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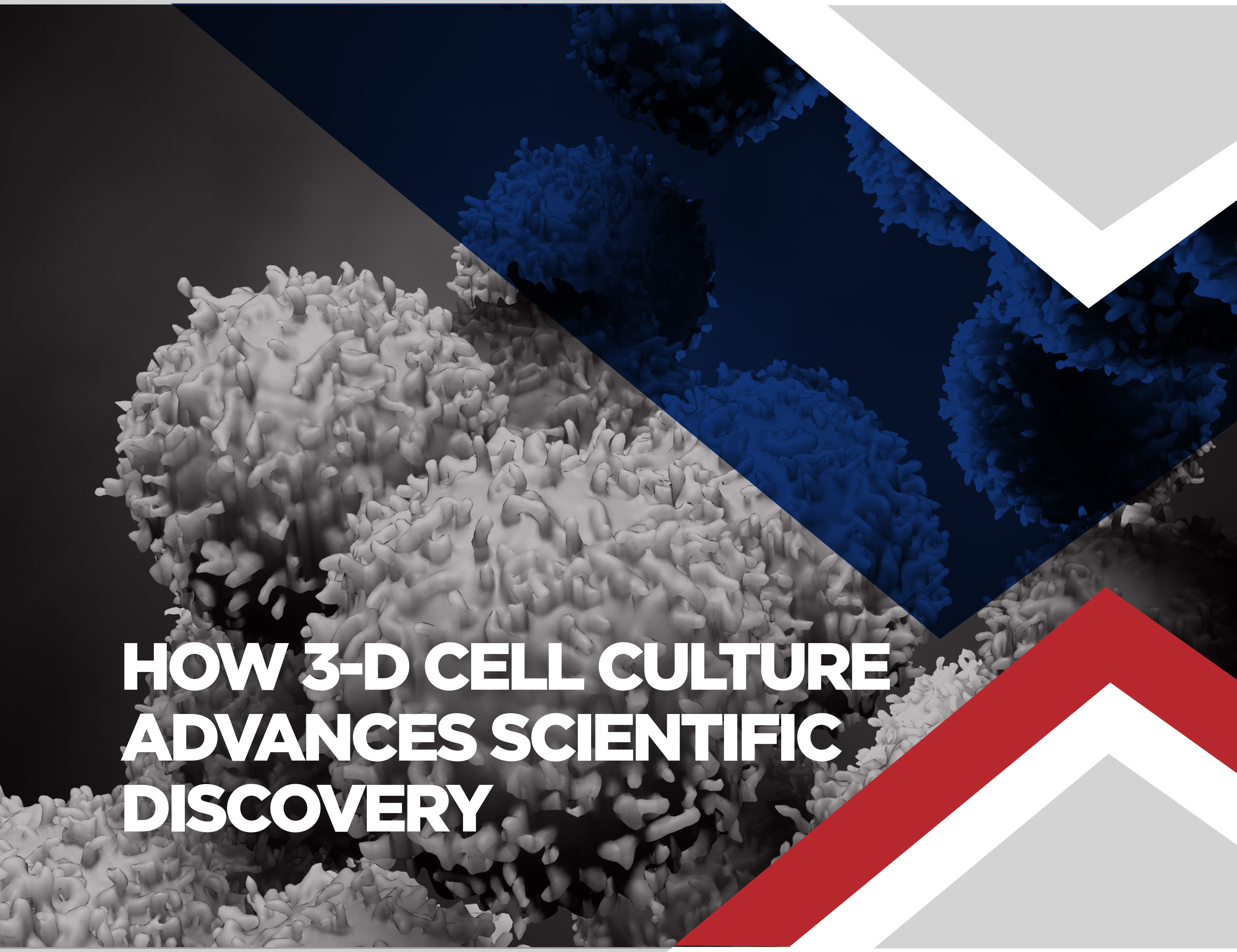

HOW 3-D CELL CULTURE ADVANCES SCIENTIFIC DISCOVERY

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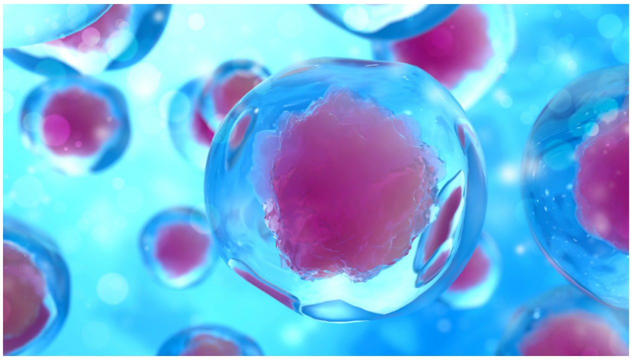
The Advantages of 3-D Cell Culture



Cell culture has been extensively used in life science laboratories for well over a century, providing scientists with experimental tools and models that have led to tremendous discoveries and advances. One of cell culture's major drawbacks is that it does not adequately capture the features of in vivo environments. A cell's behavior is strongly controlled by external cues from the surrounding structure and environment. However, the majority of cultured cells are grown in a two-dimensional monolayer format.

In order to rectify this weakness, scientists are exploring, developing, and adopting three-dimensional cell culture systems. The ideal 3-D cell culture system encapsulates key in vivo aspects absent from conventional 2-D culture approaches. It allows for interactions between multiple cell types, it forms ordered physiological structures (including vascular structures, extracellular matrix elements, and basement membranes), and it integrates flow mechanics and microfluidics. Most importantly, 3-D cell culture systems can capture the varied and dynamic conditions within a tissue or organ structure, in contrast to the uniform conditions found in 2-D culture systems.

Types of 3-D Culture Systems

There are multiple 3-D cell culture methods currently in use, each designed to assemble and sustain physiologically relevant multicellular structures. Arguably the easiest to implement are spheroids—cellular aggregations that form in low- or no-adhesion cell culture conditions. Spheroid structures are scalable and highly reproducible, but they lack the compositional and architectural complexity found in other culture techniques[1]. Organoids differ from spheroids in that they develop from stem cells or organ progenitors that self-organize in a manner similar to that found in vivo, enabling them to present realistic microanatomy. Organoids may or may not contain stromal structures depending on the progenitors used to create them, and they tend to lack vasculature—a major drawback that scientists aim to rectify[1,2].

Cultureware is a key parameter when using spheroids and organoids. Since cells must be free-floating in order to form 3-D structures, cultureware for 3-D cell culture is coated with hydrophilic polymers to limit cell adherence. Additionally, well-bottom geometry affects cell aggregates by potentially confining their physical growth. Well bottoms can be flat, U-shaped, M-shaped, or V-shaped—with the latter two facilitating the formation of tighter, more compact cellular structures. Finally, cultureware impacts data acquisition and analysis. Not only does the number of wells on a microplate create a limiting factor for throughput, but the properties of the plate itself, such as color and geometry, can determine how well individual cell cultures can be visualized using various modalities.

For scaffold techniques, researchers seed cells within natural or synthetic materials meant to provide an anchor point and to simulate the extracellular matrix. Polymer hydrogels such as those comprised of cellulose, collagen, and chitosan are well-established scaffold materials, but ceramics, recombinant proteins, and metal composites have also been used[3]. Scaffold characteristics directly affect the properties and behavior of the implanted cells, and issues with scaffold porosity can limit the complexity of cultured tissues[1].

Organ-on-chip models use microfabrication techniques to create an artificial miniature model of a human organ on a microfluidic cell culture chip. Made with great precision, chips contain well-defined structures, patterns, and scaffolds, including integrated fluidic channels. In this way, the position, shape, function, and environmental properties of the cells cultured on the chip can be tightly controlled. Organ-on-chip models are excellent at emulating the structural and functional complexities of living human organs. While overall detail levels can be limited by practicality and cost restraints, organs-on-chips capably reproduce clinically relevant disease phenotypes and enable researchers to gauge pharmacological responses[1].

Finally, researchers can bioprint 3-D living tissues layer-by-layer, with the desired cellular architecture, topology, and functionality. 3-D bioprinted models can comprise not only cells, but also biocompatible materials and supporting components such as scaffolds. 3-D bioprinting has generated functional tissues, including skin, bone, and vascular grafts for transplantation. Researchers have also used it to create scaffolds for other 3-D cell culture techniques. However, 3-D bioprinting holds challenges related to cell and material requirements and tissue maturation[1].

Multifaceted Models for Multifaceted Diseases

3-D cell culture offers researchers models that more closely mimic in vivo conditions, something that is critical for studying diseases and pathologies that involve complex environments, networks, and signaling cascades. In particular, the superior translatability provided by 3-D cultures is vital for cancer research, owing to cancer's heterogeneity and plasticity. Scientists need models that replicate the structures and interactions that occur within the body in order to devise therapeutic options that maintain efficacy when moved into the clinic.

See references on page 8.

TROUBLESHOOTING 3-D CULTURE

Switching to 3-D culture can be challenging for researchers who are used to the procedures and practices of 2-D culture. Fortunately, advanced technologies address these concerns, making 3-D culture more accessible.

COST, THROUGHPUT, AND SCALING

3-D culture techniques are more resource intensive than 2-D culture techniques. Encouraging cellular growth is typically easier in 2-D culture, as proper structural formation is not necessary. 2-D culture is therefore currently capable of higher throughputs and greater scaling.

With increased attention and use, 3-D culture techniques are being refined and improved so that they are more accessible. Newer 3-D culture techniques such as organs-on-chips can be microplate compatible, permitting automation and higher throughput.

IMAGING AND ANALYSIS DIFFICULTIES

The larger sizes associated with 3-D cell cultures can complicate analysis. General imaging with light microscopy can be particularly challenging because it relies on light penetration. Analysis of interior areas may require dissection.

Techniques such as confocal microscopy, multiphoton microscopy, and optical coherence tomography can penetrate thicker specimens, allowing for non-destructive and repeated analyses over time.

CONTROLLING CULTURE CONDITIONS

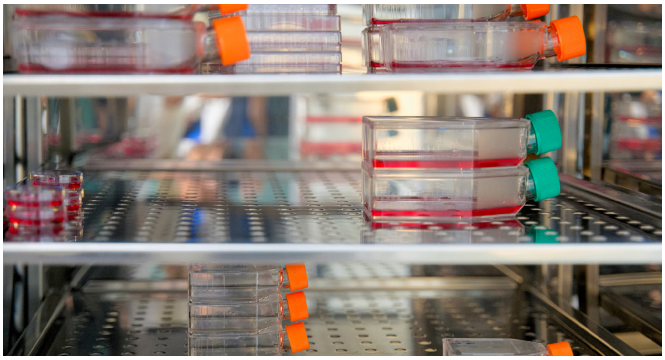
Architectural and structural diversity means that environmental conditions such as local temperatures and pH levels can vary abnormally from region to region within a 3-D cell culture. This is exacerbated by external factors such as poor perfusion and inconsistent culture conditions.

Incubator conditions should be constantly monitored and cell cultures inspected to identify signs of necrosis or poor cell health.

NUTRIENT AND OXYGEN DELIVERY

Delivering the appropriate amounts of oxygen and nutrients to all cells and removing waste products is more difficult within a 3-D cell culture structure compared to a 2-D cell monolayer.

Newer 3-D cell culture techniques use microfluidic channels to aid proper perfusion and resource distribution in physiologically-relevant manners.



Controlling Humidity in 3-D Cell Culture

Cell culture, whether 2-D or 3-D, aims to mimic in vivo environmental conditions. Three key parameters—temperature, gas tension, and humidity—govern the health and growth of cultured cells. Between the three, maintaining and restoring humidity levels is the most difficult, even with modern incubation systems.

The Importance of Water

Water accounts for 70% of overall cell mass in animal cells[1], so it is natural that regulating environmental moisture is a large part of cell culture. Humidity is instrumental in maintaining cellular homeostasis, largely through limiting the rate of culture media evaporation. Water evaporation from culture media elevates concentrations of salts and minerals, creating osmolality shifts that affect cellular metabolism and function and can result in toxicity and cell death. Moreover, the changes in liquid volumes brought about by evaporation can affect the consistency and reproducibility of experimental assays, especially ones that rely on colorimetric readings[2].

Small changes in humidity can have significant effects on evaporation, with rates four times greater at 80% relative humidity versus 93% or higher[3]. As such, cell culture incubators typically strive to maintain 93% relative humidity or greater by deriving moisture from a pan of water placed at the bottom of the incubator or water automatically introduced using a sensor-monitored and regulated system. The high humidity environment not only benefits the growth of the cultured cells, but also foreign entities such as bacteria and fungi. Water pans are especially susceptible to contamination, and while fungicides and other agents can be placed in the water in response, the evaporation of these agents may produce volatile fumes that affect cell growth. Regular inspection and cleaning of the incubator chamber and water pan is important for minimizing contamination.

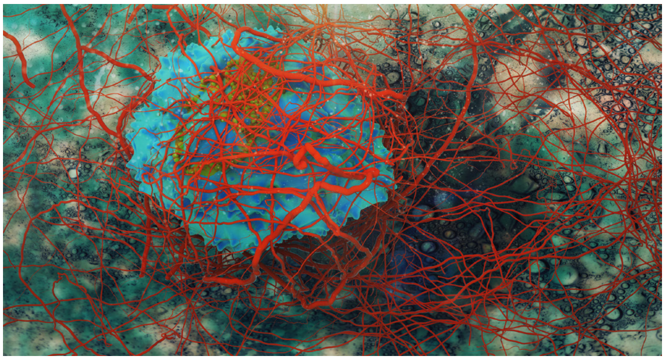
Humidity, Evaporation, and 3-D Culture Techniques

Abnormal humidity levels affect the uniformity, reproducibility, and success of 3-D cell cultures. Multi-well microplate cultures are particularly susceptible. Inconsistent humidity results in variations between wells, resulting in the "edge effect," where wells along the outside edges of the plate experience greater evaporation than centrally positioned wells[4]. Additionally, gel-based scaffolding materials commonly used for 3-D culture applications may present different evaporation kinetics than water. Matrigel®, for example, evaporates faster than water under normal laboratory conditions, making low-volume operations, such as the construction of thin-layer 3-D cell culture constructs, difficult[5].

Handling Humidity

Scientists rely on incubator technology to lessen humidity-related perturbations to their cells. To optimize incubator function, researchers must minimize the number of times that doors need to be opened. They rely on features such as internal sensors to provide external readouts and water reservoirs that can be monitored and refilled from the outside. Nonetheless, at some point, scientists need to take out their cells to work with them. Facilitating accelerated restoration of desired humidity levels following environmental disruption is therefore essential. Incubators can further promote environmental heterogeneity by eliminating potential contamination sources such as wall condensation. With constant monitoring, regular maintenance, and a strong understanding of what humidity does to cells, scientists can limit disturbances to their cells and strengthen data consistency and reliability.

See references on page 8.



Recent Advances Powered by 3-D Cell Culture

Because 3-D cell culture provides a better representation of what occurs inside the body, it allows researchers to uncover new secrets and grasp a better understanding of the mechanisms underlying health and disease. 3-D cell culture has been especially useful in scenarios where cellular behavior is dictated by an array of signals from multiple different sources, such as within tumors and during stem cell proliferation and differentiation. It has also benefitted studies of flow and penetration dynamics, aiding drug delivery investigations.

3-D Cell Culture and Cancer Research

Cancer has always presented a challenge for scientists. The inherent complexity of tumor structures, complicated by the variable behaviors of a multitude of different cell types, made it difficult for researchers to access pre-clinical models that sufficiently mimicked in vivo disease states. 3-D cell culture techniques enabled the creation of spheroid and organoid models that bridged the gap. Now, cancer development, growth, and metastasis are being studied using organ-on-chip models which encapsulate the tissue-tissue interactions, flow dynamics, and mechanical cues that the first 3-D cell culture models could not capture[1].

Scientists have been using organ-on-chip models for cancer research since 2010, with chips specifically designed for examining vasculo/angiogenesis and intra/extravasation developed since then. The presence of separate parenchymal and vascular microfluidic systems, combined with the ability to seed tumor, epithelial, and stromal cells in distinct compartments separated by extracellular matrix components and basement membranes, allow researchers to visualize tumor invasion, formation, and metastatic processes as they happen. Today, chips can also be designed to mimic specific oncogenic pathologies such as breast and lung cancer, even to the extent of using vacuums to simulate inhalation and exhalation[1].

3-D Cell Culture and Stem Cell Research

Scientists hoped that induced pluripotent stem cells (iPSCs) would result in new disease models and facilitate new therapeutic avenues. However, as with embryonic stem cells, properly directing iPSC differentiation is a challenge. Differentiation patterns and mechanisms observed during early studies using 2-D culture models do not fully translate to 3-D situations, while 3-D models also brought additional levels of detail. For example, while 2-D models recapitulated action potential and contractility phenotypes in iPSC-differentiated cardiomyocytes, researchers additionally observed self-organization and responses to biophysical cues in these cells in 3-D cell culture[2].

Today, 3-D cell culture structures such as organoids and spheroids are used to identify disease-causing mutations and find associated pathogenic phenotypes. They help scientists examine host-pathogen interactions, including modeling viral and bacterial infection responses. Cellular structures created using 3-D culture techniques can also be engrafted into animal models, creating chimeras. These chimeras offer a model for studying tissue regeneration and restoring lost functionality, such as for pancreatic β-cell insulin production[2]. 3-D models also replicate the hierarchical order of cancer cells[3], helping researchers understand cancer stem cell niches: where they are located, how they function, and how they can be targeted.

3-D Cell Culture and Drug Discovery Research

Scientists are using 3-D cell culture to better understand drug dynamics. 3-D models possess flow systems, letting researchers examine the distribution and clearance kinetics of circulating agents. They also allow researchers to observe penetration to see if physical obstacles are limiting drug delivery efficacy. Moreover, models such as spheroids and organoids can be scaled up more readily than animal models, allowing for higher throughput drug screening, hit identification, testing, and profiling[4,5]. These advantages are tremendously useful not only for cancer drug development[4], but also for identifying potential agents for organ-specific disorders, neurological conditions, regenerative medicine, and infectious diseases[5].

See references on page 8.

Article 1 – The Advantages of 3-D Cell Culture

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Article 2 – Controlling Humidity in 3-D Cell Culture

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Article 3 – Recent Advances Powered by 3-D Cell Culture

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