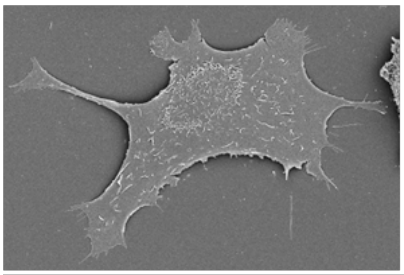
NanoCulture® Plate

Innovative Scaffold-based 3-Dimensional Cell

Culture System for Oncology Drug Discovery

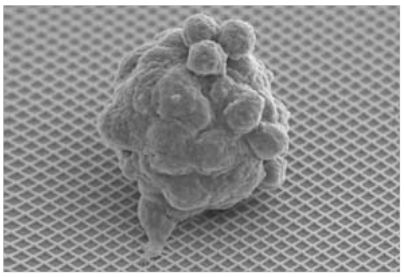
What is 3D Cell Culture?



Morphology of monolayer cultured cell

Three-dimensional (3D) culture is an established method to form multi-cellular clusters (spheroids) that are driven by cell-cell and cell-matrix contacts, rather than cell-plastic contacts. As a result, cell morphology, and by extension, the underlying biology is different compared to conventional culture method using polystyrene cultureware (two-dimensional or monolayer culture). It is understood that spheroids more closely reflect actual human and tumor biology and are therefore more relevant for basic research and drug screening than traditional 2D monolayers.

Indeed, 3D cell culture research publications have tripled in the past 10 years, and its importance both as a basic research and discovery tool is now being fully appreciated. The applications of 3D cell culture are finding utility in several areas of research and drug development, such as basic cancer research, target discovery and drug screening as well as in ancillary areas such as hepatotoxicity, and stem cell research.



Morphology of multi-cellular spheroid on NCP

The primary motivation behind culturing cells and cell lines as three dimensional spheroids is to better reconstitute the microenvironment that cell would find in the body or in a tumor. By providing cells with a more relevant physiological context, it can be expected that the emergent biology of those cells will more closely approximate *in vivo* biology and that the cells will respond to drugs in a more representative manner. Integration of the array of signals mediated by both cell–cell and cell–matrix interactions is required to regulate many aspects of cell behavior, including cell polarity, proliferation, adhesion, and

survival, all of which will dictate drug response.

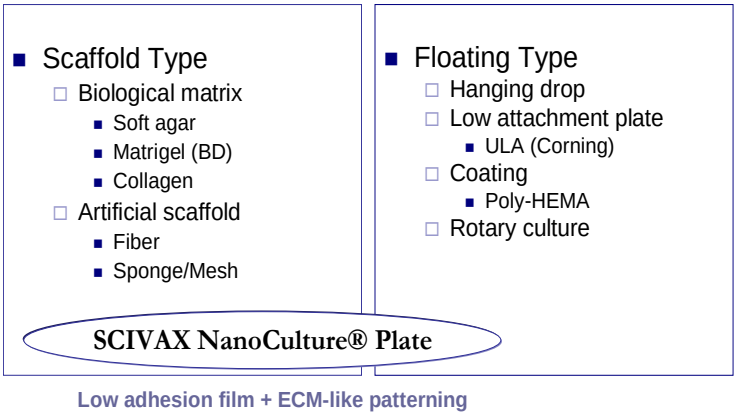
Furthermore, it is being recognized that differentiated cells derived from stem cells gain *in vivo* like function with the biological tissue/organ, and primary cells maintain sustained in vivo-like function when cultured in 3D.

Methods of 3D Cell Culture

While the current technologies for spheroid formation differ, the concept is essentially the same. By attenuating the ability of a cell's ability to strongly attach to a substrate, cell-cell contacts will drive the formation of stable spheroids. Basically, there are two ways to accomplish this: 1) growing the cells in a scaffold and 2) forcing cells to grow in suspension. Originally, soft agar assays were used to detect neoplastic transformation-induced anchorage independent growth which led to idea of culturing cells in 3D. Though soft agar is still being used as a basic research tool for 3D culture, soft agar is not suitable for drug screening given its cumbersome procedure, low throughput, and incompatibility with other experimental methods. In response to these shortcomings, multiple 3D cell culture methods have been proposed and developed.

Early on, it was observed that contact-inhibited, anchorage-dependent cell lines that grew as mono-layers in culture would become organized into 3-dimensional tubes or hollow spheroids when exposed to physiological exogenous matrices that mimic normal basement membrane. A commonly used product is known as Matrigel™ (BD Biosciences, Franklin Lakes, NJ, USA), a mixture of collagen and laminin that is derived from the Engelbreth–Holm–Swarm (EHS) murine tumor. Other preparations of basement membrane components (primarily collagen and laminin) are available from a number of commercial sources including EMD Millipore (Darmstadt, Germany), TAP Biosystems (Royston U.K.) and Life Technologies (Foster City California USA)

On the other end of the spectrum are the so called 'suspension' or 'floating' platforms. Floating technologies include hanging drop method which culture the cells within a droplet using low cell adhesion-type culture vessels (3D Biomatrix, Ann Arbor MI USA and InSphero, Zurich, Switzerland), hydrophilic coatings and the use of magnetic particles to maintain the cells in suspension (Nano3D, Houston TX USA)



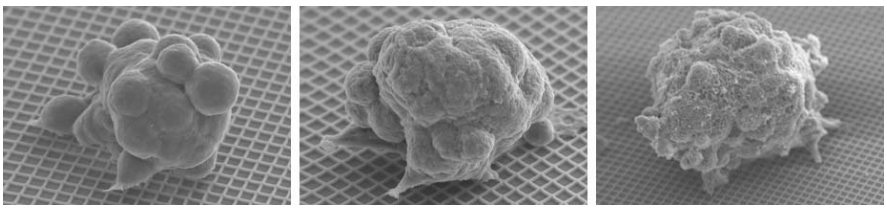
All of these methods have strengths and weaknesses and though scaffold technologies have disadvantages in handling, throughput, reproducibility, and ease of observation, spheroid quality (including cell viability) is quite good, which has been reported in numerous publications. Suspension culture, on the other hand, is more compatible with large scale projects. However, , growth rates are often slow because the cells are not attached to any substrate, and cells tend to aggregate by gravity or convection, causing problems such as rapid over-growth of the spheroids, resulting in cell death due to low nutrition and oxygen. Dehydration can also be an issue for longer incubation cycles.

NanoCulture® Plate (NCP)

SCIVAX NanoCulture® Plate (NCP) is a novel technology for 3D cell culture that combines the advantages of scaffold-based technologies with the ease of use found in other platforms. A bio-mimetic “structure” pattern that mimics structures observed in normal extracellular matrix supports the development of spheroids in a consistent and reproducible manner. Cells can be propagated using normal tissue culture techniques and then seeded into NCP plates for spheroid development. Once seeded, plates are handled in a similar manner as for conventional cell culture including visualization, automated liquid handling and harvesting of cells for analysis. Scivax has demonstrated that over 100 cell types produce robust spheroids on NCP including primary tumor cells, tumor-derived cell lines, fibroblasts, adipocytes, osteoblasts, stem cells, hepatocytes making NCP technology a versatile and easy to use technology.

Scaffold-based 3D cell culture

As with other scaffold-based technologies, cells attach to the pattern imprinted on the bottom of the NCP plate and begin to proliferate and migrate. Because the cells do not adhere to the NCP patterned plates as strongly as compared with traditional cell culture plastics such as polystyrene, the cells preferentially form cell-cell contacts and grow as a spheroid while maintaining a cell-matrix attachment component.



Electron microscopic images at day 2, 4 and 6

No biological matrix

In most cases, NCP spheroids can be cultured with normal FBS-containing liquid media recommended by ATCC for each specific cell type. In certain cases, adding trace amounts of biological matrices (such as Matrigel) has improved spheroid formation.

No lot-to-lot variation

NCP is made 100% of synthetic material, with minimal lot-to-lot variation providing a sound basis for large scale drug screening projects.

Ready-to-use and easy-to-use

NCP plates are packaged ready to go. Plates will need to be briefly conditioned with media prior to seeding cells and are otherwise handled the same at traditional polystyrene plates.

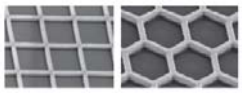
Easy to observe

The NCP well is flat and clear making the spheroids amenable to standard visualization methods including confocal microscopy.

Easy to harvest

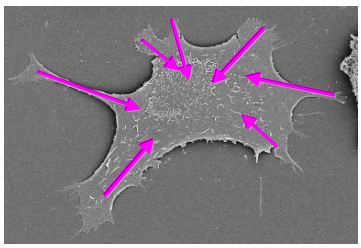
Because the NCP spheroids are so weakly attached to the well bottom, harvesting cells is a simple matter of gentle pipetting and transfer for analysis.

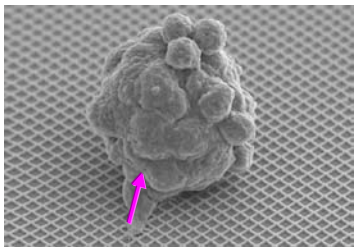
NCP is available in SBS format 24-well and 96-well plate (384-well plate and 35mm dish will be available in December 2012), with 2 different pattern types: square and honeycomb. The preference of certain cell types for a particular NCP pattern is determined empirically. Most cells and cell lines form spheroids equally well on both square or honeycomb patterns, but some cell lines exhibit a strong preference for one or the other.

NanoCulture® Plate	
Format	**Well Pattern**
	
24-well microplate	Square / Honeycomb
96-well microplate	Square / Honeycomb
384-well microplate (Available December 2012)	Square / Honeycomb
35 mm dish (Available December 2012)	Square / Honeycomb

Applications of NCP

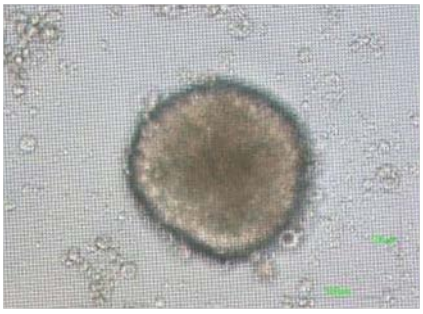
Signal Transduction Research

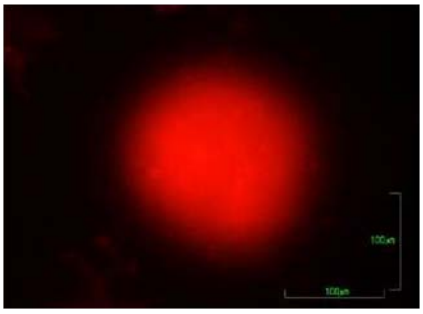




Cells growing on conventional cell culture ware are attached to polystyrene substrate primarily through integrin-mediated binding. Unfortunately, integrin signals through many of the same pathways that are being studied for modulation by potential anti-tumor agents. This strong integrin signal can actually obscure signal transduction effects of other growth factors and signal transduction inhibitors. Since spheroid structure is driven by cell-cell and cell-matrix contacts, similar to what is found is tumor, spheroids provide an improved basis for indentifying novel pharmacological agents that can be developed into more effective anti-cancer drugs.

Hypoxia research





The spherical morphology and tight cell-cell contact of NCP spheroids create a hypoxic core within 3 days. This creates a structure similar to solid tumor that is defined by a hypoxic core surrounded by viable but quiescent cells surrounded by proliferating cells. This stratification of cells within the spheroids leads to a number of biological effects driven by differential gene expression within the spheroid including the up-regulation of genes such as VEGF and other vascularization related genes due to hypoxia . The hypoxic region inside the spheroids can be easily detected with the Scivax hypoxia probe, LOX-1. Simply add this non-toxic reagent to your NCP cultures 24-hours prior to visualization. Cells with hypoxic cores will fluoresce red and is compatible with any standard fluorescent microscope helping to confirm spheroid formation.

High Throughput Drug Screening

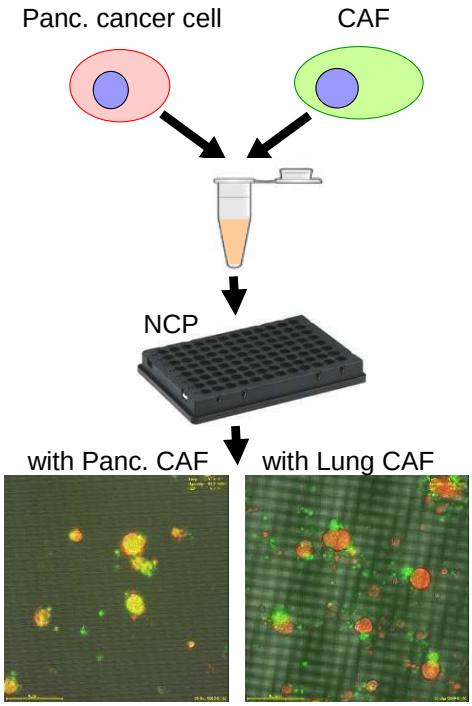
3D cell culture drug screening is becoming increasingly routine in high throughput, cell-based assays. Because NCP can form 3D spheroids simply by seeding cells, it is readily adaptable to automated liquid handling and visualization. Further, 384-well plate is under development, which will make the high through-put 3D cell culture based cell based assay much efficient and cost effective.

Spheroid morphology change is another criterion uniquely associated with 3D cell culture. Conventional anticancer drug screenings evaluated compounds by anti-proliferative or pro-apoptotic activity, while screening of compounds which cause morphology change without affecting the cell survival rate becomes a straight forward excercise by combining NCP and imaging equipment.

Co-culture

As cells do not grow as 2-dimensional monolayers, nor do they grow homogeneously in the body or in a tumor. NCP technology offers a unique approach to co-culture more than one cell type in a stable spheroid. Results from one such experiment in which pancreatic cancer cells were successfully co-cultured with pancreatic cancer-associated fibroblasts was presents at the 2012 AACR conference (Chicago). In this experiment, pancreatic cancer cells (red dye) were co-cultured with cancer-associated fibroblasts

from pancreatic cancer or lung cancer (green dye). As seen, pancreatic cancer cells formed stable spheroids (yellow) with pancreatic cancer associated fibroblasts but not with lung cancer fibroblasts. Access to such stable co-cultures provides new insights into tumor biology and a new basis for cell-based higher content drug screening.



Conclusion

SCIVAX NanoCulture® Plate is the most simple, robust and reproducible 3D cell culture system available today. 3D cell culture is fast becoming a "must use" tool for drug discovery, and NCP can lower the entry barrier for those who intend to introduce 3D cell culture into the drug discovery process. For more information, please visit http://www.scivax.com/usa/index.html, or please contact usa-contact@scivax.com.

References

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