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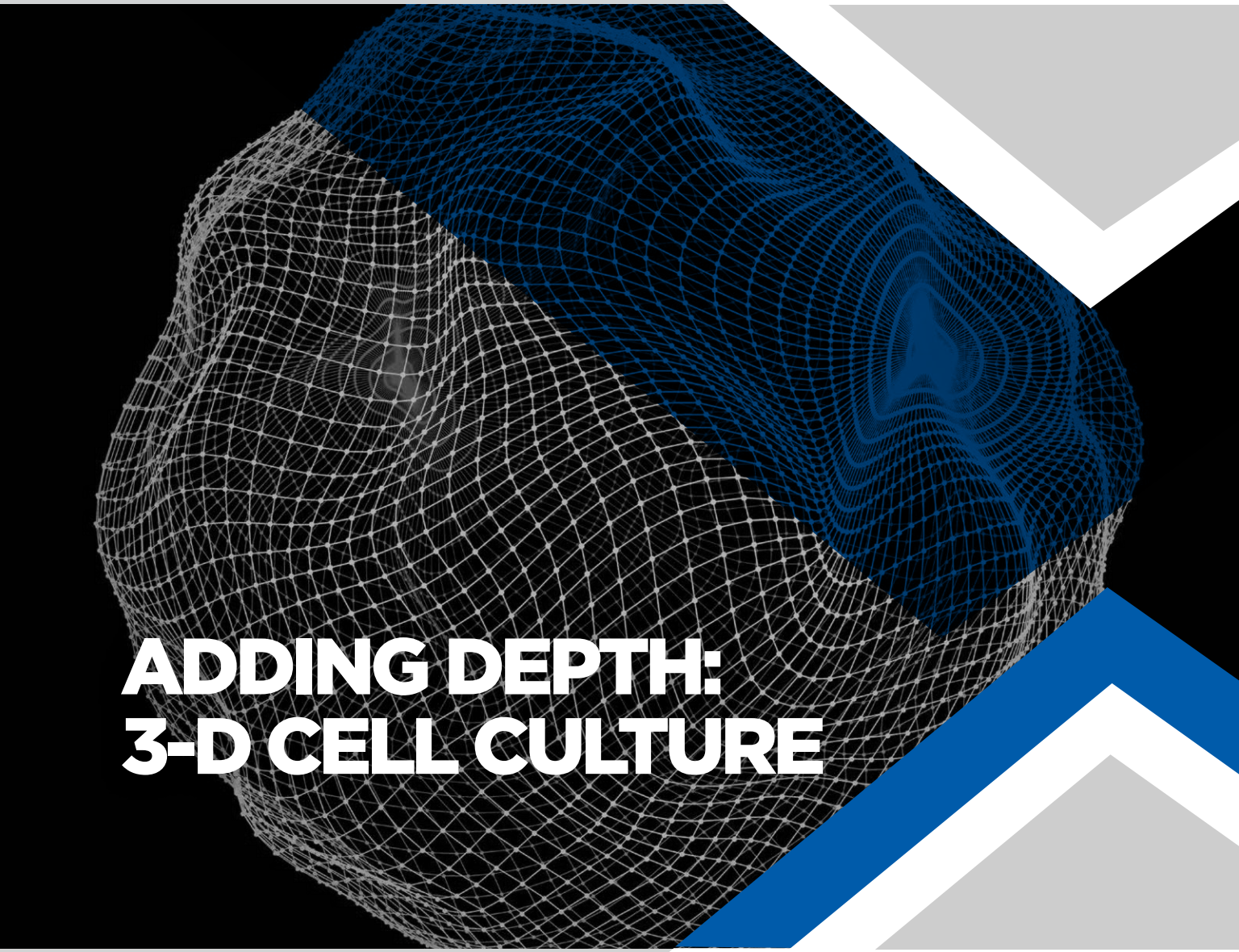

ADDING DEPTH: 3-D CELL CULTURE

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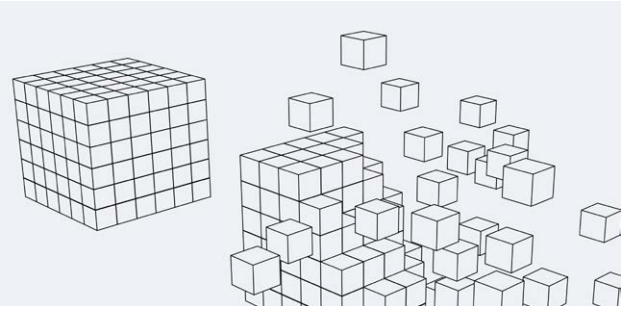
A More Realistic Representation: How the Third Dimension Affects Cell Behavior



"The ability of 3-D cultures to mimic the microenvironment of in vivo tissues sets the technique apart."

Physiologically relevant models were, in recent history, thought of as the holy grail in life science research. However, with three-dimensional (3-D) cell culture models now better able to mimic in vivo conditions, living-organism replicas are within sight.

Two-Dimensional Cultures: Falling Flat

Cell culture is an indispensable tool in a wide range of in vitro research. Classically, adherent monolayers of cells are grown on the surface of a dish or plate creating what's known as 2-D cell cultures. These cultures certainly have their place in research, but they lack the structural and functional idiosyncrasies of the natural environment and consequently are not ideal for more complex analyses. In terms of phenotype, activity, and behavior, 2-D cultures are often poorly suited for many scientific research activities.

Three Dimensions: A New Paradigm

It has been well documented that cells are highly responsive to their surroundings. In response to signals indicating environmental change, cells have evolved mechanisms to survive in diverse conditions. This quality lends well to 3-D culturing techniques.[1]

Early work in 3-D culturing focused on creating multicellular disease models for various diseases such as cancer but was constrained by a lack of biocompatible materials.[2] However, the 3-D culture paradigm has shifted in the past decade with the introduction of better matrices and scaffolds, leading to 2-D culture slowly being supplanted by the newer technology.

Bridging the Gap: Scientific Benefits to 3-D Culture

Cell-based assays are a pillar of the drug discovery process and are increasingly being used to bridge the gap between in vitro and in vivo research. But 2-D cultures are unable to mimic the in vivo extracellular microenvironment. Consequently, 3-D cell culture is becoming pivotal in drug discovery, pharmacology, cytotoxicity and genotoxicity research, apoptosis, cell signaling, differentiation, gene and protein expression research, as well as for studying developmental changes. Furthermore, 3-D co-cultures are important in cell-cell interaction studies.[3]

The ability of 3-D cultures to mimic the microenvironment of in vivo tissues sets the technique apart. Compared with 2-D cultures, 3-D cellular models better represent intricate cell-cell interactions, more closely resemble the exquisite nature of cellular communication, and have matrices that are analogous to in vivo structures.

Better Behavior: 3-D Cultures Mimic in vivo Conditions

3-D cell cultures affect in vitro cell behavior for the better compared with 2-D cultures. Although they are not perfect representations of in vivo structures, matrices or scaffolds used for 3-D culture allow good representations of cell-to-cell and cell-to-matrix interactions, as well as cellular microenvironments. Scaffold-free 3-D cultures also better mimic natural conditions compared with 2-D cultures.

Commonly used materials for 3-D cell culture scaffolds or matrices include hydrogel, agarose, collagen, fibronectin, gelatin, laminin, and vitronectin, generated from various natural or synthetic polymers.[3] These mimic the extracellular matrix; different matrices are suited to different applications.

Scaffold-free techniques include microfluidic cell culture, hanging-drop, low-adhesion plate and micropatterned plate culture, bioreactor culture, and 3-D printing with bioactive synthetic materials to create spheroids or other 3-D models. Scaffold-free 3-D cultures can be manipulated in various ways. For example, microfluidic 3-D cell culture enables spatial control of single cultures and spatially controlled co-cultures.[4]

Scaffold-based or not, 3-D cultures better allow cells to move freely in their cellular environment, generally have a longer lifespan, allow integrin ligation, intracellular signaling, and more physiologically relevant solute diffusion and gene expression compared to 2-D cultures.[5]

3-D cell culture still has some limitations, but as a tool in drug discovery, cancer research, and many other areas, it surpasses 2-D culture in mimicking in vivo conditions.

For references, please see page 7.

Comparing and Contrasting 2-D and 3-D Cell Culture

"Choosing the right method for 3-D cell culture is not trivial. The type of analysis to be carried out has to be carefully considered before a method is chosen."

Both 2-D and 3-D cell cultures are similar in goal and principle – they were developed to mimic in vivo structures and conditions. However, they vary considerably when it comes to execution.

The Good, the Bad, and the Ugly: Cell Culture Pros and Cons

While 3-D cell culture is generally considered a better model for in vivo experiments, there are pros and cons to any kind of cell culture technique.

2-D cultures don't require specialized equipment or reagents, extensive literature about certain cell lines is available, they can be maintained and stored easily, require little technical expertise, are easy to sequence, and they won't break the bank in terms of maintaining them.

However, 2-D cell cultures are notoriously affected by mutations (extensively described for HeLa cells) and contamination (most notably by mycoplasma). Some cell lines have unknown origins and carry inherent variabilities, they are generally less resistant to apoptotic factors compared to 3-D cultures, and, perhaps most importantly, they don't mimic the cell-cell and cell-matrix interactions seen in vivo.[1]

3-D cultures in contrast do better at mimicking the extracellular matrix of in vivo tissues but require greater technical expertise and specialized equipment, can be challenging to maintain, and there is a lack of reliable literature about their responses. Many of the tools used to visualize 2-D cell cultures, such as phase microscopy, are also impractical for imaging certain 3-D cell cultures since they rely on light being transmitted through the sample.[2] Other challenges include the creation of tissue-tissue interfaces, mechanical microenvironments, and controlling spatiotemporal distributions of oxygen, nutrients, and metabolic waste, to better mimic in vivo conditions.[1]

Furthermore, 3-D cultures have been shown to acquire different phenotypes during growth after addition of certain reagents. For example, tumor cells may revert to a non-cancerous cell type after the addition of antibodies. This effect has been shown to be less likely with 2-D monolayers of cells.[3]

In Preparation: Considerations for 3-D Culture

Choosing the right method for 3-D cell culture is not trivial. The type of analysis to be carried out has to be carefully considered before a method is chosen.

Spheroids for example are excellent models of drug resistance and provide clinically relevant tumor models, but it can be challenging to develop, reproduce, and maintain spheroids of uniform size. Thought must also be given to the temperature, pH, and levels of carbon dioxide and oxygen at which the spheroids are grown.[4]

Organoids are patient-specific with in vivo-like structures, but they lack vasculature, are difficult to image, and can be highly variable.[5]

Hydrogel-scaffold systems are amenable to high-throughput screening, can be used for co-cultures, and are relatively easy to culture, but suffer from variability between lots.[5]

3-D bioprinted cultures can be co-cultured, be custom made with high-throughput production, and can contain chemical and physical gradients, but they also lack vasculature, are difficult to image, and have issues with tissue maturation.[5]

Spheroids, hydrogel-scaffold systems, and 3-D bioprinted cultures also have simplified architecture in comparison to other 3-D models, making them less suited to certain assays requiring particular in vivo-like conditions.[5]

3-D culture methods also require well-trained staff and high levels of expertise to achieve good in vitro models, particularly when it comes to building complex, multilayer models.

In Tandem for Comprehensive Data

By combining 2-D and 3-D cell cultures, researchers can reap the benefits of both methods. 3-D cell cultures are likely to usurp 2-D cultures for a variety of life science activities; however, 2-D cell cultures are still useful in numerous biological assays. Substrates for 2-D and 3-D cultures that are currently in development will bring new capabilities. For new therapeutic targets before pre-clinical assessment, a good approach is to combine the best-adapted 3-D cell culture method with a classic 2-D culture.[6]

Cell culture will remain an indispensable tool and it will be exciting to see where 3-D cell culture techniques take life science research next.

For references, please see page 7.

LIFE-LIKE: COMMON 3-D CELL CULTURE MODELS

SCAFFOLD-FREE TECHNIQUES

Hanging drops concentrate cell suspensions via gravity. Forced-floating promotes cell-cell interaction by preventing cell adhesion to a surface. Bioreactors use rotation to gradually turn cell suspensions into aggregates.

Advantages: Methods are relatively easy to perform. Hanging drops and forced-floating creates homogenous, easily accessible spheroids. Bioreactors form many spheroids of a range of sizes. Spheroids can be used for co-cultures.

Disadvantages: Hanging drops and forced-floating sometimes require specialized plates. Bioreactors can be expensive.

Hanging Drop

Forced-Floating

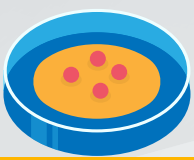
MATRICES AND SCAFFOLDS

Most experiments use hydrogels, which are networks of polymers that form a gel when incubated. Non-gel scaffolds are often made of nanofibers.

Advantages: Hydrogels mimic the extracellular environment. Scaffolds can be used for tissue engineering.

Disadvantages: Complex to set up. Requires specialized equipment. Non-uniform 3-D cultures. Expensive. Hydrogel batch-to-batch variability.

Hydrogels



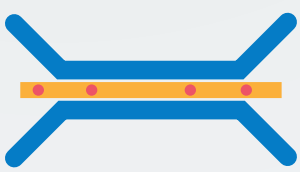
Non-gel Scaffolds

MICROFLUIDICS

Microfluidic devices consist of microchannels filled with gel that allow 3-D cell models to be formed. Avascular microfluidic devices are hybrid devices used to culture tumor cells in a gradient.

Advantages: Increased control of microenvironment. Good imaging capabilities of whole device. Fine-tuning of growth conditions possible. Fast 3-D cell model formation.

Disadvantages: Requires specialized equipment. Difficult to collect cells for analysis. Avascular microfluidic devices lack vasculature and 3-D environment.

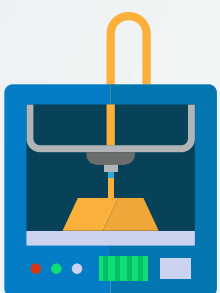


3-D BIOPRINTING

3-D bioprinting is a computer-assisted technique allowing precise spatial positioning of biomaterials to create 3-D cultures and organoids including vessels, bone, skin, heart, liver, and nerve systems.

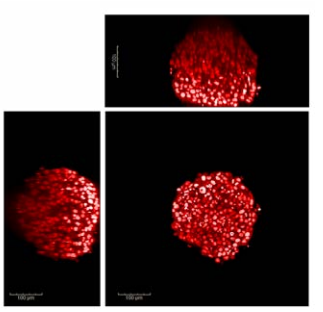
Advantages: Allows 3-D printing of various tissue architectures.

Disadvantages: Requires expensive, specialized equipment and expertise.



Penetrating the Darkness: Imaging and Analyzing 3-D Cell Models

"Newer platforms offer improved imaging and analysis capabilities that enable a fuller appreciation for the cell-cell architectures at play."



Water immersion objectives improve 3D image quality.[7]

Traditional 2-D cultures are more easily imaged with standard equipment and techniques than are their more complex, 3-D counterparts. However, thanks to the boom in 3-D cell culture, newer platforms offer improved imaging and analysis capabilities that enable a fuller appreciation for the cell-cell architectures at play.

In Focus: Microscopy

Many microscopy techniques that work well for cell monolayers are poorly suited for imaging thick, highly scattering 3-D cultures. Imaging techniques that allow reflected light to be collected (epi-illumination) including confocal microscopy and multiphoton microscopy are better for thicker specimens.

Confocal microscopy can be used in fluorescence or reflectance mode and has a penetration depth of approximately 100 µM.[1] Confocal laser scanning microscopy is the most commonly used type of confocal microscopy for 3-D tissues and is based on point illumination of the sample with a laser followed by filtering of the return beam to block light originating outside the focus.[2]

Multi-photon microscopy (MPM) can achieve greater depths compared to confocal microscopy, with at least a twofold improvement. It is most commonly used for samples labeled with fluorescent dyes. MPM is preferred over confocal microscopy for imaging 3-D cell cultures when they are thick or scatter light to a greater extent, since imaging penetration is better, and little absorption or thermal effects are seen.[2]

Light sheet microscopy is another option. The technique is ideal for light-sensitive specimens as it illuminates just one focal plane at a time. The technique has a good penetration depth, as it enables imaging of millimetre-sized samples in their entirety.[3]

However, all of these microscopy techniques allow imaging of just a single or a few samples at a time, as they are slow and some require manual sample preparation such as agarose embedding in specialized sample holders. Researchers using conventional microscopes typically observe their samples qualitatively, without quantifying the information contained in the images.

Automated Imaging and Analysis: High Content Screening

High-content screening (HCS) provides researchers with high-throughput analysis while also enabling quantification and maintaining physiological relevance.

HCS is a fast, powerful method for analyzing cells that makes use of robotics and automation alongside fluorescence imaging and flexible algorithms. Multiparametric cellular data can be procured through HCS, which can be used to quantify phenotypes. Consequently, HCS enables a greater depth of analysis that facilitates comparisons between individual cells, predictions of functional relationships, and associations of morphological changes with genes.[4]

Analysis of 3-D cultures is a relatively new player in the HCS arena, but is anticipated to take 3-D culture analysis to the next level through increasing physiological relevance while maintaining the ability to interrogate a large number of compounds or genes.

Some HCS systems incorporate multiple cameras for simultaneous imaging of fluorescent channels. Furthermore, water immersion objectives allow fast and high-quality imaging of 3-D samples.

The current bottleneck in HCS is the lack of software for the analysis of 3-D images. However, this is a manageable problem, as multiple solutions are currently in development.[4]

Real-Time: Live imaging

Although fluorescent confocal microscopy is the most commonly used analytical tool for examining 3-D cultures, it doesn't provide realtime information so it has its limitations, for example in kinetic studies.

Live imaging of 3-D cultures is challenging. Many systems for both live and fixed cell imaging use a spinning disk confocal microscopy approach, although these can suffer from "pinhole crosstalk", which causes image blurriness and increases background haziness.[5] However, spinning disk systems are preferred for live cell imaging compared to laser scanning systems, as they are much faster and cause less photobleaching and phototoxicity.

Real-time 3-D culture analysis introduces further challenges; processing the large data volumes generated can be logistically difficult, while prolonged single-cell imaging may be susceptible to the introduction of experimental variability on a cell-by-cell basis. Solving these challenges will allow a deeper understanding into how co-cultures grow and interact, as well as be useful for the study of kinetics and dynamics of fluorescently labeled therapeutics over a long period of time.[6]

For references, please see page 7.

Article 1 - A More Realistic Representation: How the Third Dimension Affects Cell Behavior

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