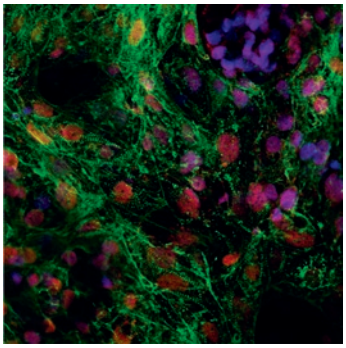
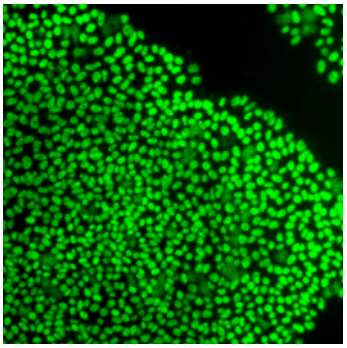
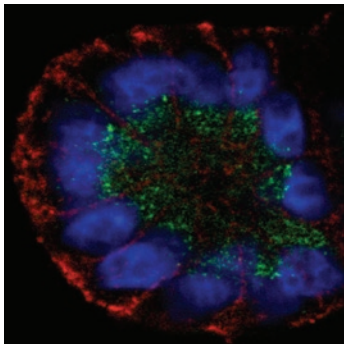

CORNING

Culture and Assay Systems Used for 3D Cell Culture

Review Article

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Introduction

Mammalian cell culture has served as an invaluable tool in cell biology for several decades. Monolayers of adherent cells grown on flat and rigid two-dimensional (2D) substrates, such as polystyrene or glass, have evolved as the mainstay in conventional cell culture systems. Two-dimensional cell culture studies have played a pivotal role in furthering our understanding of developmental biology, tissue morphogenesis, disease mechanisms, drug discovery, large-scale protein production, tissue engineering, and regenerative medicine. Simultaneously, a multitude of inadequacies associated with 2D culture systems have also emerged, especially with respect to their inability to emulate *in vivo* conditions and providing physiological relevance.

In the body, nearly all cells in tissues reside in an extracellular matrix (ECM) consisting of a complex three-dimensional (3D) architecture and interact with neighboring cells through biochemical and mechanical cues[1]. Cell-cell and cell-ECM interactions establish a 3D communication network that maintains the specificity and homeostasis of the tissue[2]. Key events in the life cycle of a cell are regulated by organizing principles that are determined by the surrounding cellular microenvironment[3]. The inability of cells to achieve *in vivo*-like structural organization and connectivity in 2D cell culture assays can limit or diminish properties such as cellular morphology, viability, proliferation, differentiation, gene and protein expression, response to stimuli, drug metabolism, and general cell function.

These limitations of 2D cultures have contributed to poor predictive power of preclinical cell-based drug and toxicity screening assays. More than 90% of drugs that pass through *in vitro* preclinical studies fail to meet the desired efficacy or safety margins required in subsequent clinical trials[4]. The failure rate is even higher for cancer drugs[5,12] as 2D culture systems are often inadequate to effectively model tumor biology[6,7]. Furthermore, there is a strong dependence on concomitant use of animal models for bioavailability and toxicological studies during preclinical drug development when 2D cell culture models are utilized. The high rates of failed drugs suggest that animal models may not be suitable and/or representative for safety assessment of therapeutics intended for human use[4,16,17].

To overcome some of these shortfalls, numerous 3D cell culture models have been developed over the past two decades. These developments are further fueled by the optimism that 3D models may significantly accelerate translational research in cancer biology, tissue engineering, and regenerative medicine. To this end, concerted multidisciplinary efforts have been put forth by cell biologists, material scientists, biomedical engineers, and others with a vision of developing more useful, *in vivo*-like 3D models to bridge the gap between 2D cell culture and live tissues. Emerging evidence strongly suggests that 3D cell cultures that establish physiological cell-cell and cell-ECM interactions can mimic the specificity of native tissue with greater physiological relevance than conventional 2D cultures[8-10]. This is particularly evident in applications such as stem cell culture and differentiation, cancer biology, drug and toxicity screening, and tissue engineering.

The spectrum of 3D cell culture models is vast and varied owing to the diverse requirements of different cell types and applications. Each model comes with its own set of advantages and limitations, and one distinct model is not suitable for all applications. Nevertheless, some methodologies have gained wider applicability than others especially for *in vitro* 3D culture. According to a 3D cell culture trends report[11], natural ECM-based hydrogels (e.g., Corning® Matrigel® matrix and Collagen) and 3D cell aggregates known as spheroids are among the most widely used models for 3D cell culture *in vitro*. These 3D models have been used to demonstrate increased physiological representation for diverse cell types and have been extensively applied to stem cell differentiation, tumorigenesis, and drug discovery. Spheroids and/or natural hydrogels have an added advantage in that they can be easily manipulated into more complex *in vivo*-like co-culture models that incorporate multiple cell types. Furthermore, devices such as permeable supports (e.g., Transwell® inserts) can be incorporated into 3D culture models with a hydrogel substrate(s) to facilitate the study of interactions between different cell types, soluble factors, and the culture microenvironment.

This review article will focus on general principles, advantages and caveats of *in vitro* 3D cell culture systems, with particular attention to the following three areas: 1) Cell Biology and Tissue Modeling, 2) Drug Discovery, and 3) Drug Toxicity. In this perspective, particular emphasis will be placed on the most commonly used matrices for *in vitro* 3D culture such as ECM-based hydrogels and their application.

1. 2D versus 3D Cell Culture Models

Fundamental differences in the microenvironment of 2D and 3D cell culture systems influence various cellular behaviors including the way in which cells attach, spread and grow, their morphology and polarity, gene and protein expression, viability, proliferation, motility, differentiation, response to stimuli, cellular metabolism, and overall function. One of the primary differences observed when comparing cells in 2D and 3D cultures is the dissimilarity in cell morphology. Cells adopt 2D or 3D shapes primarily based on the orientation of integrin-mediated adhesions to the extracellular matrix. In the case of 2D cultures, cell attachment occurs on one side of the cell (that which is in contact with the 2D surface), whereas in 3D cultures, cell attachment occurs around the entire surface of the cell[13]. In general, cell attachment and spreading on restraint-free 2D substrates occurs within minutes. In contrast, cell attachment and spreading in 3D cultures is preceded by proteolytic degradation of their physical environment, which can occur over hours, and, in some instances, over the course of a number of days[18].

It has been suggested that the degree of cell spreading can impact cell proliferation, apoptosis and differentiation[19-22]. Many cells, when isolated from tissues and placed onto planar cell culture surfaces, become progressively flatter, divide aberrantly, and lose their differentiated phenotype[23,24]. Interestingly, some of these cell types can regain their physiological form and function when embedded in a 3D culture environment. For instance, encapsulation of dedifferentiated chondrocytes in 3D cultures restores their physiological phenotype, including cell shape and the expression of cartilaginous markers[25]. Similarly, mammary epithelial cells embedded in a 3D environment halt uncontrolled division, assemble into acinar-like structures, and establish a *de novo* basement membrane[24,26,27].

Apart from cell spreading, the morphology adopted by a cell can also impact its functionality[28-32]. For example, glandular epithelial cell organization, signaling, and secretion more closely resemble the properties observed *in vivo* when cultured in a 3D environment, in contrast to the behavior that occurs on 2D surfaces[33,34]. The morphologies of fibroblasts, including cytoskeletal organization and the types of cell adhesions, are also more similar to their *in vivo* behavior when the fibroblasts are grown in a 3D matrix as compared to 2D[35]. Moreover, a 3D environment has been found to be optimal for supporting fibroblast intracellular signaling characteristics[36-38].

Another important physiological attribute conferred by 3D models is appropriate cell polarity. Polarity *in vivo* depends both on the cell type and the tissue microenvironment. Epithelial cells are often polarized, with apical and basolateral surfaces that are important for tissue organization and directional secretion of bioactive molecules. Tissue organization is lost when these cells are explanted onto flat 2D tissue culture substrates. When they are returned to appropriate 3D culture conditions, epithelial cells generally regain apical-basolateral polarity, and glandular cells form a lumen into which cellular factors are secreted[35]. Permeable supports, such as Transwell Inserts, have been particularly useful for recreating 3D models of epithelial cells with native tissue-like cell morphology, cell-cell interaction, polarity, and secretory function[39-42]. An example of a 3D *in vitro* model for endometrial glands is shown in Figure 1. In this model, endometrial epithelial cells were cultured in medium containing Corning® Matrigel® matrix for 7 to 8 days followed by immunofluorescence staining of various

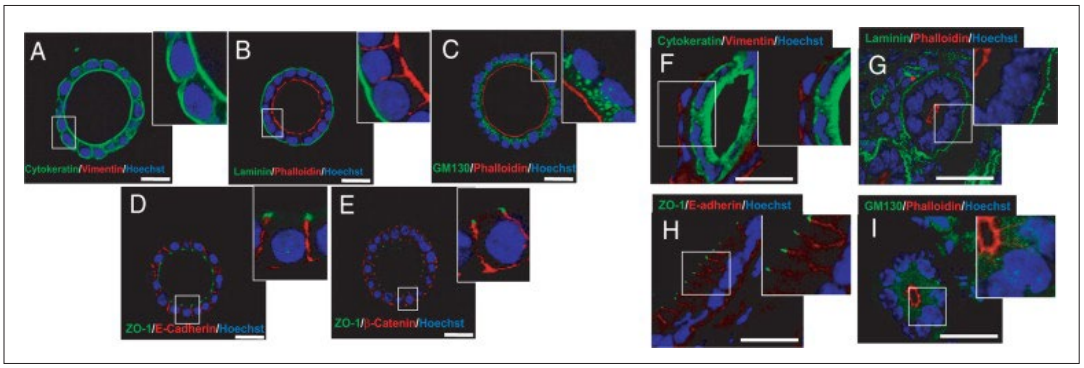

Figure 1. *In vitro* developed endometrial glands display epithelial apicobasal polarity. Double immunostaining of either *in vitro* developed glands cultured for 8 days in BIE medium (A-E) or cryostat sections obtained from mouse endometrium (F-I). A and F: Cytokeratin (green) and vimentin (red). B and G: Laminin (green) and phalloidin (red). C and I: GM130 (green) and phalloidin (red). D and H: ZO-1 (green) and E-cadherin (red). E: ZO-1 (green) and β-catenin (red). White scale bar = 20 mm. BIE medium = basal medium supplemented with 5 ng/mL EGF and 1:100 dilution of ITS supplement and 3% fresh Corning® Matrigel® matrix; EGF = epidermal growth factor; ITS = insulin transferrin selenium. (Eritja, et al., 2010)[143].

polarity markers. Marker expression analysis confirmed that the glandular structures were composed exclusively of cells of epithelial origin (Figure 1A and 1F). Furthermore, spheroids representing endometrial glands displayed correct apical-basolateral polarity (Figure 1B and 1G), positioning of Golgi apparatus (Figure 1C and 1I), adherent junctions (Figure 1D and 1H), and tight junctions (Figure 1E).

Gene expression and mRNA splicing patterns can also vary considerably depending on whether cells are cultured under 2D versus 3D conditions[43,44]. For example, melanoma cells exhibit distinct gene expression patterns when cultured on flat substrates, when compared to melanoma cell spheroids that are observed in a 3D environment. The genes that are up-regulated in the 3D spheroids are also found to be up-regulated in tumors *in vivo*[45]. Other studies have demonstrated that properties of the cell culture substrate influence the expression of integrin mRNA and protein biosynthesis. Mammary epithelial cells that are cultured on flat 2D plastic substrates, exhibit a dramatic up regulation of α1 integrin mRNA expression. In contrast, when the same cells are cultured under 3D conditions using a reconstituted basement membrane such as Corning Matrigel matrix, the cells exhibit mRNA expression levels comparable with those observed in native breast tissue(reviewed in 13,46).

The differentiation niches of primary cells and stem cells are inherently 3D, and their biochemistry and topology have been found to dramatically affect the differentiation process[47]. For example, primary hepatocytes cultured as a monolayer dedifferentiate and die within a few days[48]. One of the first functions lost in dedifferentiated hepatocytes is the biosynthesis of drug-metabolizing enzymes known to be essential for toxicity assays in pharmaceutical research[48]. The deficits in hepatocyte function observed on 2D surfaces can be overcome by embedding primary hepatic cells within 3D matrices such as Collagen I[49], Corning Matrigel matrix[49], synthetic peptide scaffolds[50], or by maintaining them under conditions of perfusion flow[51].

Collectively, these observations demonstrate that 3D cell culture systems are capable of supporting varying degrees of cell complexity and functionality that are observed *in vivo*, which is dependent on the cell type and culture conditions. In contrast, in most cases, 2D culture models support limited cell differentiation and *in vivo*-like functionality.

2. 3D Cell Culture Techniques

One of the most critical aspects of *in vitro* 3D models is the need to mimic specific aspects of *in vivo* cell behavior to enable the accurate prediction of tissue development and morphogenesis, cellular differentiation, genotypic, and/or phenotypic response to compounds in drug and toxicity screening assays. Some of the more basic 3D models have involved the culture of cellular aggregates in suspension without the use of matrix-based substrata. However, the majority of more complex 3D cell culture models utilize either hydrogel-based matrices or solid scaffolds. A vast array of materials and fabrication techniques has been employed to develop scaffolds with varying physical and biological characteristics to address the requirements of different cell types in the body. Of the various methods available, naturally derived ECM-based hydrogels are most commonly used for *in vitro* applications of 3D cell culture.

3D Spheroid Cultures

Cellular spheroids are simple 3D models that can be generated from a wide range of cell types, which form spheroids because of the tendency of adherent cells to aggregate. Common examples of spheroids include embryoid bodies, mammospheres, tumor spheroids, hepatospheres, and neurospheres. Adherent cells have a natural tendency to aggregate and form spheroids under circumstances that impede adhesion to cell culture substrates. Common matrix-free methods employed for generating spheroids include the use of attachment resistant cell culture surfaces such as the Corning® Ultra-Low Attachment surface, or by maintaining the cells as suspension cultures in media (e.g., hanging drop technology, rotary cultures, and bioreactors). Several cell types also form spheroids in 3D hydrogels, and to a limited extent, in some solid scaffolds depending on the structural and physical properties of the material. The overall size of spheroids is limited to a few hundred micrometers, beyond which, necrosis ensues within the core of the spheroids[52].

Spheroids naturally mimic various aspects of solid tissues and are equipped with inherent gradients for efficient diffusion of oxygen and nutrients as well as the removal of metabolic wastes. These cellular aggregates can emulate avascular, solid tumor behavior more effectively than standard 2D environments because spheroids, much like tumors, usually contain a heterogeneous population of surface-exposed and deeply buried cells, proliferating and non-proliferating cells, and well-oxygenated and hypoxic cells[53]. Additionally, differentiation of pluripotent stem cells (PSCs) typically involves formation of spherical structures called embryoid bodies, an important step for subsequent cell differentiation studies *in vitro*. Spheroids thus represent an especially good physiological 3D model for studying solid tumorigenesis and stem cell differentiation. In addition, spheroids can be readily analyzed by imaging using light, fluorescence, and confocal microscopy, which is an advantage over more complex 3D cell culture models. Furthermore, it is relatively simple to mass-produce uniformly sized 3D spheroids making them highly amenable for many *in vitro* high throughput and toxicity screening applications.

3D Cultures using Hydrogels and Extracellular Matrices

Hydrogels are comprised of networks of cross-linked polymer chains or complex protein molecules of natural or synthetic origin. Due to their significant water content, hydrogels possess biophysical characteristics very similar to natural tissue, and serve as highly effective matrices for 3D cell culture. Hydrogels can be used as stand-alone 3D matrices or combined with other technologies, such as solid scaffolds, permeable supports, cellular microarrays, and microfluidics devices.

Hydrogels can be employed in 3D culture systems in a variety of ways. They can be used as a coating reagent for various cell culture surfaces including solid scaffolds. Alternatively, cells can be encapsulated in or sandwiched between these matrices. The morphology, growth and functionality of cells within the hydrogel matrices depend on the presentation of biophysical and biochemical cues, as well as physical properties such as permeability and matrix stiffness.

Natural Hydrogels and Extracellular Matrices (ECMs)

Naturally derived hydrogels for cell culture are typically formed of proteins and ECM components such as collagen, laminin, fibrin, hyaluronic acid, chitosan, or Corning Matrigel® matrix. Derived from natural sources, these gels are inherently biocompatible and bioactive[54]. They also promote many cellular functions due to the presence of various endogenous factors, which can be advantageous for supporting viability, proliferation, function, and development of many cell types[55].

The ECM surrounding a cell performs several critical functions. To begin with, it provides a complex, nanoscale architecture of structural proteins such as collagen, laminin, and fibronectin to create the mechanical properties inherent in the cellular microenvironment[90,91]. Cells sense these mechanics through their cell surface integrins, and bind to specific adhesion motifs present on the ECM proteins. As described earlier in this review, cell adhesion in a 3D system leads to and influences a series of subsequent cellular responses that are more physiologically relevant compared to cells grown on 2D surfaces[13]. Furthermore, the ECM is vital for sequestering soluble biomolecules and growth factors, and releasing these signaling molecules with spatial-temporal control to guide processes such as cell migration, matrix degradation and deposition[90,91]. ECM remodeling is imperative for achieving tissue homeostasis and is particularly pronounced during development and diseases. Thus, to truly mimic the ECM *in vivo*, it is necessary to develop 3D culture models that exhibit the mechanical and chemical properties of the ECM, not only at the initial stage of cell seeding, but rather, in a dynamic and tunable manner as the cells grow and develop.

Corning® Matrigel® matrix is an ECM-based natural hydrogels that has been used extensively for 3D cell cultures *in vitro* and *in vivo*. This reconstituted basement membrane is extracted from Engelbreth-Holm-Swarm (EHS) mouse tumors and contains all of the common ECM molecules found in basement membrane (i.e., laminin, collagen IV, heparin sulfate proteoglycan, and nidogen/entactin)[56]. The ECM components of Matrigel matrix activate various signaling pathways in cancer cells that control angiogenesis[57,58], cancer cell motility[59], and drug sensitivity[60]. Because it mimics an *in vivo* basement membrane, Matrigel matrix is often used for studying cancer cells that resemble the cells that reside in epithelial tissues[61]. Dr. Mina Bissell's pioneering research on mammary epithelial cells and breast cancer has utilized Matrigel matrix, or an equivalent, which demonstrated the enabling power of 3D culture for creating *in vivo*-like model systems[62] and the importance of integrin signaling in cancer[63,64].

Collagen Type I is another commonly used natural hydrogel for 3D cell culture. Collagen I is a common ECM molecule found in stromal compartments and bone. It can be isolated from various biological sources including bovine skin, rat tail tendon, and human placenta. Collagen I can also be electrospun into membranes[65,66], and can support 3D cell growth and differentiation. Additionally, Collagen I interacts with integrin receptors to modulate gene expression[67]. Target genes include those that alter the production of matrix metalloproteinases (MMPs), enzymes that degrade ECM components and allow tumor cell invasion[68], and those that affect cell sensitivity to anti-cancer drugs[69], cell proliferation, and cell migration[70,71].

Natural hydrogels do present some disadvantages, including their isolation from animal-derived sources, and inherent batch-to-batch variability in composition. Also, they contain endogenous bioactive components such as growth factors that can be advantageous for creating some 3D models, but in other instances, can confound the specific cell behavior or response that is under investigation.

To circumvent some of the issues associated with animal-derived biomaterials, matrices have been developed in organisms that are animal-free, or derived from recombinant nucleic acid technology. Hyaluronic acid (hyaluronan or HA) is an increasingly popular biologically derived matrix[72-74]. Most commercial grade HA is of bacterial origin, and characterized by high purity and homogeneous quality. These gels may be modified by the addition of ECM components for improving cell attachment and growth properties.

Synthetic Hydrogels

Synthetic hydrogels are a good choice for 3D cell culture applications when naturally derived biological matrices are unsuitable. Synthetic hydrogels are comprised of purely non-natural molecules (reviewed in 75) such as poly(ethylene glycol) (PEG)[76], poly(vinyl alcohol)[77], and poly(2-hydroxy ethyl methacrylate)[78]. They are biologically inert, but provide structural support for various cell types.

PEG hydrogels have been shown to maintain the viability of encapsulated cells while allowing for ECM deposition as the hydrogel degrades[79], thereby demonstrating that synthetic gels can function as 3D cell culture platforms in the absence of integrin-binding ligands. Such inert gels are highly reproducible, allow for facile tuning of the mechanical properties, and are simple to process and manufacture.

It is also possible to modify inert synthetic hydrogels with appropriate biological components. An example of a synthetic hydrogel that is tunable is Corning PuraMatrix™ peptide hydrogel, a self-assembling, synthetic oligopeptide that exhibits nanometer scale fibers. Nanometer-sized fibres and pores are essential to ensure a true 3D environment for the cell[80,81]. An additional advantage of PuraMatrix peptide hydrogel is the ability to customize the material with specific peptide sequences to improve cell attachment, cell homing, and other behaviors[82]. To achieve optimal cell growth and differentiation using PuraMatrix peptide hydrogel, it is necessary to supplement the hydrogel with appropriate bioactive molecules (e.g., growth factors, ECM proteins, and/or other molecules). An example of this is shown in Figure 2, where functionalized PuraMatrix peptide hydrogel was used to support neuronal cell proliferation and differentiation. In this study, Ortinau and colleagues used PuraMatrix peptide hydrogel supplemented with laminin (PML 0.25%) for neuronal differentiation of human neural progenitor cells. Using transmission light microscopy (Figure 2A and 2B) and scanning electron microscopy (Figure 2C and 2D) the researchers showed that neural progenitor cells can develop into a dense network of neuronal processes (Figure 2B and 2D). Furthermore, immunocytochemistry revealed that after 7 days of differentiation in the Corning PuraMatrix-laminin scaffold, the neuronal precursor cells began to express neuronal markers (βIII-tubulin and tyrosine hydroxylase

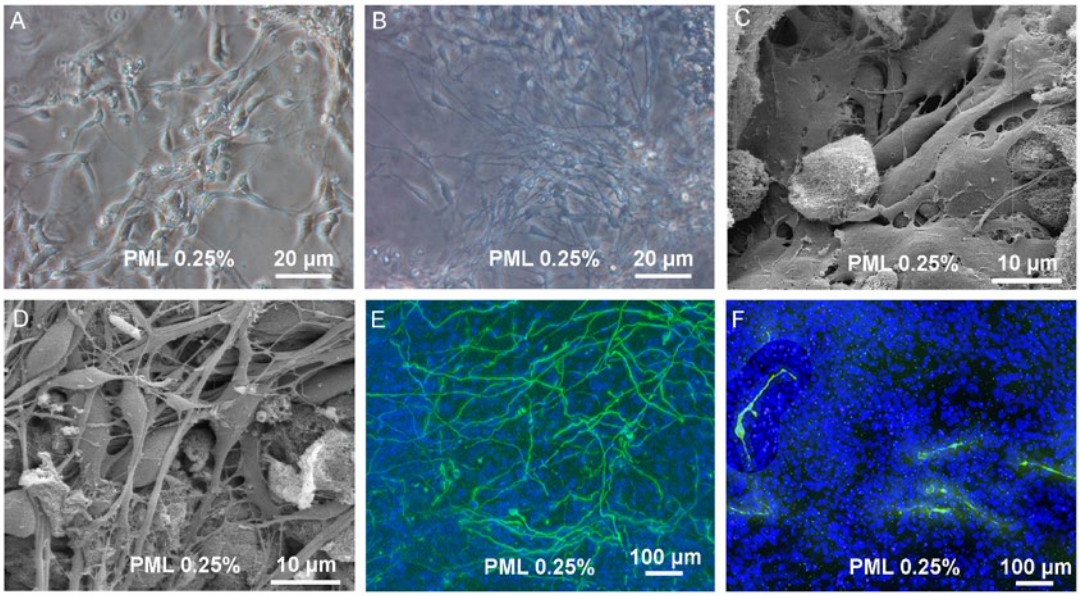

Figure 2. Proliferation and differentiation in 3D scaffold. (A, B) Transmission light picture of proliferating cells in Corning PuraMatrix peptide hyrdrogel with 0.25% laminin (PML) and differentiating cells in PML 0.25%. (C, D) Scanning electron microscope picture of proliferating cells and differentiated cells in PML 0.25%. (B, D) Upon induction of differentiation one observes the development of a dense 3 dimensional network of processes. (E) Immunocytochemistry for βIII-tubulin and TH of uninduced cells in PML 0.25% and (F) Cells after 7 days of differentiation revealed a dense network of βIII-tubulin positive cells. TH+ cells were found to possess processes, but without building up a dense network (Ortinau, et al., 2010)[133].

[TH]) (Figure 2E and 2F). These observations demonstrate how functionalization with appropriate biological factors can convert inert synthetic matrices such as Corning® PuraMatrix™ peptide hydrogel into useful *in vitro* models for supporting differentiation of stem and progenitor cells. As this field advances, there will be a need for matrices with combined properties of natural and synthetic hydrogels.

Solid Scaffolds

Solid scaffolds for 3D cell culture are fabricated with a broad range of materials including metal, ceramics, glass, and polymers. In particular, polymers are a common choice for generating solid scaffolds of diverse size, varying structure, stiffness, porosity, and permeability[61]. A multitude of fabrication techniques are being utilized to generate solid scaffolds for 3D cell culture, including soft-lithography, electrospinning, microarray, bio-printing, and many others. The major drawbacks of using solid scaffolds are limited scope for cell imaging and difficulties that are encountered when recovering cells from the matrix.

An important consideration for designing scaffolds for 3D cell culture is the scale and topography of the internal structures within the scaffold. In the body, the ECM provides an intricate nanoscale infrastructure to support cells, and presents an instructive background that governs cell behavior[83-87]. Cells binding to scaffolds that exhibit microscale architectures may flatten and spread out as if cultured on flat surfaces[88]. Even minute nanoscale level alterations in topography of the cell's environment can elicit diverse effects on cell behavior[89]. Apart from scale and structure, the material used for constructing the scaffold, the surface chemical properties, matrix stiffness, permeability, and mechanical force can significantly impact cell adhesion, growth, and behavior[10].

3. Applications of 3D Cultures *In Vitro*

Despite the emergence of a myriad of 3D culture systems, the use of natural ECM-based hydrogels continues to be the predominant method for *in vitro* 3D cell culture[11]. Many of the newer 3D culture systems have been developed for specific cell types, and have not been optimized or validated for a wide variety of applications. Furthermore, many of the complex biological processes involved in the progression of cell differentiation and tumor formation are still not well established, and thus cannot be easily recreated using culture systems that require specific biological components that need to be identified and added exogenously.

Cell Biology and Tissue Models

Stem Cell Differentiation and Organoid Generation

Remarkable advances in culture and differentiation of stem cells and committed progenitor cells have been realized with the use of 3D cell culture systems. Stem cells, particularly pluripotent stem cells (PSCs), have tremendous potential for generating pure populations of any cell type in the body. Pure populations of progenitor or terminally differentiated cells, especially those that are difficult to isolate from tissues, could be invaluable for enabling drug discovery, cell therapy, and tissue regeneration.

Major breakthroughs have been recently achieved in the area of stem cell differentiation using 3D culture systems that recapitulate *in vivo* development and temporal control of signaling pathways (Table 1). Notably, in a sophisticated study by Koehler and colleagues, mouse embryonic stem cells (mESCs) were successfully differentiated to inner ear sensory epithelia when cultured as floating cell aggregates in media containing Corning® Matrigel® matrix[92]. From the sensory epithelia, hair cells with structural and functional properties of native mechanosensitive hair cells in the inner ear were shown to spontaneously arise[92]. This novel approach has enabled elucidation of complex mechanisms underlying inner ear development, and may be useful for *in vitro* disease modeling, drug discovery, or

Table 1. Tissue and Disease Models

Application	Cell Type	3D Model	Culture Substrate and Matrix	Reference
STEM CELL DIFFERENTIATION	Human PSCs	Differentiation to ureteric-bud committed renal progenitor cells	Corning® Matrigel® matrix	94
	Human PSCs	Differentiation to cardiac microtissues	Aligned collagen I	124
	Human PSCs	Differentiation to Endoderm	Collagen I	125
	Human ESCs	Differentiation to hepatocytes	Collagen I scaffold; 2D collagen I-coated dish	126
	Human ESCs	Differentiation to smooth muscle, neurons, and hepatocyte-like cells	Polymer scaffold with ECM coating	127
	Human ESCs	Differentiation to neural precursors	Hyaluronic acid matrix	128
	Mouse ESCs	Differentiation to renal tubular cells	Corning Matrigel matrix; Ultra-Low Attachment plates	93
	Mouse ESCs	Differentiation to inner ear sensory epithelia	Corning Matrigel matrix	92
	Mouse ESCs	Differentiation to neurons	Collagen 1 scaffold	129
	Mouse ESCs	Diffrentiation to neurons and astrocytes	Fibrin scaffold	130
	Mouse ESCs	Differentiation to chondrocytes	PEG scaffold	131
	Human NPCs	Differentiation to neuronal cells	Corning PuraMatrix™ (RADA-16) scaffold	132
	Human NPCs	Differentiation to neuronal cells	Corning PuraMatrix ± functionalized matrix (laminin I)	133
	Mouse primary neuronal cells	Differentiation to osteogenic lineage cells in 2D and 3D systems	PLLA and polystyrene polymer scaffolds coated with Collagen I	134
	Human MSCs	Differentiation to osteocytes	Corning PuraMatrix peptide hydrogel	135
	Canine AdMSCs	Differentiation osteogenic lineage cells in 2D, 3D, and *in vivo* (implantation in dog) systems	Corning Matrigel matrix	136
	Human dental pulp stem cells	Differentiation to osteocytes	Corning Matrigel matrix; Collagen sponge	137
ORGANOID GENERATION	Human PSCs	Intestinal organoids	Corning Matrigel matrix	95, 96
	Mouse ESCs	Retinal optic-cup structure	Corning Matrigel matrix	97
	Mouse embryonic pancreatic progenitors	Pancreatic organoid	Corning Matrigel matrix	98
	Mouse epitheilia	Mammary organoid	Corning Matrigel matrix	138
	Mouse primary colon tumor cells	Primary mouse colon tumor organoids	Corning Matrigel matrix	139
	Mouse ESCs	Functional thyroid follicular cells	Corning Matrigel matrix	140
	Human ESCs	Cerebral organoid model of human brain development and microcephaly	Corning Matrigel matrix	141

cell therapy.

Recent studies have enabled the derivation of renal lineage from stem cells. In a recent study by Morizane, et al., it was illustrated that mESCs could differentiate into complex renal tubular cells when grown as a 3D culture using Corning® Matrigel matrix[93]. In another study by Xia, et al., hPSCs were differentiated into renal progenitor-like cells[94]. Further maturation of these cells into ureteric bud structures was accomplished by establishing a 3D culture system in which differentiated human cells assembled and integrated alongside murine cells to form chimeric ureteric buds[94]. Both of these studies identified key steps in modeling kidney development, and collectively highlight the importance of 3D systems for supporting the differentiation of cells associated with highly organized organs.

Another major advancement in stem cell differentiation is the formation of self-organizing 3D mini "organs" known as organoids. Organoids can recapitulate incredible histological details and provide functional representation of multiple cell types that are present within the native organs. Organoids representative of intestinal[95,96], retinal[97], pancreatic[98], mammary[99], colonic[100], and cerebral tissues[101]

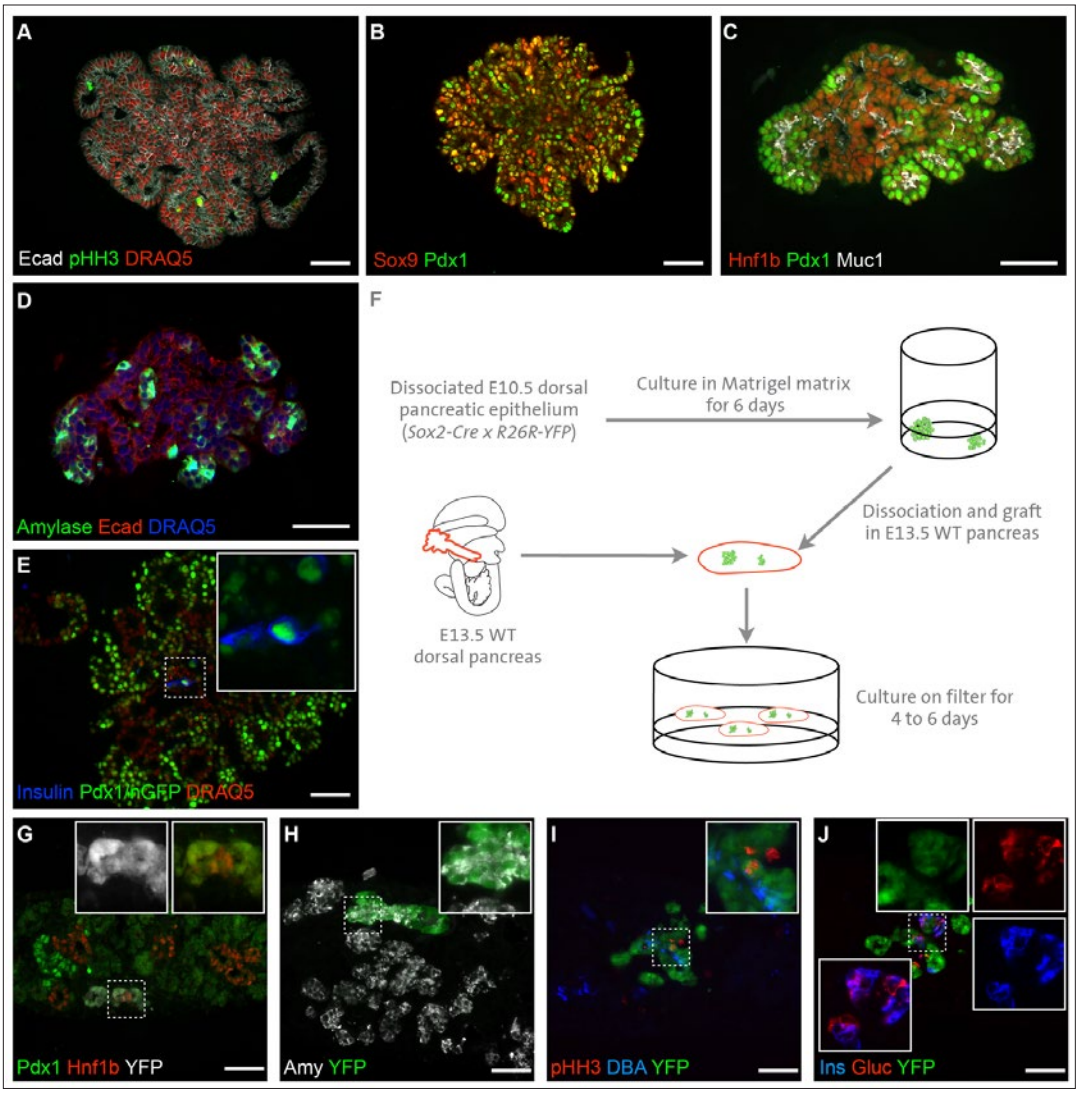

Figure 3. Organoids recapitulate progenitor expansion and organized differentiation. (A-E) Immunohistochemistry on sections of 7-day organoids showing that (A) all cells (DRAQ5, red nuclei) are epithelial (E-cadherin) and many proliferate [phospho-histone H3 (pHH3)] and (B, C) retain pancreatic markers PDX1, SOX9 and HNF1B. (C) Cells polarize and form tubes lined by mucin 1. (D) Exocrine differentiation (amylase) is seen at the periphery. (E) Endocrine differentiation (insulin) is detected in the center. The section in B is close to that in A, and the section in D is close to that in C. (F) Experimental scheme to test endocrine differentiation after back-transplantation of cells grown in organoid in a pancreatic niche. WT, wild type. (G-J) The cells that were first grown *in vitro* integrate into the host epithelium (white in G and green in H-J). Some remain progenitors (G; HNF1B), some become acinar (H; amylase) or ductal (I; DBA) and others become endocrine (glucagon or insulin). Insets are magnifications of the dashed boxes. Scale bars: 50 µm (Greggio, et al., 2013)[98].

have been developed recently using 3D models comprised of Corning Matrigel matrix (Table 1). Figure 3 illustrates a schematic of 3D culture conditions that enabled efficient expansion of dissociated mouse embryonic pancreatic progenitors[98]. By manipulating the medium composition, researchers generated either hollow spheres, which are mainly composed of pancreatic progenitors, or complex organoids that spontaneously undergo pancreatic morphogenesis and differentiation. Another example is shown in Figure 4, where Lancaster, et al., generated cerebral organoids with remarkable structural similarity to brain tissue[101]. These cerebral organoids grew larger than typical cellular spheroids and survived for weeks to months. *In vitro* culture of organoids is a major step toward elucidating the principles of organ development and the mechanisms responsible for genetic diseases.

Cancer and Tumor Cell Biology

Perhaps the most notable application of Corning® Matrigel® matrix for 3D cell culture is in the field of cancer biology. Since Matrigel matrix is a reconstituted basement membrane isolated from mouse tumors, this physiological material appears to be well equipped with microenvironment characteristics that are important for mimicking key steps that are involved in the progression of tumor growth, vascularization, and metastasis. Tumor cells typically form spheroids when grown in 3D suspension cultures or within hydrogels. Cells within the spheroids are heterogeneous and organized in a manner that enables gradient formation and diffusion dynamics that correspond more closely to tumors formed *in vivo*[102]. The inner core of the spheroids exhibits a hollow lumen resembling the necrotic areas of *in vivo* cancers[102]. These areas are hypoxic and are usually observed at a distance from nutrient and oxygen supplies. In addition, the proliferation of tumor cells cultured in 3D is typically slower than that observed in monolayer cultures[102,103].

Tumor cells of epithelial origin that are cultured in 3D have been shown to change shape and lose polarity, a feature typically associated with tumor progression *in vivo*[104]. Other parameters, such as proliferation, gene expression, and drug-sensitivity of tumor cells cultured in 3D environments, are also more representative of *in vivo* tumors compared to those cultured on 2D surfaces (reviewed in

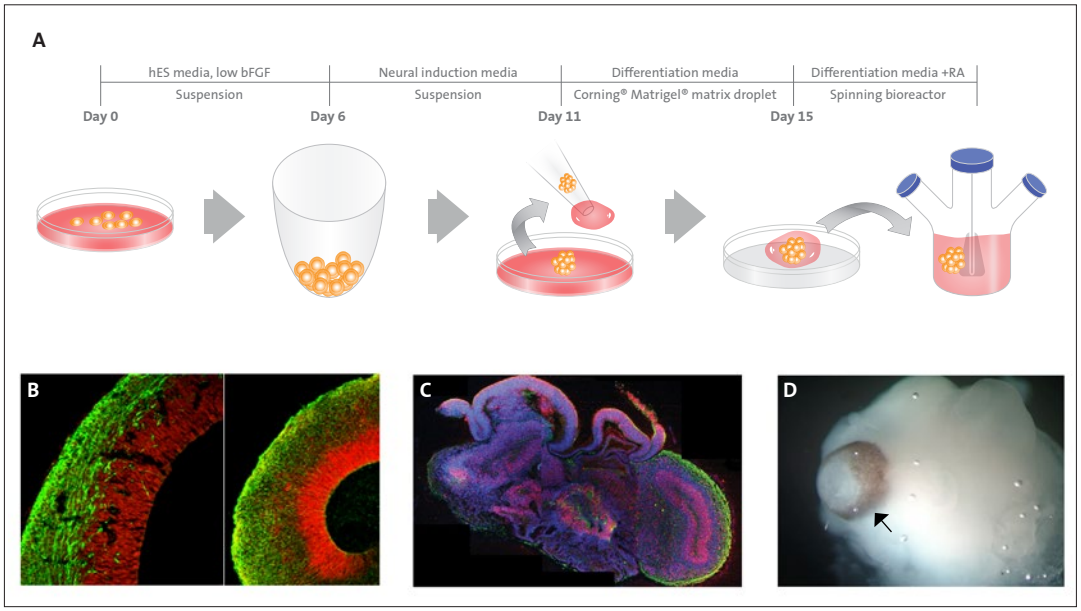

Figure 4. Description of cerebral organoid culture system. (A) Schematic of the culture system used to generate cerebral organoids. Example images of each stage are shown. bFGF, basic fibroblast growth factor; hES, human embryonic stem cell; hPSCs, human pluripotent stem cells; RA, retinoic acid. (B) A comparison between organoid and mouse brain structure demonstrates recapitulation of dorsal cortical organization. Immunohistochemistry for neurons (TUJ1, green) and radial glial stem cells (PAX6, red) in a large dorsal cortical region. (C), Sectioning and immunohistochemistry revealed complex morphology with heterogeneous regions containing neural progenitors (SOX2, red) and neurons (TUJ1, green) (arrow). (D) Low-magnification bright-field images revealing fluid-filled cavities reminiscent of ventricles (white arrow) and retina tissue, as indicated by retinal pigmented epithelium (black arrow). (Lancaster, et al., 2013)[141].

61,105) (Table 2). For example, Cichon, et al., demonstrated that lung cancer cells adopt a flattened morphology when grown on 2D tissue culture plastic, but can form smooth, round spheroids, grape-like structures, or invasive, branching structures when grown in 3D Corning® Matrigel® matrix[106]. Gene expression analysis revealed differences that distinguish lung cell lines that grow as smooth versus branched structures in this 3D model. This type of information may be useful for understanding differential prognostic outcomes of adenocarcinoma patients[106]. In another study, Harma, et al., illustrated that primary and non-transformed prostrate epithelial cells as well as some prostate cancer epithelial (PrC) cell lines formed well-differentiated round spheroids, while most PrC lines formed poorly differentiated spheroids or aggressively invading structures in 3D Matrigel matrix cultures[107]. Additionally, specific signaling pathways were activated in the invasive PrC aggregates, suggesting potential targets for blocking invasion of tumor cells in 3D models[107]. Yet another group investigated the impact of 3D microenvironments on phenotype, gene expression, and EGRF inhibition of common colorectal cancer cell lines[108]. Culture of PrC cell lines in 3D Matrigel matrix resulted in a drastically altered phenotype and significant changes in gene expression patterns compared to 2D culture, which was consistent with sensitivity to anti-EGFR targeted therapy *in vivo*[108]. The ability to model phenotypic and genotypic changes in cancer cells that are present in live tumors (especially tumors that are malignant or metastatic) is critical for developing more effective and predictive screening assays for chemotherapeutics.

Cancer Co-culture Models

Most *in vitro* cell culture models involve growing a single cell type to enable investigation of its growth and behavior in isolation. In the body however, communication between different cell types is critical to maintaining tissue homeostasis. Simulating these interactions between two or more pertinent cell types through co-culture can improve the overall biological relevance of cell culture models.

Table 2. 3D Cancer and Tumor Cell Models

Application	Cell Type	3D Model	Culture Substrate and Matrix	Comments/Benefits of 3D Model	Reference
CANCER AND TUMOR CELL BIOLOGY	Normal and breast cancer cell lines	A model to evaluate effects of BMP4 on breast cancer cells in a 3D system	Corning® Matrigel® matrix; PEG	*In vivo*-like morphology, polarity observed in Matrigel matrix but not PEG gel	142
	Lung cancer cell lines	Compared cell morphology and gene expression in 2D and 3D systems	Corning Matrigel matrix	Gene expression profile and tumor cell morphology varied for different cancer cell lines in 3D but not 2D model	106
	Primary mouse endometrial epithelial cells	Evaluated effects of growth factors, drugs, and RNAi-induced gene alterations on cell proliferation, polarization, and glandular formation	Corning Matrigel matrix	Single-lumened, polarized glandular structures resembling endometrial carcinogenic tissue *in vivo*. Model suitable for studying effects of drugs, growth factors, and gene alterations.	143
	Prostate cancer cell line (panel)	Compared tumor morphogenesis, gene expresssion, and metabolism in 2D and 3D models	Corning Matrigel matrix	Some cancer cell lines demonstrated differences in signaling pathway activation and poorly differentiated spheroids and invasive structures that could be blocked in 3D but not 2D systems	107
	Breast adinocarcinoma fibroblast-like, epithelial-like, and human fibrosarcoma cell lines	Developed an XTT proliferation assay in 2D and 3D systems for HTP drug screening	Corning Matrigel matrix; Collagen 1	Cell proliferation kinetics, anti-proliferative and cytotoxic effects of drugs were established in a more physiologically relevant 3D model compared to 2D systems	144
	Mouse breast cancer cell line + stromal fibroblasts	Co-culture model to study the effects of stromal cells on breast cancer progression.	Corning Matrigel matrix	Fibroblasts confered chemoresistance and protected cancer cells from cytotoxic compounds in 3D cultures	109
	Colorectal cancer cells	Investigated impact of 3D microenvironment on phenotype, gene expression and EGFR inhibition of common colorectal cancer cell lines	Corning Matrigel matrix	Significant changes in cell phenotype and gene expression was observed in 3D but not 2D model; data from 3D studies correlated with sensitivity to anti-EGFR targeted therapy *in vivo*	108
	Human intestinal cancer cell line (Caco-2)	Studied the effects of CLCA1 on cancer proliferation and differentiation	Corning Matrigel matrix	Found that CLCA1 may contribute to spontaenous differentiation and reduce proliferation of Caco-2	145

Three-dimensional co-culture models have been very useful for studying complex interactions between cancer cells and other cell types, and elucidating their contribution to tumor growth, vascularization, and metastasis (Table 3). For example, stromal cells have been shown to induce chemo-resistance and protect tumor cells from the toxic effects of anti-cancer drugs[109]. Endothelial cells and the associated vasculature provide a blood supply that enables tumor growth and survival, which is also responsible for carrying therapeutic compounds to the cancer cells[110]. In a recent co-culture study it was shown that endothelial cells can sensitize tumor spheroids to the toxic effects of chemotherapeutic drugs *in vitro*, and can induce angiogenesis and metastasis following implantation *in vivo*[111].

Tissue Co-culture Models

Permeable supports (e.g., Transwell® inserts) offer unique advantages for co-culture studies and are thus widely incorporated into these systems (Table 3). The pore-containing membranes of permeable supports are available with small pore sizes (e.g., 0.4 micron) that allow the passage of bioactive factors or soluble cues across the membrane, yet serves to physically separate cells located on opposite sides of the membrane. In two recent studies, permeable supports were used to investigate the role of endothelial cells in the growth of breast epithelial cells[112] and human lung epithelial cells[42] embedded within 3D Corning® Matrigel® matrix. In both instances, the endothelial cells were found

Table 3. 3D Co-culture Models

Application	Cell Type	3D Model	Culture Substrate and Matrix	Reference
CANCER CO-CULTURE MODELS	Mouse mammary tumor cells + endothelial cells	Co-culture of tumor spheroid to study impact of endothelial cells on tumor growth, vascularization and metastasis *in vitro* and *in vivo*	Hanging drop; implantation in nude mice	111
	Primary breast epithelial cells + endothelial cells	Co-culture model to study the role of endothelial cells in growth of normal and cancerous breast epithelial cells in 3D cultures	Transwell® filters (0.4 µm); Corning® Matrigel® matrix	112
	Mesenchymal stem cells + ovarian cancer cells (OCC)	Co-culture model to study the influence of MSCs on OCC migration and invasion in an amniochorionic membrane model	Transwell filters; Ultra-Low Attachment plates; Corning Matrigel matrix; Amniotic membrane scaffold	114
	Mouse breast cancer cell line + stromal fibroblasts	Co-culture model to study the effects of stromal cells on breast cancer progression	Corning Matrigel matrix	109
	Keratinocytes + melanocytes + dermal stem cells + melanoma cells	A 3D skin reconstruct model to study melanoma progression in human skin	Bovine Collagen I	150
	Human melanoma cells + keratinocytes	Organotypic skin melanoma spheroid model for *in vitro* drug testing	Collagen I	151
MULTI-CELLULAR CO-CULTURE MODELS	Human primary brain endothelial cells + human primary pericytes + human primary astrocytes	Blood-brain barrier model	Transwell inserts (0.4 µm)	146
	Hepatocytes + mouse fibroblasts or bovine endothelial cells	Micropatterned co-culture of hepatocyte spheroids	Micropatterned PEG hydrogel; gelatin	113
	Human bronchial epithelial cells + endothelial cells	A model to recapitulate lung morphogenesis	Transwell inserts (0.4 µm); Corning Matrigel matrix	42
	Primary rat hepatocytes + rat liver endothelial cells	A physiologically relevant co-culture model of hepatic sinusoids	Collagen I	147
	Alveolar cell line + macrophage-like cells + mast cells + endothelial cells	Tetraculture model mimicking alveolar barrier to study toxic effects of particles on the lungs; cells cultured at air-liquid interface (ALI)	Transwell inserts (0.4 µm)	39
	Mesenchymal stem cells + alveolar epithelial cells	Novel collagen-drop cell migration assay for wound repair model	Transwell insert (3 µm); Collagen I	148
	Human retinal progenitor cells + endothelial cells	Hypoxia induced retinal neovascularization with and without endothelial cells	Transwell inserts (0.4 µm); Corning Matrigel matrix	41
	Human mammary epithelial cells + human fibroblasts + adipocytes	A physiologically relevant tri-culture system to model human breast tissue	Corning Matrigel matrix + Collagen I; porous silk protein scaffold	149
	Human bronchial epithelial cells	Differentiation of cells to glandular acini on ALI	Transwell Collagen IV-coated; Corning Matrigel matrix	152
	Polarized airway epithelial cells	Infection of polarized epithelial cells from normal and cystic fibrosis patients. Cells were grown at ALI.	Transwell inserts (0.4 µm)	40

to induce growth and morphological changes of the cancer epithelial cells, which could be achieved, at least in part, through secretion of soluble cues. In another study, fibroblasts and endothelial cells were co-cultured with hepatocyte spheroids, which were found to significantly enhance hepatocyte function[113]. Using permeable supports, it was further determined that endothelial cells required direct cell-cell contact to enhance the function of hepatocytes, while fibroblasts were able to accomplish this through the action of soluble factors (paracrine regulation) or when the cells were cultured with direct contact. In a more complex permeable support co-culture model, four cell types (alveolar, macrophage-like, mast, and endothelial cells) were grown together to generate a model that mimics the alveolar barrier to study the potential toxic effects of particles on the lung[39]. In this study, the membrane played a central role by providing support for the complete tetraculture system, and enabled 3D organization of cells that closely resembled *in vivo* histology of the alveolar barrier (Figure 5). Here, endothelial cells were seeded on the basolateral side of the microporous membrane and the other three cell types were seeded in the apical compartment and cultivated at the air-liquid interface (ALI)[39]. In addition to cell permeation responses, permeable supports are also used to study cell migration and invasion. In an interesting 3D co-culture model, mesenchymal stem cells within an amnionic membrane scaffold were found to enhance the migration and invasion of ovarian cancer cells via the action of MSC-secreted IL-6[114]. These findings using permeable support co-culture models suggest that such approaches may significantly improve existing drug screening and tissue engineering methodologies.

Drug Discovery

The cost of drug development is becoming unsustainable due to the high attrition rate of drugs in late-stage human clinical trials, or in some cases, post-marketing[14,16]. The predominant reasons for drug failure are lack of efficacy, poor pharmacokinetics, and adverse or toxic side effects that are not identified during early preclinical studies involving cell-based assays and animal testing[4,12,15]. These challenges of drug discovery predicate the need for improved preclinical models; those that can better recapitulate pathobiological processes underlying diseases of specific tissues and organs in humans, and also more accurately predict physiological responses to therapeutic compounds and toxicity screens.

Physiologically relevant 3D cell culture models are ideally suited to bridge the gap between conventional 2D preclinical models and *in vivo* clinical studies in humans. Great strides have already been achieved over the past few decades in this field, yet there remain many practical limitations before

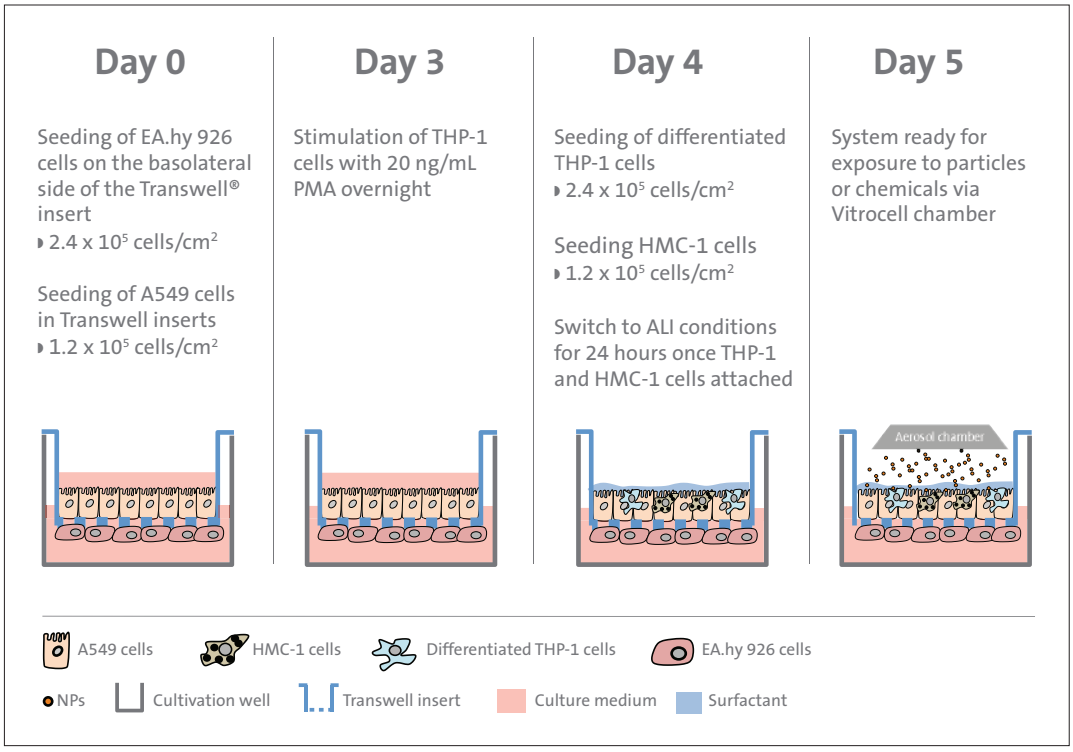

Figure 5. Workflow to setup an aerosol exposure experiment with the tetraculture system. (Klein, et al., 2013)[39].

3D cell culture models can be widely implemented in drug discovery. To begin with, many of the3D models are novel, and data generated using these methods needs to be validated against established *in vivo* responses to avoid misinterpretation of the observed results. Additionally, the outcome of 3D assays can be highly variable owing to a lack of standardized protocols and the use of heterogeneous, and sometimes multiple, cell populations. Many of the 3D assays can be cumbersome, technically challenging, and may require highly specialized and uncommon detection methods. Also, some 3D matrices have limited permeability or poor diffusion dynamics that can impact cell viability and function. Furthermore, cell recovery and visualization can be greatly restricted in certain 3D scaffolds. Another major limitation is that many of the 3D culture models cannot be easily automated or scaled for high throughput screening. Apart from these reasons, the cost of some 3D cell culture systems can be prohibitively high and hinder their utilization for drug discovery. These limitations however, are progressively and mindfully being overcome with emerging technologies and improved 3D *in vitro* assays.

Hanging drop and magnetic cell levitation technologies have recently been utilized for commercializing 3D *in vitro* assays with matrix-free cell cultures in suspension. The liquid medium in these assays facilitates visualization of cells with conventional microscopes. Drug permeation and diffusion of soluble factors are also easily achieved using these models.

Of the natural ECM-based hydrogels, Corning® Matrigel® matrix has been the longstanding gold standard material for 3D cell culture, especially for recreating tumor models. The most common *in vitro* 3D applications for this matrix are tumor cell invasion and angiogenesis (i.e., endothelial cell tube formation) assays in cancer drug development. One of the issues limiting the use of Matrigel matrix in drug discovery is dispensing small volumes of this viscous gel reproducibly in high throughput 3D assays. In a recent study however, Hongisto, et al., successfully generated a high throughput 3D screening assay using Matrigel matrix in a 384 well format that was filled with a diluted preparation of Matrigel matrix (1:3 dilution) using a liquid handling system[115]. When 63 prominent cancer drugs were analyzed using this Matrigel matrix system, the sensitivity and the gene expression profile for breast cancer cells (JIMT1) was found to closely match those of tumor xenografts[115]. In the same study, breast cancer cells grown in polyHEMA-induced anchorage independent 3D culture failed to show similar drug sensitivity or gene expression profiles, and instead, displayed results that more closely resembled those of cells cultured in 2D conformation. This study exemplifies the types of assays that may be more predictive and effective compared to existing 2D preclinical models, if they can be successfully translated into drug discovery.

Recent improvements in microscale engineering techniques such as fabrication of microarrays and microfluidics devices have the potential to transform the current drug discovery landscape. Microarrays consist of a solid support wherein small volumes of different biomolecules and cells can be positioned in defined locations, allowing multiplexed investigation of living cells and their responses to stimuli. Microfluidics devices manipulate small volumes (10^{-9} to 10^{-6} L) to generate and precisely control dynamic fluid flow and spatio-temporal gradients, as well as deliver nutrients and other chemical cues to cells in a controlled manner[116-118]. Microfluidic networks have been incorporated directly within cell embedded hydrogels, as well as several other matrices, to enable efficient convective transport of nutrients and other soluble cues throughout the 3D scaffolds[117]. Use of these technologies can greatly improve viability and functionality of cell types such as hepatocytes that are impoverished when grown in static cultures. Moreover, these technologies can increase throughput and significantly reduce the cost of culture due to low reagent volume requirements.

Toxicity Screening

State-of-the-art drug metabolism and toxicity screening assays often use difficult to source and expensive primary cells such as hepatocytes, cardiomyocytes, and neurons in 2D cell culture models. Long-term toxicity tests are usually performed in animal models. As discussed earlier, these models can be highly inaccurate and misleading in predicting the *in vivo* human responses to drugs[4,16,17]. Several 3D cell culture models have the potential to improve the predictive outcome of preclinical toxicity studies.

Microfabrication and microfluidics technologies have been used to generate several organ-on-chip models (reviewed in 117). These are essentially miniaturized 3D models of organs with *in vivo*-like functionality, which may prove to be very useful for toxicity testing (Table 4). For example, Toh, et al. developed a 3D hepatocyte chip (3D HepaTox Chip) for *in vitro* toxicity testing. In this model, the

hepatocytes retain their synthetic and metabolic functions, and multiple drugs can be tested simultaneously using this system[119]. The IC_{50} values of drugs tested in this model closely correlated with reported *in vivo* LD_{50} values. Lee, et al., created another 3D microarray for HTP screening of drug candidates and their cytochrome p450-generated metabolites[120]. This microarray chip consists of cells encapsulated in collagen or alginate gels arrayed on a functionalized glass slide and a complementary human P450-containing microarray, and is also suitable for screening multiple compounds simultaneously[120].

Culturing cells at an air-liquid interface (ALI) using permeable supports has enabled the development of many *in vitro* 3D models used in drug discovery (Table 4). One of the well-known applications of ALI cultures is 3D modeling of lung epithelium. These cellular models mimic native tissues and demonstrate apical-basal polarity, cilia on the apical end, and are capable of secreting mucus (reviewed in 121). ALI cultures made from freshly isolated nasal and bronchial biopsies are commercially available as MucilAir™ (Epithelix) and is useful for studying inhalation based toxic responses to nanoparticles, gases, smoke and viral infections. Using cells from diseased donors for MucilAil™ enables modeling pulmonary disorders such as asthma, allerigies, viral infections, and cystic fibrosis. The ALI cultures

Table 4. Drug Discovery and Toxicity Models

Application	Cell Type	3D Model	Culture Substrate and Matrix	Comments/Benefits of 3D Model	Reference
DRUG DISCOVERY AND TOXICITY	Human epithelial-like breast cancer cell line (JIMT1)	High throughput 3D screening assay	Corning® Matrigel® matrix; polyHEMA	Using a liquid handling system and diluted Matrigel matrix (1:3) drug sensitivity and gene expression of 63 drugs were compared simultaneously. Drug response in Matrigel matrix closely matched tumor xenografts while those on polyHEMA anchorage-independent 3D culture resembled profiles closer to 2D cell culture.	115
	Rat primary hepatocytes	A microfluidic 3D hepatocyte chip for drug toxicity testing	3D matrix made with positively charged methylated collagen + negatively charged HEMA-MMA-MAA terpolymer; microfluidic perfusion enabled	High throughput model for testing multiple drugs simultaneously in perfused hepatocytes that retain synthetic and metabolic function	119
	Human alveolar epithelial cells	A human lung-on-a-chip microdevice for nano-toxicology testing	PDMS microscaffold coated with fibronectin and collagen for supporting cells	Mimicked organ-level lung function for studing effects of nanotoxic particles on lung; also able to model impact of mechanical strain on lung function and inflammation	117
	Human breast cancer cell line (MCF7) and human hepatoma cell line (Hep3B)	Microarray for HTP toxicology assay screening of drugs and their p450-generated metabolites	Cells encapsulated in collagen I or alginate gel arrayed on a functionalized glass slide with complementary p450-containing microarray	Enabled testing of multiple compounds and their metabolites generated by CP1A2, CYP2D6, CYP3A4, and a mixture of the three p450s	120
	EGFP-expressing colon cancer and mouse ES cells	High throughput 3D proliferation and cytotoxic assay	Microbioreactors were made from multiwell plates. PET fibrous scaffold disks were fitted in the microwells within the bioreactors. Scaffolds were coated with fibronectin.	Significantly increased fluorescence signals and high signal-to-noise ratio (10-fold) in this 3D system compared to 2D cultures. Could be used with conventional fluorescence microplate readers.	153
	Human primary nasal or bronchial epithelial cells	MucilAir™ (Epithelix Sarl)-human airway epithelium model for toxicity testing	Cells cultured using ALI method on Transwell® inserts (0.4 µm)	Mimics physiological characteristics of lung epitheliuma (e.g., morphology, apicobasal polarity, cilia formation, and mucus secretion). Validated model.	121
	Alveolar cell line + macrophage-like cells + mast cells + endothelial cells	Tetraculture model mimicking alveolar barrier to study toxic effects of particles on the lungs; cells cultured at air-liquid interface (ALI)	Transwell inserts (0.4 µm)	In this study, the Transwell membrane played a central role by providing support for the complete tetraculture system and enabled 3D organization of cells that closely resembled *in vivo* histology of the alveolar barrier.	39

can be maintained in a homeostatic state for long periods of time (3 to 4 weeks), which is typically only feasible in animal models and not *in vitro* 2D cell culture models[121]. Another commercially available ALI culture is EpiDerm™ (MatTek), a model for human epidermis derived by differentiating epidermal keratinocytes on chemically modified collagen-coated cell culture inserts. This *in vitro* skin model reproduces morphological, structural, and barrier function properties of normal human skin, and is used for studying dermal toxicity. This same technology has been used by MatTek to generate other models for toxicity testing such as EpiOcular™ (corneal), EpiAirway™ (lung), EpiOral™ (inner cheek) etc., all representing tissue models for potential points of entry for toxins.

Another potential method for improving drug discovery is through production of PSC-derived differentiated cells. PSCs can provide an unlimited source of scarce or difficult to isolate cells for evaluating disease mechanisms, drug screening and toxicity testing. In particular, differentiated cardiomyocytes, hepatocytes, and neuronal cells are highly sought after for toxicity screens. One critical issue that hinders the use of PSCs is that they are susceptible to apoptosis upon enzymatic dissociation[122], which limits production of large-scale cultures of these cells. Faulkner-Jones, et al. recently demonstrated for the first time that ESCs could be bio-printed into micro-droplets that can be cultured in hanging drops to generate uniform spheroids while maintaining viability and function[123]. These findings suggest there is potential for high throughput screening assays using directed differentiation of pluripotent stems cells. Using hPSC-derived cells could circumvent the problems of species-specific differences in drug response that are often encountered when using animal models to predict toxicity in humans.

Future Directions

In vitro 3D cell culture models have become increasingly sophisticated, and their usefulness in supporting cell growth, tissue morphogenesis, stem cell differentiation, disease modeling, drug discovery, and toxicity testing is well established. It is also evident that different 3D models with varying characteristics are required to meet the needs of specific cell types or applications. To date, many complex 3D models of tumorigenesis, stem cell differentiation, and organoid formation make use of natural ECM-based hydrogels, such as Corning® Matrigel® matrix or collagen. Combining newer technologies such as microarray on a chip, microfluidics, or bio-printing with biologically relevant materials, such as Matrigel matrix, could help to scale-up and drive some of these complex 3D cell culture models to highly predictive and relevant drug screening assays and toxicity tests, as well as providing novel systems and models for basic research.

References

1. Lodish, H. et al. Molecular Cell Biology. New York: W.H. Freeman and Company, (2002).
2. Kleinman, H. K. et al. Curr. Opin. Biotech. 14:526-532 (2003).
3. Bissell, M. J. et al. Differentiation 70:537-546 (2002).
4. van der Worp, H. B. et al. PLoS medicine 7(3): e1000245 (2010).
5. Hutchinson, L. and Kirk, R. Nat. Rev. Clin. Onc. 8(4):189-190 (2011).
6. Aggarwal, B. B. et al. Biochem. Pharmacol. 78(9):1083-1094 (2009).
7. Hait, W. N. Nat. Rev. Drug Disc. 9(4): 253-254 (2010).
8. Pampaloni, F. et al. Nat. Rev. Mol. Cell Biol. 8:839-845 (2007).
9. Lee, J. et al. Tissue Eng. 14(1):61-86 (2008).
10. Haycock, J. W. Methods Mol. Biol. 2011; 695:1-15 (2011).
11. 3D Cell Culture Trends 2010 Report, published by HTStec Limited, Cambridge, UK, February 2010.
12. Westhouse, R. A. Toxicologic Pathol., 38:165-168 (2010).
13. Baker, B.M. and Chen, C.S. J. Cell Sci. 125(13):3015-3024 (2012).
14. Kola, I. Clin. Pharmacol. Ther. 83(2):227-230 (2008).
15. Singh, S. S. Curr, Drug Metab. 7(2):165-182 (2006).
16. Li, A. P. Chem. Biol. Interact. 150(1):3-7 (2004).
17. Polson, A. G. and Fuji, R. N. Br. J. Pharmacol. 166(5):1600-1602 (2012).
18. Khetan, S. and Burdick, J. A. Biomaterials 31:8228-8234 (2010).
19. Singhvi, R. et al. Science 264:696-698 (1994).
20. Chen, C. S. et al. Science 276:1425-1428 (1997).
21. Thomas, C. H. et al. Proc. Natl. Acad. Sci. USA 99:1972-1977 (2002).
22. McBeath, R. et al. Dev. Cell 6:483-495 (2004).
23. von der Mark, K. et al. Nature 267:531-532 (1977).
24. Petersen, O. W. et al. Proc. Natl. Acad. Sci. USA 89:9064-9068 (1992).

25. Benya, P. D. and Shaffer, J. D. Cell 30:215-224 (1982).
26. Emerman, J. T. and Pitelka, D. R. In Vitro 13:316-328 (1977).
27. Lee, E. Y. et al. J. Cell Biol. 98:146-155 (1984).
28. Brock, A. et al. Langmuir 19:1611-1617 (2003).
29. The Théry, M. et al. Proc. Natl. Acad. Sci. USA 103:19771-19776 (2006).
30. The Théry, M. et al. Nature 447:493-496 (2007).
31. Mahmud, G. et al. Nat. Phys. 5:606-612 (2009).
32. Kilian, K. A. et al. Proc. Natl. Acad. Sci. USA 107:4872-4877 (2010).
33. Debnath, J. and Brugge, J. S. Nat. Rev. Cancer 5:675-688 (2005).
34. Nelson, C. M. and Bissell, M. J. Annu. Rev. Cell Dev. Biol. 22:287-309 (2006).
35. Yamada, K. M. and Cukierman, E. Cell 130:601-610 (2007).
36. Cukierman, E. et al. Science 294:1708-1712 (2001).
37. Grinnell, F. Trends Cell Biol. 13:264-269 (2003).
38. Walpita, D. and Hay, E. Nat. Rev. Mol. Cell Biol. 3:137-141 (2002).
39. Klein, S. G. et al. Particle and Fibre Tox. 10:31-47 (2013).
40. Mitchell, G. et al. Infection and Immunity 79(9):3542-3551 (2011).
41. Kumar, R. et al. Vascular Cell, 3:27-41 (2011).
42. Franzdóttir, S. R. et al. Respir. Res. 11:162-171 (2010).
43. Birgersdotter, A. et al. Semin. Cancer Biol. 15:405-412 (2005).
44. Li, C. et al. Cancer Res. 66:1990-1999 (2006).
45. Ghosh, S. et al. J. Cell Physiol. 204:522-531 (2005).
46. Delcommenne, M. and Streuli, C. H. J. Biol. Chem. 270:26794-26801 (1995).
47. Fuchs, E. et al. Cell 116:769 (2004).
48. Gómez-Lechón, M. J. et al. J. Cell Physiol. 177:553-562 (1998).
49. Berthiaume, F. et al. FASEB J. 10:1471-1484 (1996).
50. Semino, C. E. et al. Differentiation 71:262-270 (2003).
51. Powers, M. J. et al. Tissue Eng. 8:499-513 (2002).
52. Mueller-Klieser, M. et al. Br. J. Cancer 53:345-353 (1986).
53. Frieboes, H.B. et al. Cancer Res. 66:1597-1604 (2006).
54. Dawson E. et al. Adv. Drug. Deliv. Rev. 60(2):215-228 (2008).
55. Tibbitt, M. W. and Anseth, K. S. Biotechnol. Bioeng. 103(4):655 (2009).
56. Kleinman, H. K. and Martin G. R. Semin Cancer Biol. 15:378-386 (2005).
57. Languino, L. R. et al. J Cell Biol. 109:2455-2462 (1989).
58. Zhou, Z. et al. Cancer Res. 64:4699-4702 (2004).
59. Carpenter, P. M. et al. Mol. Cancer Res. 7:462-475 (2009).
60. Miyamoto, H. et al. Pancreas 28:38-44 (2004).
61. Gurski, L. A. et al. Oncology Issues 25:20-25 (2010).
62. Barcellos-Hoff, M. H. Development 105:223-235 (1989).
63. Weaver, V.M. J. Cell Biol. 137:231-245 (1997).
64. Bissell, M. J. et al. Curr. Opin. Cell Biol. 15:753-762 (2003).
65. Sisson, K. et al. Biomacromolecules 10(7):1675-1680 (2009).
66. Hartman, O. et al. Biomacromolecules 10(8):2019-2032 (2009).
67. Kiefer, J. A. and Farach-Carson, M. C. Matrix Biol. 20:429-437 (2001).
68. Ellerbroek, S. M. et al. J. Biol. Chem. 276:24833-24842 (2001).
69. Kim, Y. J. et al. Int. J. Biol. Macromol. 45:65-71 (2009).
70. Menke, A. et al. Cancer Res. 61:3508-3517 (2001).
71. Hall, C. L. et al. Neoplasia. (New York, NY) 10:797-803 (2008).
72. Gurski, L. A., et al. Biomaterials 30:6076-6085 (2009).
73. David, L. et al. Acta Biomater. 4:256-263 (2008).
74. Chen, C. et al. Leukemia Lymphoma 46:1785-1795 (2005).
75. DeVolder, R. and Kong, H. J. Wiley Interdiscip. Rev. Syst. Biol. Med. 4(4):351-365 (2012).
76. Sawhney, A. S. et al. Macromolecules 26(4):581-587 (1993).
77. Martens, P. and Anseth K. S. Polymer 41(21): 7715-7722 (2000).
78. Chirila, T. V. et al. Biomaterials 14(1):26-38 (1993).
79. Bryant, S. J. and Anseth, K. S. J. Biomed. Mater. Res. 59(1):63-72 (2002).
80. Gelain, F. et al. PLoS ONE 1: e119 (2006).
81. Horii, A. et al. PLoS ONE 2(2): e190 (2007).

82. Zhang, S. Nature Biotech. 22:151-152 (2004).
83. Zagris, N. Micron 32:427-438 (2001).
84. Gullberg, D. and Ekblom, P. Int. J. Dev. Biol. 39:845-854 (1995).
85. Aumailley, M and Gayraud, B. J. Mol. Med. 76:253-265 (1998).
86. Scott, J. E. J. Anat. 187:259-269 (1995).
87. Wallner, E. I. et al. Am. J. Physiol. 275:F467-F477 (1998).
88. Stevens, M. M. and George, J. H. Science 310:1135-1138 (2005).
89. Curtis, A and Wilkinson, C. Biochem. Soc. Symp. 65:15-26 (1999).
90. Hynes, O. Science 326:1216-1219 (2009).
91. Daley, W.P. et al. J. Cell Science 121(3):255-264 (2008).
92. Koehler, K.R. et al. Nature 500:217-221 (2013).
93. Morizane, R. et al. PLoS One 8(6):e64843 (2013).
94. Xia, Y. et al. Nat. Cell Biol. 15(12):1507-1515 (2013).
95. Spence, J. R. et al. Nature 470:105-109 (2011).
96. McCracken, K. W. et al. Nat. Protoc. 6(12):1920-1928 (2011).
97. Eiraku, M. et al. Nature 472:51-56 (2011).
98. Greggio, C. et al. Development 140:4452-4462 (2013).
99. Gray, R. S. et al. Curr. Opin. Cell Biol. 22(5): 640-650 (2010).
100. Wang, S. et al. Lab Chip 13(23):4625-4634 (2013).
101. Bershteyn, M. and Kriegstein, A. R. Cell 155(1):19-20 (2013).
102. Feder-Mengus, C. et al. Trends Mol. Med. 14(8):333-340 (2008).
103. Gorlach, A. et al. Int. J. Cancer 56, 249–254 (1994).
104. Yamada, K. M. and Cukierman, E. Cell 130(4):601-610 (2007).
105. Kim, J. B. Semin. Cancer Biol. 15(5):365-377 (2005).
106. Cichon, M. A. et al. Integr. Biol. (Camb). 4(4):440-448 (2012).
107. Härmä, V. et al. PLoS One 5(5):e10431 (2010).
108. Luca, A. C. et al. PLoS One 8(3):e59689 (2013).
109. Li, L. and Lu, Y. J. Cancer 2:458-466 (2011).
110. Carmeliet, P. and Jain, R. K. Nature 407:249-257 (2000).
111. Upreti, M. et al. Transl. Oncol. 4(6):365-376 (2011).
112. Ingthorsson, S. et al. BMC Res. Notes 3:184 (2010).
113. Otsuka, H. et al. Sci. Technol. Adv. Mater. 14:065003 (2013).
114. Touboul, C. et al. J. Transl. Med. 11:28 (2013).
115. Hongisto, V. et al. PLoS One 8(10):e77232 (2013).
116. Zang, R. et al. Intl. J. Biotechnol. for Wellness Industries 1:31-51 (2012).
117. Huh, D. et al. Trends Cell Biol. 21(12):745-754 (2011).
118. Whitesides, G. M. Nature 442:368–373 (2006).
119. Toh, Y.-C. et al. Lab Chip 9(14):2026-2035 (2009).
120. Lee, M.-Y. et al. Proc. Natl. Acad. Sci. USA 105(1):59-63 (2008).
121. Huang, S. et al. The Use of In Vitro 3D Cell Models in Drug Development for Respiratory Diseases, Drug Discover and Development - Present and Future, Dr. Izet Kapetanovi (Ed.), ISBN: 978-953-307-615-7 (2011).
122. Watanabe, K., et al. Nat. Biotechnol. 25:681-686 (2007).
123. Faulkner-Jones, A. et al. Biofabrication 5:015013 (2013).
124. Thavandiran, N. et al. Proc. Natl. Acad. Sci. USA 110(49):E4698-4707 (2013).
125. Turovets, N. et al. Cell Transplantation 21:217-234 (2012).
126. Baharvand, H. et al. Int. J. Dev. Biol. 50(7):645-652 (2006).
127. Carlson, A. L. et al. FASEB J. 26:3240-3251 (2012).
128. Brannvall, K. et al. J. Neurosci. Res. 85:2138-2146 (2007).
129. Li, X. et al. Prog. Polymer Sci. 37(8):1105-1129 (2012).
130. Willerth, S. M. et al. Biomaterials 27:5990-6003 (2006).
131. Hwang, N. S. et al. Stem Cells 24:284-291 (2006).
132. Liedmann, A. et al. Biores. Open Access. 1(1):16-24 (2012).
133. Ortinau, S. et al. Biomed. Eng. Online 9:70-87 (2010).
134. Lai, Y. et al. PLoS One 7(9):e45074 (2012).
135. Chen, J. et al. Tissue Eng. Part A. 19(5-6):716-728 (2013).
136. Kang, B.-J. et al. J. Vet. Med. Sci. 74(7):827-836 (2012).
137. Riccio, M. et al. Eur. J. Histochem. 54(4):e46 (2010).

138. Ewald, A. J. et al. Dev. Cell. 14(4):570-581 (2008).
139. Xue, X. and Shah, Y. M. J. Vis. Exp. 75:e50210 (2013).
140. Antonica, F., et al. Nature 49:66-71 (2012).
141. Lancaster, M. A. et al. Nature 501:373-379 (2013).
142. Ampuja, M. et al. BMC Cancer 13:429 (2013).
143. Eritja, N. et al. Am. J. Pathol. 176(6):2722-2731 (2010).
144. Huyck, L. et al. Assay Drug Dev. Technol. 10(4):382-392 (2012).
145. Yang, B. et al. PLoS One 8(4):e60861 (2013).
146. Urich, E. et al. Sci. Rep. 3:1500 (2013).
147. Kim, Y. and Rajagopalan, P. PLoS One 5(11):e15456 (2010).
148. Akram, K. M. et al. Resp. Res. 14:9 (2013).
149. Wang, X. et al. Biomaterials 31(14):3920-3929 (2010).
150. Li, L. et al. J. Vis. Exp. 54:e2937 (2011).
151. Vörsmann, H. et al. Cell Death Dis. 4(7): e719 (2013).
152. Wu, X. et al. Am. J. Respir. Cell Mol. Biol. 44:914-921 (2011).
153. Zhang, X. and Yang, S.-T. J. Biotech. 151:186-193 (2011).

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