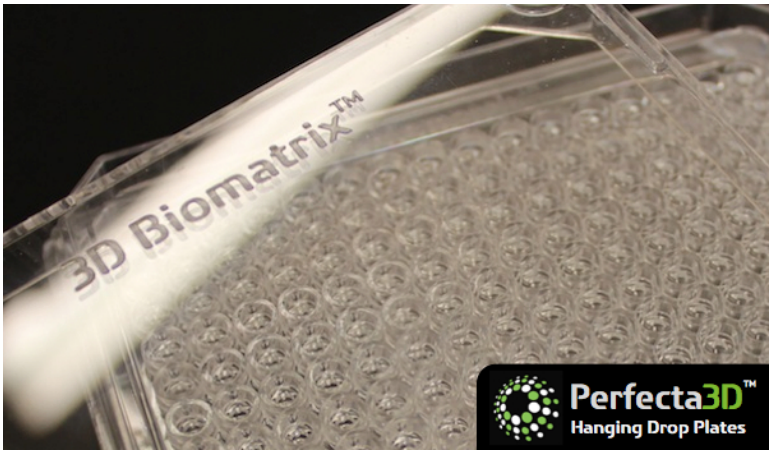
3D Biomatrix's Perfecta3D™ Hanging Drop Plates are a new 3D cell culture platform that enables uniformly-sized spheroids to be generated and tested in 384-well microplate format and using high throughput screening instruments, such as plate readers, liquid handling robots, and automated imaging and analysis systems.

3D cell culture combined with high-throughput screening increases accuracy during pharmaceutical development

A novel 3D cell culture platform as straightforward to perform as conventional 2D culture plates

3D Biomatrix's Perfecta3D™ Hanging Drop Plate was invented by Dr. Shuichi Takayama, a professor of biomedical engineering at the University of Michigan, whose research objective has been the development of microfluidics and micro- and nanotechnology platforms capable of testing cells. His research aims to create physiologically relevant in vitro environments that mimic the *in vivo* environment to allow better understanding of cell behavior and function in healthy and diseased states.

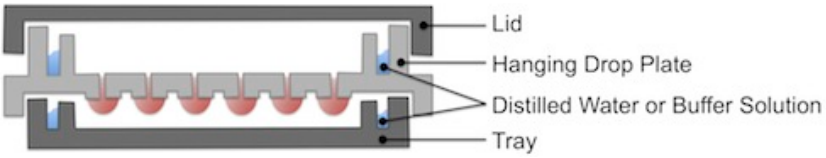
Invented at the University of Michigan



"After struggling to generate reproducible spheroids in spinner cultures and agarose-coated plates, the Perfecta3D™ Hanging Drop Plates have finally given me consistent cell growth and morphology, and the spheroids size and shape are remarkably reproducible."

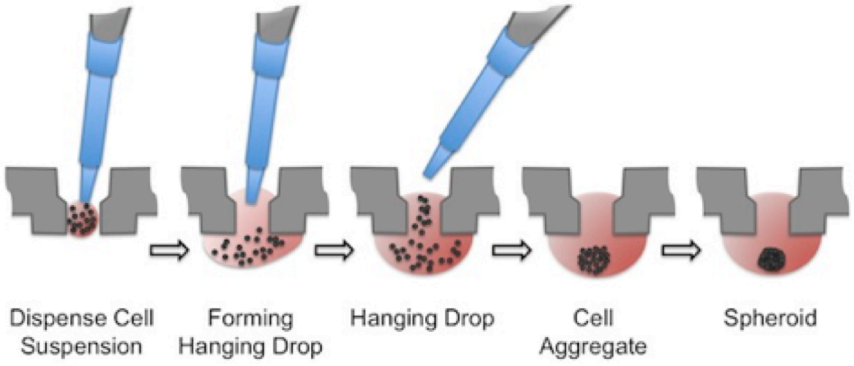
Research scientist at a major Canadian university

The Perfecta3D™ Hanging Drop Plate is a novel cell culture device that makes formation, testing, and analysis of 3D multicellular spheroids as straightforward to perform as conventional 2D cell culture plates. The device consists of the main hanging drop culture plate and a complementary lid and tray, which serve to maintain sterility and reduce evaporation. The main culture plate comprises access holes that allow manipulation of fluids and spheroids from the topside. A water reservoir is constructed around the periphery of the culture plate, alleviating the commonly encountered hanging drop evaporation problem. The dimensions of the entire system meet ANSI/SBS standards and are thus compatible with conventional liquid handling robots and plate readers.



Designed to enable consistent formation of spheroids for life sciences research and drug screening

The Perfecta3D™ Hanging Drop Plate was designed to enable consistent formation of spheroids using conventional liquid handling tools. Hanging drops can be created easily and simply by dispensing small volumes of cell suspension through pipette tips inserted into the access holes of the plate from the topside, just like pipetting into conventional multi-well plates. The hanging drops are stabilized by a plateau structure on the bottom of the plate. Reagents and drugs can be similarly added or removed to each hanging drop.



Colorimetric, fluorescence, and luminescence assays can be easily performed by inserting the Perfecta3D™ Hanging Drop Plate into a plate reader. Microscopic imaging of spheroids can be performed directly with the plate lid and tray attached.

This new 3D cell culture platform has two important advantages that will catapult adoption. First, the device is user-friendly and in the standard 384-well microplate format that researchers are already familiar with. Second, the platform offers simplified liquid handling procedures and compatibility with high-throughput screening instruments, such as plate readers and liquid handling robots.

The Perfecta3D™ Hanging Drop Plates are expected boost the pre-animal and pre-clinical selection of promising drug candidates and novel treatment modalities. This new platform is set to be an integral tool for drug screening and life sciences research, potentially revolutionizing the drug discovery process as 2D microplates did in the 1980s.

Advantages

- Physiological and non-expensive spheroid culture system
- Efficient formation of uniform-size spheroids
- Compatible with high-throughput screening (HTS) instruments
- Easily maintained from the top of the plate
- Suitable for long-term culture
- Standardized plate format
- Reduced consumption of media and reagents.

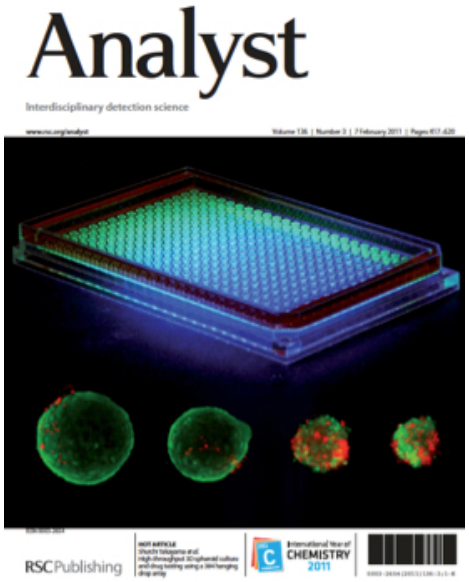
Specifications

- Polystyrene
- Untreated
- Sterile (gamma irradiation)
- Individually packaged
- Standard 384-well format with lid and tray
- Meets ANSI/SBS standards
- Stackable
- Recommended reservoir capacity: Plate 2 mL, Tray 1.5 mL

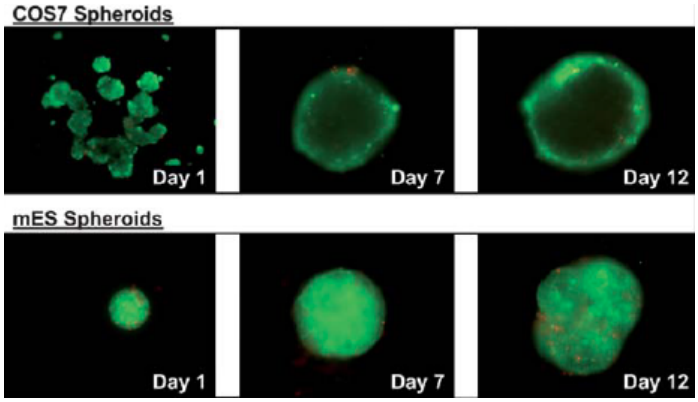
As seen on the cover of *Analyst*

Assessment of Chemosensitivity using Perfecta3D™ Hanging Drop Culture Plates

Experimental results obtained using the Perfecta3D™ Hanging Drop Plates were first published in 2011. In this study, which was featured on the cover of the journal *Analyst*, Takayama and his team of researchers at the University of Michigan demonstrated that drugs with different modes of action produce distinct responses in the physiological 3D spheroids compared to conventional 2D cell monolayers.

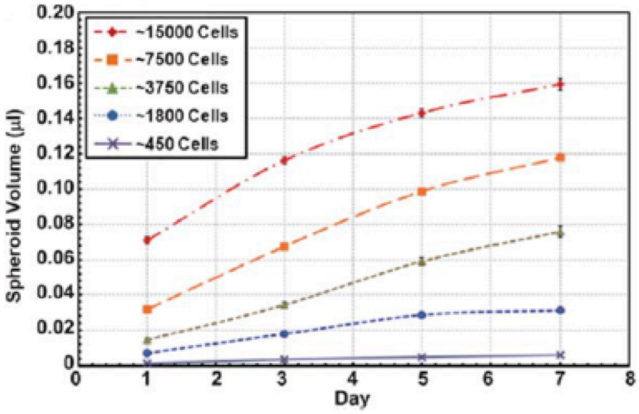


Spheroid formation. COS7 African green monkey kidney fibroblasts, ES-D3 murine embryonic stem (mES) cells, and A431.H9 human epithelial carcinoma cells were cultured in hanging drops using the Perfecta3D™ Hanging Drop Plates to form spheroids. While mES cells readily formed spheroids on Day 1, the COS7 cells formed smaller aggregates initially that later assembled into a larger spheroid. Live/dead assay indicated that more than 90% of the cells remained alive after 12 days of culture, demonstrating that the Perfecta3D™ Hanging Drop Plates are suitable for long-term spheroid cultures.

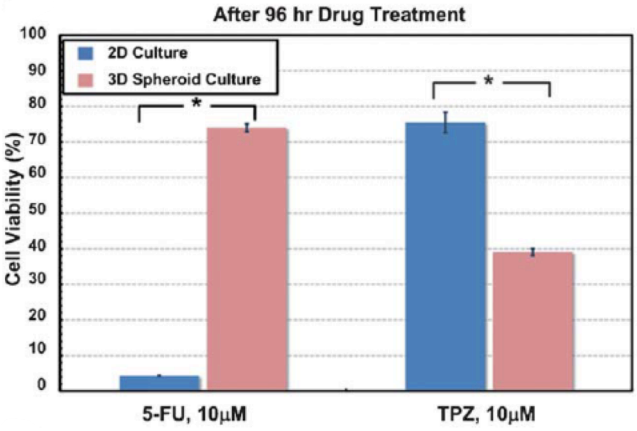


Data adapted from Tung et al. [7]

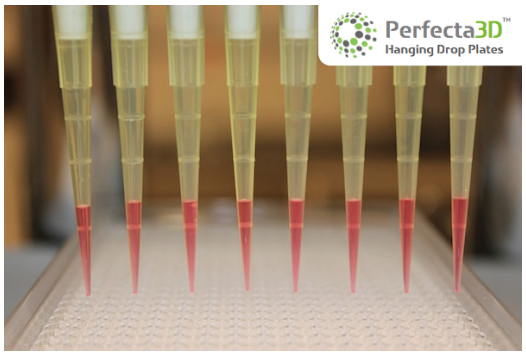
A431.H9 cells were seeded at various cell densities and the size of the spheroids was recorded over a 7-day culture period. The data shows that cells remained proliferative by the end of the culture period. Moreover, it shows that spheroid size can be fine-tuned by altering the cell seeding density and the length of culture period. This is important since the size of cell colonies has been reported to affect the differentiation of stem cells. [8]



2D versus 3D. Two anti-cancer drugs with distinctly different activity profiles were used to assess chemosensitivity: 5-fluorouracil (5-FU) is a conventional compound that inhibits cellular proliferation, and tirapazamine (TPZ) is a hypoxia-trigger cytotoxin that causes DNA damage. Our data shows that A431.H9 cells are more resistant to 5-FU under 3D spheroid than 2D culture conditions. However, the opposite is true for treatment with TPZ. Our data indicates that A431.H9 cells are more resistant to TPZ when cultured under 2D than 3D spheroid culture conditions.



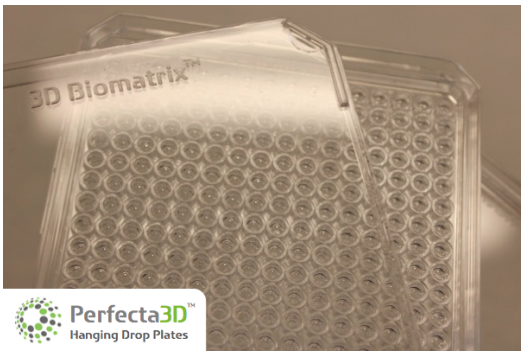
5-FU primarily targets proliferating cells. Therefore, it is more effective against the rapidly proliferating cells in 2D monolayer culture and would not kill the quiescent cells in the spheroids. In contrast, TPZ is a hypoxia-activated cytotoxin, so it is more effective in the spheroids, where active oxygen consumption by cells and limits in diffusive oxygen transport create a hypoxic core similar to that of solid tumors. [5]



Examples of cell types that the Perfecta3D™ Hanging Drop Plates have been successfully used with:

- A431.H9 human epithelial carcinoma cell line
- BT-20 human breast cancer cell line
- NF human fibroblast cell line
- COS7 African green monkey kidney fibroblast cell line
- DU-145 human prostate carcinoma cell line
- ES-D3 murine embryonic stem cell line
- HBME human bone marrow endothelial cell line
- HEK-293 human embryonic kidney cell line
- HepG2 human hepatocellular liver carcinoma cell line
- hFOB human fetal osteoblast cell line
- HUVEC human umbilical endothelial cell line
- MCF-7 human breast adenocarcinoma cell line
- MDA-MB-231 human breast adenocarcinoma cell line
- MT3C3-E1 mouse osteoblastic cell line
- SUM-159 human breast anaplastic carcinoma cell line

Data adapted from Tung et al. [7]



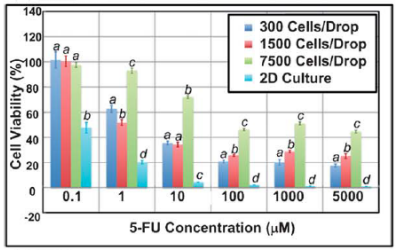
Applications

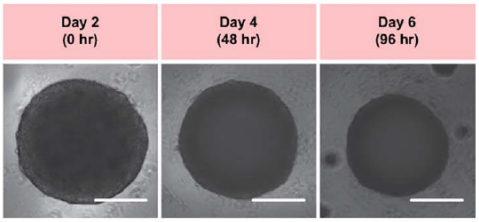
- Tissue Engineering
- Stem Cell Research
- Cancer Research
- Drug Discovery and Testing
- High Throughput Screening
- Chemotherapy and Radiotherapy Research

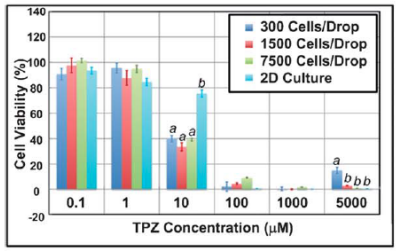
Assays

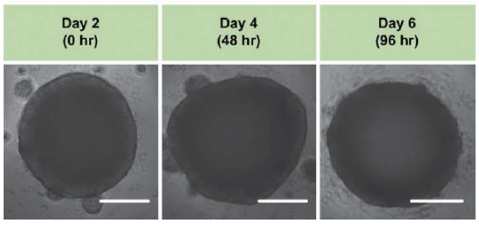
- Efficacy Testing
- Cell Migration
- Toxicity Testing
- Cell Differentiation
- Embryoid Body Formation
- Angiogenesis
- Colony Formation
- Cell Expansion
- Spheroid Formation
- Cell-to-Cell Interactions
- Genomic Expressions
- Cell-to-ECM Interactions
- Proteomic Expressions

Chemosensitivity testing. The IC_{50} of A431.H9 cells treated with FU-5 is about 0.1 μM in 2D condition and 1 to 100 μM, depending on cell density, in 3D spheroid culture. For the TPZ treated cells, the IC_{50} is about 50 μM when cultured in 2D and 8 μM for all spheroid sizes. Such distinctly and dramatically different responses from the same cells to the same drugs tested under different culture conditions illustrates the importance and unmistaken need of using 3D models in drug screening and testing.

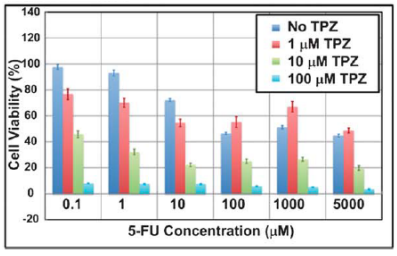


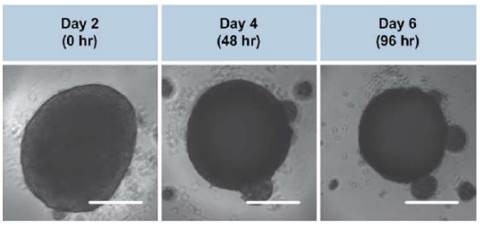






Combination drug treatment. The combined treatment of 5-FU and TPZ is more effective at killing the A431.h9 cells. At a drug concentration of 10 μM, cell viability decreased from 75% and 40% when treated with 5-FU and TPZ, respectively, to only 20% when the spheroids received the combined treatment. By combining two drugs of distinctly different mechanisms, both the proliferating cells in the peripheral layer and cells in the hypoxic core of the spheroids can be targeted effectively.

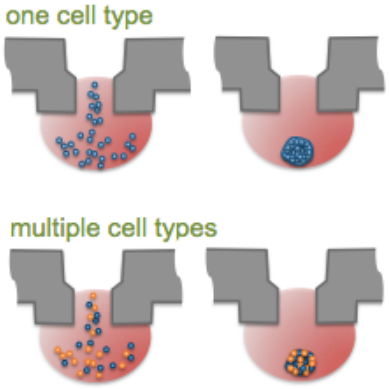


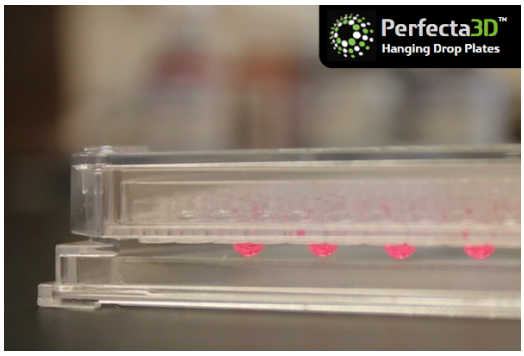


At the American Association for Cancer Research (AACR) meeting in April 2011, Rational Therapeutics also reported that cancer drugs that target different pathways are more effective when used in combination than alone. By targeting more than one pathway, cancer cells are trapped and killed more easily. Rational Therapeutics is using 3D spheroid culture to provide clinical laboratory-based treatments for cancer patients. Tumor samples are collected from patients, maintained as spheroids, and then exposed to different drugs and combinations to identify treatments that are most effective at killing the cancer cells.

Data adapted from Tung et al. [7]

Modes of 3D spheroid culture. Perfecta3D™ Hanging Drop Plates allow spheroids of pre-designed compositions and architectures to be produced to meet various experimental needs. For example, multiple cell types can be employed to control the composition and spatial distribution of different cell types in the spheroid. Co-cultured spheroids have been shown to be more realistic 3D tumor models and can be used to study cellular interactions, such as migration and invasion, between different cell populations.





Perfecta3D™ Hanging Drop Plates

Case of 8 Plates (HDP1384-8)

384-Well Format with Lid and Tray

Individually Packaged

Untreated

Polystyrene

Sterile

Visit www.3DBiomatrix.com to order

Conclusion

Perfecta3D™ Hanging Drop Plates are a highly versatile and simple-to-use 3D cell culture platform for life sciences research and drug testing and screening applications. Our customers include major academic laboratories and pharmaceutical companies in the United States and Europe. Perfecta3D™ Hanging Drop Plates have greatly simplified and streamlined our customers' 3D cell culture assays and have produced excellent results that helped to move their projects forward.

We invite you to discover the powerful 3D cell culture experience offered by the Perfecta3D™ Hanging Drop Plates!

Please visit www.3DBiomatrix.com to learn more and to order.

"We are getting excellent results using the Perfecta3D™ Hanging Drop Plates. This elegant 3D tissue culture system provide more authentic models that can be expected to decrease the high incidence of false positive leads in drug screening."

Senior research scientist at a major US medical research institute

3D Biomatrix
www.3DBiomatrix.com
service@3DBiomatrix.com
Phone: 734.272.4688
Fax: 734.818.1999

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3D Biomatrix
1600 Huron Parkway
Building 520, 2nd Floor
Ann Arbor, MI 48109-2590
United States of America