3D Biomatrix™
Three-Dimensional Cell Culture

3D Cell Culture 101: An Introduction to 3D Cell Culture Tools and Techniques

White Paper

If you are new to 3D, what do you choose?

In this white paper

Introduction

For decades, three-dimensional (3D) cell culture has been employed by tissue engineers, stem cell scientists, cancer researchers and cell biologists, largely in university settings. The development of new materials or methods has been driven by the desire of these scientists to incorporate experimental systems that better represent the *in vivo* environment into their research. Early adopters of 3D cell culture technology have reaped the benefits of better data with groundbreaking knowledge of tissue and cancer behavior.

3D cell culture methods were once expensive, messy, laborious, and difficult to adapt to existing procedures. Today, researchers can pick among an array of 3D cell culture tools, ranging from simple to complex, to fit their specific needs. Segmentation of the 3D cell culture choices into discrete categories demonstrates a new maturation in the market. As the number of 3D cell culture options has grown, publications proving their importance have exploded in numbers (Figure 1).

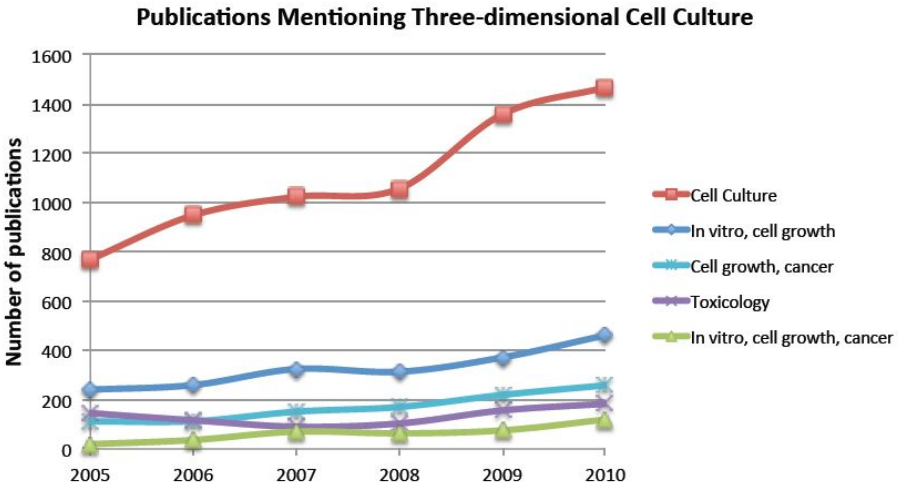
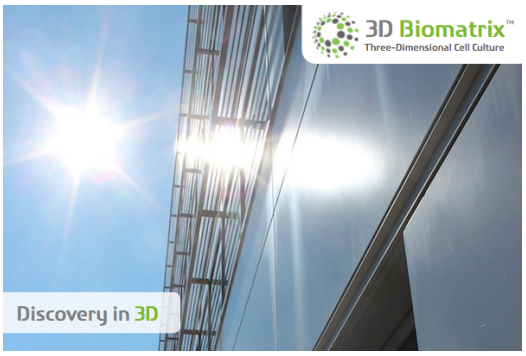


Figure 1. Publications referencing 3D cell culture have grown dramatically in the last several years. Each line on the graph represents a separate search for three-dimensional cell culture plus the additional listed search term. A small degree of overlap is possible.



3D Cell Culture 101

If you are new to 3D, what do you choose?

An important consideration in 3D cell culture is replicating or mimicking the extracellular matrix (ECM) (Figure 2). The ECM provides physical structure, sequesters and secretes growth factors, and facilitates cellular communication. Consideration of the ECM composition, structure, and density in your target tissue, or whether or not you would like to provide ECM at all, may dictate the 3D cell culture format choice.

In this tutorial, we discuss 3D cell culture methods that rely on cells to secrete their own ECM and others that utilize natural or artificial materials to mimic the ECM until cells create their own. Five types of 3D cell culture options are reviewed: scaffold-free platforms for spheroid growth, scaffolds, gels, bioreactors, and microchips. For your convenience, select review articles are included at the end of each section.

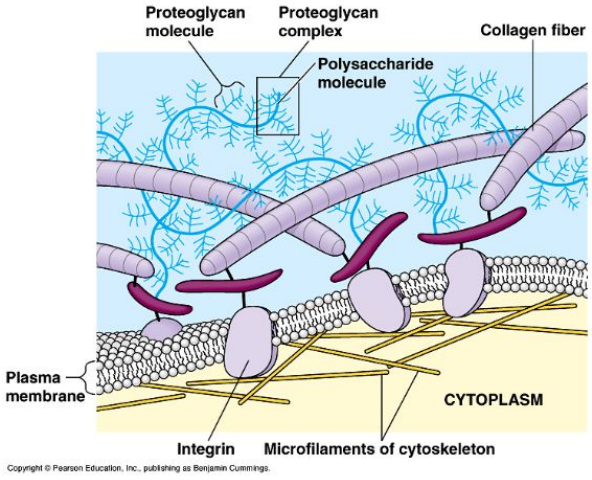


Figure 2. Schematic of various components of the ECM.

Scaffold-Free Platforms for Spheroid Growth

Scaffold-free platforms for spheroid growth

Spheroids are self-assembled spherical clusters of cell colonies. They were first documented in 1944 by Johannes Holtfreter who worked with spherical aggregates of embryonic cells. Spheroids naturally mimic solid tissues, avascular tumors, and embryoid bodies, and have found application among researchers in cancer and stem cell research. With inherent metabolic (oxygen, carbon dioxide, nutrients, wastes) and proliferative gradients, spheroids serve as excellent physiologic models.

Scaffold-free platforms for spheroid growth do not contain added biomaterials or ECM, and cells grown in them generate and organize their own 3D ECM, so spheroids closely resemble *in vivo* tissues. Co-cultures with other cell types (i.e., endothelial, stromal, epithelial cells) extend the predictive cytotoxicity capabilities of this 3D cell culture system.

Scaffold-free platforms have no support structure or porosity. The overall spheroid size is limited beyond a critical size of 500 – 600 µm in diameter, after which central secondary necrosis develops in most, but not all, spheroids grown from permanently-transformed cell lines.

Standardized mass production of 3D spheroids makes them applicable for both basic laboratory research and high-throughput screening (HTS) applications. The Perfecta3D® Hanging Drop Plate from 3D Biomatrix™ is designed to enable consistent formation of spheroids using conventional liquid handling tools. This scaffold-free platform is simple to use and generates spheroids with consistent sizes and shapes so that testing is controllable and reliable. Adjusting the seeding density, from as few as 50 cells to as many as 15,000 cells, allows production of varying spheroid sizes.

"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 plate consists of the main hanging drop culture plate and a complementary lid and tray, which serve to maintain sterility and reduce evaporation. Access holes in the culture plate allow manipulation of fluids and spheroids from the topside. A water reservoir constructed around the periphery of the culture plate also helps to alleviate evaporation (Figure 3).

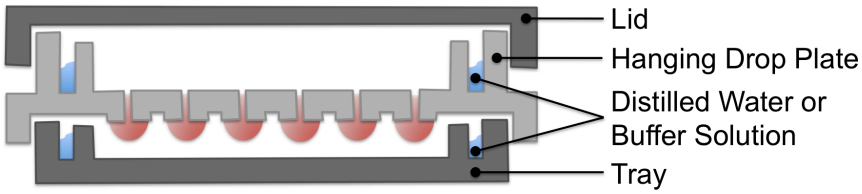


Figure 3. A schematic of the Perfecta3D Hanging Drop Plate.

Hanging drops are created by dispensing small volumes of cell suspensions, using standard pipette tips, into the access holes on the top of the plate, just like pipetting into conventional multi-well plates. In a similar fashion, reagents and drugs can be added to or removed from each hanging drop.

A plateau structure on the bottom of the plate stabilizes the hanging drops (Figure 4). The Perfecta3D Hanging Drop Plates do not have a bottom substrate for cells to eventually attach to, therefore cells in suspension aggregate into a spheroid.

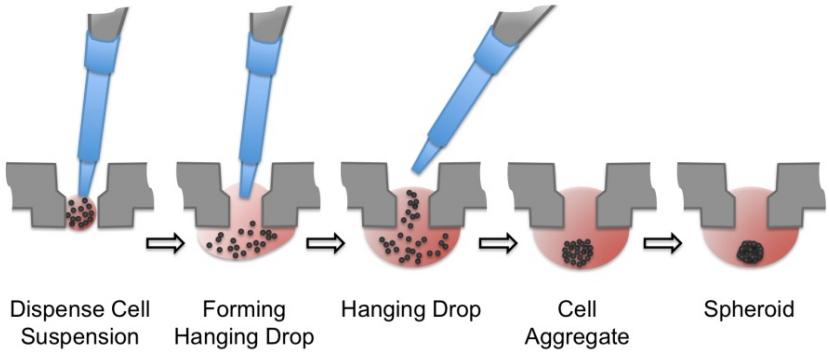


Figure 4. Spheroids are created by dispensing cell suspensions into the access holes of the Perfecta3D Hanging Drop Plate, just like pipetting into conventional multi-well plates.

Spheroids can be harvested and analyzed using colorimetric, fluorescence, and luminescence assays measured with a plate reader. Microscopic imaging of spheroids can be performed directly with the transparent plate, lid and tray assembled. The platform also offers simplified liquid handling procedures and compatibility with HTS instruments, such as liquid handling robots like the Biomek® FX and epMotion automated pipetting systems.

Articles:

The inventing University of Michigan research team discusses the Perfecta3D Hanging Drop Plate.

- Tung, YC, Hsiao, AY, Allen, SG, Torisawa, Y, Ho, M, Takayama, S, High throughput 3D spheroid culture and drug testing using a 384 hanging drop array, Analyst, 136 (2011), 473-478.

Demonstration of Z-factors and co-cultures with the Perfecta3D Hanging Drop Plate.

- Hsiao, AY, Tung, YC, Qu, X, Patel, LR, Pienta, KJ and Takayama, S, 384 hanging drop arrays give excellent Z-factors and allow versatile formation of co-culture spheroids, Biotechnology and Bioengineering, 109 (2012), 1293-1304.

An overview of 3D *in vitro* cancer models as they pertain to drug discovery, with a specific focus on those that have been developed from a tissue engineering perspective.

- Burdett E, Kasper FK, Mikos, AG and Ludwig, JA, Engineering Tumors: A Tissue Engineering Perspective in Cancer Biology, Tissue Engineering: Part B Vol 16, No. 3 (2010), 351-9.

Scaffolds

Scaffolds

Scaffolds, also commonly called 3D matrices, are available in a large variety of materials with different porosities, permeabilities and mechanical characteristics designed to reflect the *in vivo* ECM of the specific tissues being modeled. Scaffolds are manufactured using a variety of techniques, such as 3D printing, particulate leaching, or electrospinning, each of which introduces different porosities, pore sizes, scaffold materials and features.

Scaffolds are typically divided into two main application categories: functional implants for clinical and regenerative medicine applications and *in vitro* 3D scaffolds for laboratory applications.

Though the characteristics of implantable scaffolds may vary greatly depending on the tissue being mimicked, the ultimate goal for many implantable scaffolds is to provide support to a wound site and aid eventual replacement of the scaffold by natural tissue. As such, the requirements for functional implant scaffolds differ from those for *in vitro* 3D laboratory applications. Functional implants must match the defect site, support and promote desired cell growth, and biodegrade without harmful effects.

When scaffolds are used for *in vitro* laboratory applications, geometric match and biodegradability are less necessary. In fact, degradability may introduce an undesirable variable into experiments as byproducts may change the chemistry and pH of the culture system. Furthermore, as the scaffold degrades and cells re-organize the matrix, the cells may not retain their 3D configuration. *In vitro* scaffolds should represent a more stable structure and function similar to the natural *in vivo* environment.

3D scaffolds for *in vitro* laboratory applications are available in a variety of materials: metals, ceramics, polymers - natural and synthetic, and composites. Properties to consider include biocompatibility, wettability, mechanical properties, and surface chemistry. The method of fabrication must also be considered as it may introduce a random or ordered structure to the scaffold (Figure 5). When utilizing a scaffold with a random structure, such as those that result from particulate leaching methods, scaffold-to-scaffold variability, as well as isolation of areas within the scaffold, may be a problem.

Due to the variety of material and structural choices for scaffolds, they are widely used in many applications. Furthermore, as they provide a surface on which cells can grow, they can easily impart 3D growth with little alteration to cell culture procedures. The porosity of scaffolds aids mass transport of nutrients, oxygen, and wastes, allowing for larger culture growth than the scaffold-free platform discussed earlier; it can be difficult to extract all cells for analysis with increased scaffold size and tortuosity. Imaging may also become difficult depending on the scaffold size, transparency of material, and depth of the microscope.

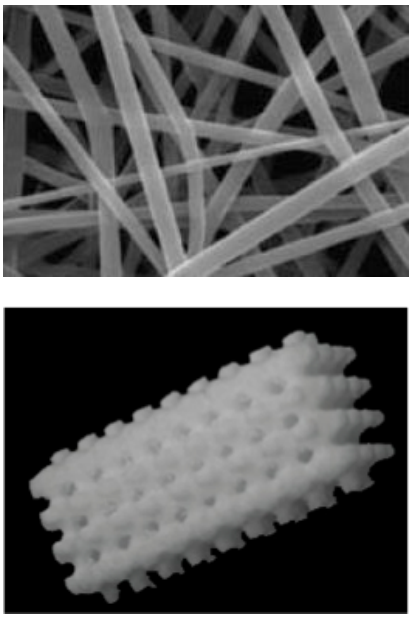


Figure 5. Examples of random (left) and ordered (right) scaffold structures. Image adapted from Lee et al.

In a 2008 review article, Lee et al. discuss the macro-, micro-and nano-scale elements of 3D scaffolds.

- Macro-scale: Overall size and shape, dependent on application.
- Micro-scale: Porosity, pore interconnectivity, pore geometry, pore size distribution and elements of surface topography. Micro-scale elements may be customized for different tissue types. Micro-scale elements facilitate mass transport, diffusion of nutrients, metabolic wastes and soluble molecules and can activate certain genes and modulate cellular behavior in differentiation and proliferation. Micro-scale features also affect the overall robustness of the scaffolds and hence the desired application, such as use in a bioreactor, multiwell plate or human body.
- Nano-scale: Nutrient supply and functional effects due to the size of many cell-signaling molecules.

Articles:

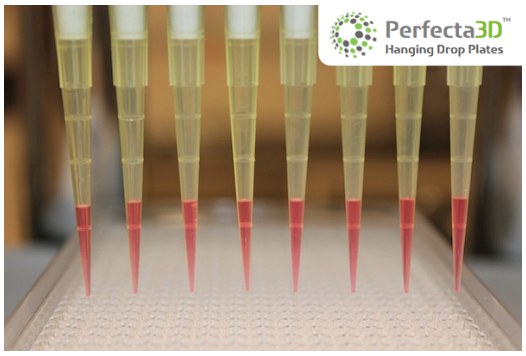
A review of 3D cell-growth techniques and scaffolds analyzed from the perspective of materials properties, manufacturing and functionality.

- Lee J, Cuddihy M and Kotov NA, Three-Dimensional Cell Culture Matrices: State of the Art, Tissue Engineering: Part B, Vol 14, Number 1 (2008), 61-86.

General review of 3D cell culture approaches and techniques.

- Haycock JW (ed.), 3D Cell Culture: Methods and Protocols, Methods in Molecular Biology, Springer Science+Business Media, LLC , Vol 695 (2011), DOI 10.1007/978-1-60761-948-0_1.

Gels



Gels

In general, gels have a soft tissue-like stiffness and aim to mimic the ECM. Gels made from ECM mixtures of natural origin, such as collagen, and alginate, have been used for decades as substrates for 3D cell culture. The most well-known gel is Matrigel™, first documented in 1972 by Hynda Kleinman. Matrigel is a reconstituted basement membrane preparation extracted from the Engelbreth-Holm-Swarm mouse sarcoma, a tumor rich in ECM proteins, such as laminin and collagen, plus growth factors and enzymes.

Gels made from animal-sourced natural ECM extracts may contain residual growth factors, undefined constituents, such as animal viruses, or non-quantified substances. Batch-to-batch variations make it difficult to compare and correlate work from different scientific groups. Gels may also change in structure over time as they are organized by cells in culture.

The desire to impart controllability in gels drove the development of synthetic gels, such as the poly-ethylene glycol (PEG)-based hydrogels from QGel™, which are modified to obtain desired characteristics. Modification of the bulk material may be performed through hybridization of natural and synthetic materials, incorporation of different types of proteins or molecules within the matrix, and hybridization of biomaterials with functional nano-materials.

A drawback to many 3D gels is the difficulty of use, which often stems from the gelling mechanism. In the case of Matrigel, the gel must be kept on ice to keep its viscosity low enough for manipulating and mixing with cells. pH-based gelling mechanisms are also common, which can expose sensitive cells to adverse conditions.

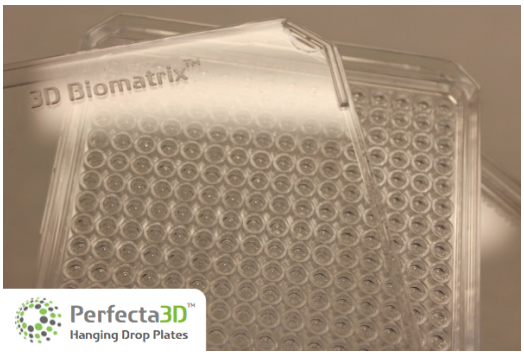
The ability to choose from a variety of natural and synthetic materials, the soft tissue-like material properties, and the ease of converting cultures to 3D have made gels widely popular. As well, 3D cell cultures in gels have led to important findings, specifically in cancer research.

Lastly, gels can be combined with other methods, such as spheroid cultures, scaffolds, and microchips.

Article:

Three current technologies are presented in this review: membranes, sponges/gels and microcarriers.

- Justice, BA, Badr, NA and Felder RA, 3D cell culture opens new dimensions in cell-based assays, Drug Discovery Today, Volume 14, Numbers 1/2 (2009), 102-107.



Bioreactors

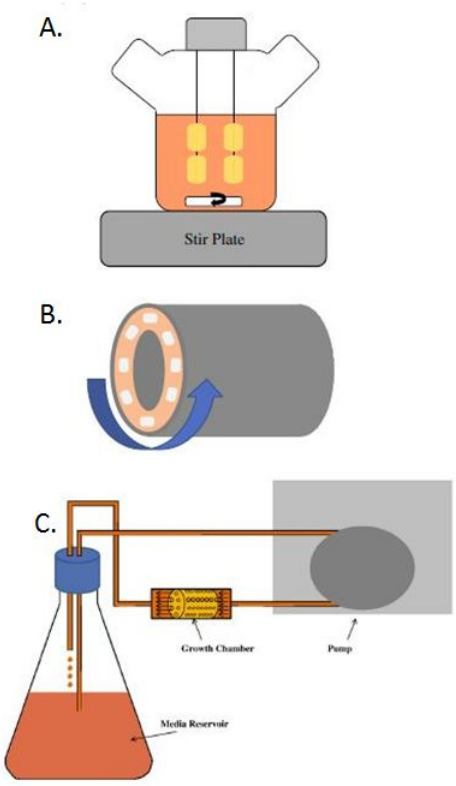


Figure 6. Examples of bioreactor systems: A. Spinner flask, B. Rotating-wall vessel and C. Perfusion system. Images adapted from Yeatts et al.

Bioreactors

Bioreactors have been adapted for 3D cell culture by the addition of scaffolds and are most ideal for high volume cell production and *ex vivo* tissue engineering applications (Figure 6). Many bioreactors include media flow, allowing for circulation of nutrients, removal of wastes, and homogeneity of the environment within the reactor. As such, they are well-suited for cell expansion applications or scaled production of cellular products, such as antibodies.

Bioreactors for 3D cell culture fall into 4 main categories:

- The spinner flask is typically composed of a glass media reservoir with side arms that can be opened and may have porous covers for gas exchange. Scaffolds are suspended on a structure or placed within the media, which is stirred by a stir bar.
- Low-shear-stress rotating wall vessels, originally designed by the National Aeronautics and Space Administration to simulate microgravity, are composed of two concentric cylinders, an inner stationary cylinder that provides for gas exchange and an outer rotating cylinder. Free-moving scaffolds and media are placed in the space between the two cylinders.
- Perfusion bioreactors use a pump system to perfuse media, directly or indirectly, through a scaffold. The basic design consists of a media reservoir, a pump, a tubing circuit, and a perfusion cartridge, which houses the scaffold. Hollow-fiber systems, a sub-group, are composed of small tube-like filters, approximately 200 µm in diameter with molecular weight cut-offs, sealed into a cartridge shell.
- When combined with scaffolds, bioreactors can be used for specialty purposes that utilize mechanical load or electrical or pulsed electrical fields.

A large variety of scaffolds or microcarriers, which can be matched to experimental and cellular needs, are available for use in bioreactors. Cells and/or supernatants must be harvested for analysis. The major restrictions for the use of bioreactors may be the cost and throughput. Although the simple spinner flask is an inexpensive option, many bioreactors require specialized equipment. Bioreactors may not be the ideal choice for HTS unless a custom automated system is designed.

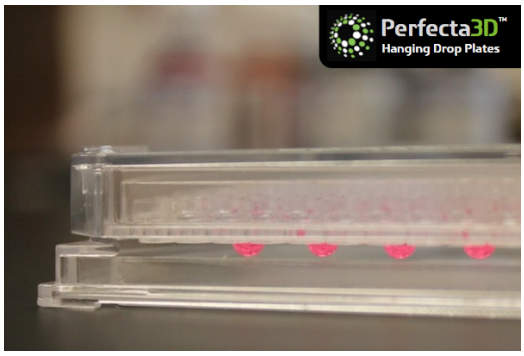
Articles:

An overview of the concepts, advantages, challenges, and potential future applications associated with current bioreactor systems for bone tissue engineering.

- Rauh J, Milan F, Gunther KP, and Stiehler, M, Bioreactor Systems for Bone Tissue Engineering, Tissue Engineering: Part B, Volume 17, Number 4 (2011), 263-280.

A summary of *in vitro* bioreactors for bone tissue engineering as well as commercial bioreactor systems.

- Yeatts AB, Fisher JP, Bone tissue engineering bioreactors: Dynamic culture and the influence of shear stress, Bone 48 (2011), 171–181.



Microchips

Microchips, frequently called 'organ on a chip' or microsystems, are the next wave of 3D cell culture models. 'Organ on a chip' integrate microfluidics technologies with cells cultured within microfabricated 3D devices, using techniques from the microchip industry.

Although not expected to be widely commercially available for some time, advances in microfabrication and microfluidic techniques open the door to this option. Recent advances include development of integrated 'organ on chip' microsystems that reproduce key architectural, functional, biochemical and mechanical features such as mechanical strain and shear forces, of living organs, including lung, liver (Figure 7), kidney, gut, bone, breast, brain and eye. The eventual goal is to link the individual organ microsystems together to develop a human-on-a-chip.

Scale-up and manufacturing of microchips is a crucial milestone, which needs to be overcome to make the technology an affordable, commercially-viable option. Today's microchips use photo or soft lithography and replica molding techniques along with silicone rubber, poly(dimethylsiloxane) (PDMS), microfluidics systems. These systems are less expensive, easier to fabricate, have high gas permeability and are optically transparent. Although PDMS has desirable properties it has poor chemical resistance to some solvents and can absorb small hydrophobic molecules limiting its effectiveness in commercial uses. Other challenges involve poorly-understood effects of polymers and microfluidics on cellular behavior. HTS options are currently limited; the minute sample volumes make collection and analysis difficult.

Although microchips may be the next wave of 3D tools, many of the current versions require considerable expertise to operate and troubleshoot, limiting widespread adoption.

Microchips

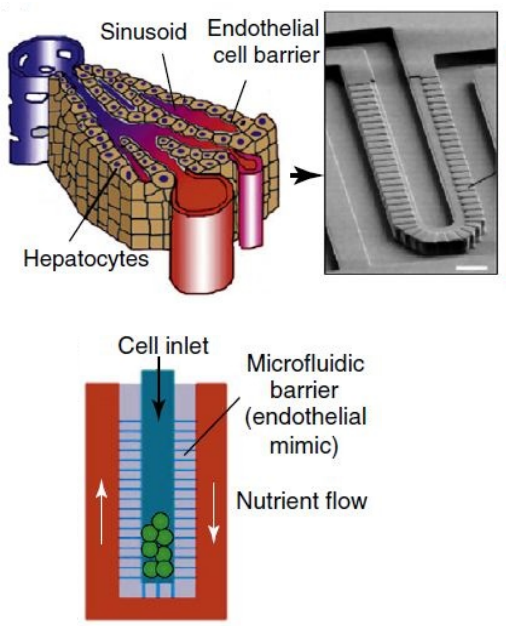


Figure 7. A micro-engineered liver-on-a-chip reconstitutes hepatic microarchitecture. The microsystem consists of a central liver-cell culture chamber and a surrounding nutrient flow channel separated by microfabricated barrier structures that mimic the highly permeable endothelial barrier between hepatocytes and the liver sinusoid. Image adapted from Huh et al.

3D Cell Culture Options

Articles:

A review of new advances in 3D culture, 'organs-on-chips'.

- Huh D, Hamilton GA and Ingber DE, From 3D cell culture to organs-on-chips, Trends in Cell Biology, Vol. 21, No. 12 (2011), 745-754

For years, scientists have struggled to reconstruct tissues and organs by combining cells and nanotechnology. These devices are now edging from cool concept to practical application.

- Baker M, A Living System on a Chip, Nature, Vol 471 (2011), 661-665

How to decide what fits your lab

If you are just starting out, deciding on the best option for your specific application can be frustrating. Each available option has pros and cons (Table 1), as well as best-suited applications.

Option	Pros	Cons	Research Stage
Scaffold-free systems	• No added materials • Consistent spheroid formation; control over size • Co-cultures possible • Transparent • HTS capable; compatible with liquid handling tools • Inexpensive	• No support or porosity • Limited flexibility • Size of spheroid limiting	• Basic research • Drug discovery • Personalized medicine
In vitro 3D scaffolds for laboratory applications	• Large variety of materials possible for desired properties • Customizable • Co-cultures possible • Medium cost	• Possible scaffold-to-scaffold variation • May not be transparent • Cell removal may be difficult • HTS options limited	• Basic research • Drug discovery • Cell expansion
Gels	• Large variety of natural or synthetic materials • Customizable • Co-cultures possible • Inexpensive	• Gelling mechanism • Gel-to-gel variation and structural changes over time • Undefined constituents in natural gels • May not be transparent • HTS options limited	• Basic research • Drug discovery
Bioreactors	• Several options available • High volume cell production • Customizable	• Cost • HTS options limited	• Basic research • Tissue engineering • Cell expansion
Microchips	• *In vitro* organ specific systems • High gas permeability • Transparent	• Commercial availability • Required expertise • Cost • HTS options limited	• Basic research • Drug discovery

Table 1. Summary of 3D cell culture options.

3D Biomatrix
1600 Huron Parkway
Building 520, 2nd Floor
Ann Arbor, MI 48109-2590
USA

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

A perusal of the review articles listed throughout this paper is a good start. Initially a comparison of one or more options may be required. The best-fit 3D cell culture option will depend on many factors (Figure 8).

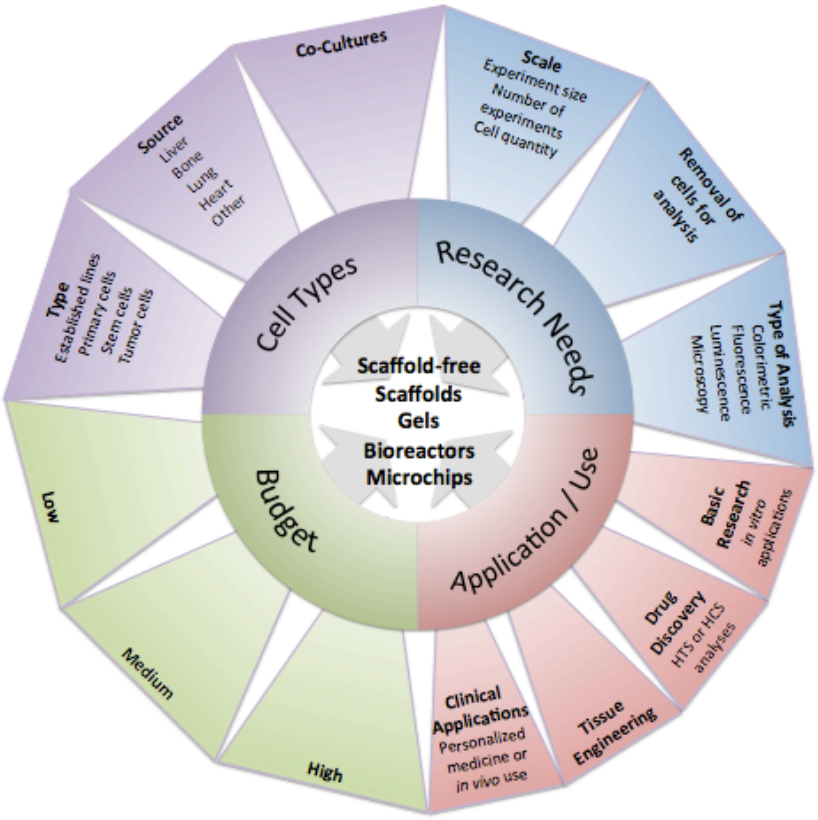


Figure 8. Factors to consider when choosing 3D cell culture options.

What are you waiting for? Take the leap to 3D cell culture today.

As we have demonstrated in this short tutorial there are many 3D cell culture options available today. One, or more, options may be right for your application.

3D cell culture options:

- Scaffold-free platforms for spheroid growth
- Scaffolds
- Gels
- Bioreactors
- Microchips

The sooner the median level of 3D cell culture adoption rises, the sooner companies will introduce new products, and the faster the technique will progress, especially in analytic techniques, such as imaging, assay validation, correlation to historical 2D culture results, and automation. 3D cell culture may have taken decades to reach its current level of growing acceptance, but there is no mistaking this: the technique is here to stay.