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# Three-Dimensional Cell Cultures in Drug Discovery and Development

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**Abstract**

The past decades have witnessed significant efforts toward the development of three-dimensional (3D) cell cultures as systems that better mimic in vivo physiology. Today, 3D cell cultures are emerging, not only as a new tool in early drug discovery but also as potential therapeutics to treat disease. In this review, we assess leading 3D cell culture technologies and their impact on drug discovery, including spheroids, organoids, scaffolds, hydrogels, organs-on-chips, and 3D bioprinting. We also discuss the implementation of these technologies in compound identification, screening, and development, ranging from disease modeling to assessment of efficacy and safety profiles.

**Keywords**

3D cell culture, 3D bioprinting, disease models, efficacy, organoids, organs-on-chips, safety, screening, multicellular spheroid, toxicity

## Introduction

Cell-based assays have been widely used in drug discovery for several decades. Historically, two-dimensional (2D) monolayer cells cultured on a variety of planar substrates were the only practical option for cell-based screening and have proven to be a convenient and effective means to discover drug candidate molecules. Nowadays, 2D cell models can be used to effectively predict in vivo drug responses for many targets and pathways and are still very useful in drug discovery. However, it is evident that these 2D cultures suffer disadvantages associated with the loss of tissue-specific architecture, mechanical and biochemical cues, and cell-to-cell and cell-to-matrix interactions,[1,2] thus making them relatively poor models to predict drug responses for certain diseases such as cancer. For instance, compared with 2D culture, colon cancer HCT-116 cells in 3D culture have been found to be more resistant to certain anticancer drugs such as melphalan, fluorouracil, oxaliplatin, and irinotecan[3]; such chemoresistance has been observed in vivo as well.[4]

The past decade has seen the accelerating implementation of 3D cell cultures in early drug discovery, principally fueled by the need to continuously improve the productivity of pharmaceutical research and development (R&D).[5–7] The use of 3D cell cultures, together with better cell models such as stem cells and primary cells, would allow greater predictability of efficacy and toxicity in humans before drugs move into clinical trials,[8,9] which, in turn, would lower the attrition rate of new molecular medicines under development. The 3D cell culture and co-culture models are advantageous in that they not only enable drug safety and efficacy assessment in a more in vivo–like context than traditional 2D cell cultures but also eliminate the species differences (vs. animal models) that often impede interpretation of the preclinical outcomes by allowing drug testing directly in human systems.

In this review, we examine the new opportunities for the application of 3D cell culture technologies in early drug discovery, such as disease modeling, target identification and validation, screening, and drug efficacy and safety assessment. We also discuss emerging opportunities of 3D cell cultures in drug development. Future directions and technical challenges for 3D cells-based drug discovery and development are also discussed.

## 3D Cell Culture Technologies

Recent advances in cell biology, microfabrication techniques, and tissue engineering have enabled the development of a wide range of 3D cell culture technologies. These include multicellular spheroids, organoids, scaffolds,

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**Table 1.** Advantages and Disadvantages of Different 3D Cell Culture Techniques.

| Technique | Advantages | Disadvantages |
|---|---|---|
| Spheroids[a] | Easy-to-use protocol<br>Scalable to different plate formats<br>Compliant with high-throughput screening (HTS)/high-content screening (HCS)<br>Co-culture ability<br>High reproducibility | Simplified architecture |
| Organoids | Patient specific<br>In vivo–like complexity<br>In vivo–like architecture | Can be variable<br>Less amenable to HTS/HCS<br>Hard to reach in vivo maturity<br>Complication in assay<br>Lack vasculature<br>May lack key cell types |
| Scaffolds/hydrogels | Applicable to microplates<br>Amenable to HTS/HCS<br>High reproducibility<br>Co-culture ability | Simplified architecture<br>Can be variable across lots |
| Organs-on-chips | In vivo–like architecture<br>In vivo–like microenvironment, chemical, physical gradients | Lack vasculature<br>Difficult to be adapted to HTS |
| 3D bioprinting | Custom-made architecture<br>Chemical, physical gradients<br>High-throughput production<br>Co-culture ability | Lack vasculature<br>Challenges with cells/materials<br>Difficult to be adapted to HTS<br>Issues with tissue maturation |

[a]Discussion is limited to low-adhesion plates.

hydrogels, organs-on-chips, and 3D bioprinting, each with its own advantages and disadvantages (see **Table 1** for a summary). These 3D cultures, although different in principle and protocols, are used to restore the morphological, functional, and microenvironmental features of human tissues and organs. This section briefly describes the key features of these technologies.

## *Spheroids*

Multicellular spheroid cultures were initially developed by Sutherland and coworkers in 1970 to recapitulate the functional phenotype of human tumor cells and their responses to radiotherapy.[10,11] Since then, spheroid cultures have been applied to many other types of cells, including stem cells, hepatocytes, and neuronal cells (**Table 1**). Furthermore, tumor spheroid monocultures or co-cultures with immune or endothelial cells have been adapted to experimental cancer research and recently to oncology drug screening (see below). The spheroid model compensates for many of the deficiencies seen in monolayer cultures. For instance, spheroids can develop gradients of oxygen, nutrients, metabolites, and soluble signals, thus creating heterogeneous cell populations (e.g., hypoxic vs. normoxic, quiescent vs. replicating cells). In addition, spheroids have a well-defined geometry and optimal physiological cell-cell and cell-extracellular matrix (ECM) interactions. However, there are several practical challenges associated with spheroid culture, including the development and maintenance of spheroids of uniform size, the formation of spheroids from a small seed number of cells, the precise control of specific ratios of different cell types in spheroid when co-culture, and the lack of reliable, simple, standardized, and high-throughput compatible assays for drug screening using spheroids.

There are four different approaches to enable spheroid cultures. The first approach is to use low-adhesion plates to promote the self-aggregation of cells into spheroids[12] (**Fig. 1a**). These plates not only have an ultralow attachment surface coating to minimize cell adherence but also possess a well-defined geometry (e.g., round, tapered, or v-shaped bottom) to drive and position a single spheroid within each well. The key advantage of this approach is to form, propagate, and assay the spheroids within the same plate, thus enabling high-throughput screening (HTS) or high-content screening (HCS).

The second approach is to use hanging drop plates (HDPs) to promote the formation of multicellular spheroids[13] (**Fig. 1b**). When cells in media are dispensed into the top of an HDP well, cells are segregated into the discrete media droplet formed below the aperture of the HDP well bottom opening, eventually forming spheroids. Similar to the low-adhesion plates, the HDP can also be used for spheroid co-culture, wherein multiple cell types are added either at the time of initial dispensing or sequentially. However, a clear caveat of this approach is that spheroids are required to transfer from the HDP to a second plate for assays.

The third approach is to use a bioreactor (e.g., spinner flask or microgravity bioreactor) to drive cells to self-aggregate into

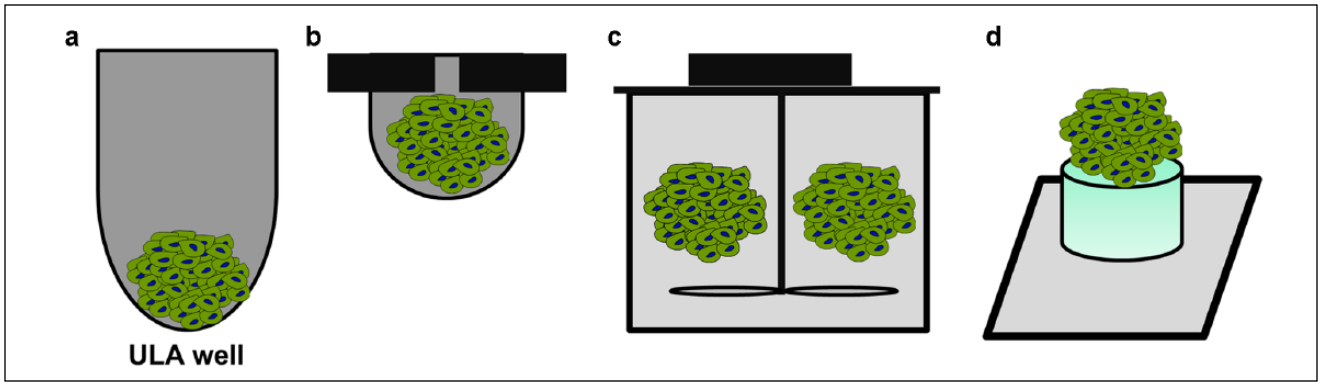


**Figure 1.** Four different techniques used for spheroid cultures. (**a**) A well of low-adhesion plates that have a round bottom with an ultralow cell attachment coating.[12] (b) A droplet of hanging drop plate where cells are partitioned and self-organized into a spheroid.[13] (**c**) Suspension culture in bioreactor where cells become self-aggregated into spheroids.[14] (**d**) A representative pillar of micropatterned plates where the cells are enriched on the top of the pillar to form a spheroid.[15]

spheroids under dynamic culture condition[14] (**Fig. 1c**). This approach permits large-scale production of spheroids. However, this approach has disadvantages associated with fluidic flow-induced shear stress, as well as nonuniformity in size of spheroids produced.

The fourth approach is to use micro-/nano-patterned surfaces as the scaffolds to control cell adhesion and migration, thus enabling spheroid cultures[15] (**Fig. 1d**). This approach offers a wide range of nanoscale scaffolds imprinted onto a flat substrate for the selection of appropriate patterns and adhesive properties for a variety of cell types. Similar to low-adhesion plates, these micropatterned plates have little well-to-well and plate-to-plate variation, which make them compliant with HTS. However, one caveat is that bubbles may easily form during the culture, and pipetting often damages the micropatterned surfaces.

## *Organoids*

Organoids, also termed *organ buds*, represent a rapidly expanding family of dish-based, 3D developing tissues that show realistic microanatomy.[16–18] An organoid is "a collection of organ-specific cell types that develops from stem cells or organ progenitors and self-organizes through cell sorting and spatially restricted lineage commitment in a manner similar to *in vivo*."[16] Organoids are classified into tissue and stem cell organoids, depending on how the organ buds are formed.[19] Tissue organoids refer to stromal cell–free (or mesenchyme-free) culture and mostly apply to epithelial cells because of their intrinsic ability to self-organize into tissue-like structures. Stem cell organoids are generated from either embryonic stem cells (ECSs) or induced pluripotent stem cells (iPSCs) or primary stem cells such as neonatal tissue stem cells or tissue-resident adult stem cells. To date, several in vitro organoids have been established to resemble various tissues, including functional organoids for thyroid,[20] pancreas,[21] liver,[22,23] stomach,[24,25] intestine,[26] vascularized cardiac patch,[27] cerebral cortex,[28] thymus,[29] kidney,[30,31] lung,[32] and retina.[33] **Table 2** summarizes key features (e.g., cell types culture techniques used, and organotypic features) of these organoids.

Numerous different approaches have been used to obtain organoids (see **Table 2** for specifics).[34] The first approach is to directly culture cells as a monolayer on a bed of feeder cells or an ECM-coated surface, so the organoids are formed after the cells differentiate. The second approach is to use a mechanically supported culture to allow the further differentiation of primary tissues. For example, human keratinocytes can further differentiate and self-assemble into a fully stratified tissue when the supported culture is in contact with an air-liquid interface over a period of weeks.[35] The third approach is to generate embryoid bodies on the low-adhesion plates or through hanging drop culture, similar to spheroid cultures. The fourth approach is to use serum-free floating culture of embryoid body-like aggregates with quick reaggregation in low-adhesion plates.

Organoids mimic some, but not all, of the structure and function of real organs.[16] First, all organoids lack vasculature, which is essential to nutrient and waste transport. Second, some organoids may lack key cell types found in vivo. Third, some organoids replicate only the early stages of organ development. For example, retinal organoids do not have the outer segments, and photoreceptors fail to fully mature to become light sensitive, whereas the cerebral organoids fail to fully develop later features, such as cortical plate layers.[16] Technical challenges still remain to produce organoids with in vivo–like complexity, increasing maturity, and screening-compatible reproducibility.

## *Scaffolds and Hydrogels*

Scaffolds refer to synthetic 3D structures made of a large variety of materials with different porosities, permeability,

**Table 2.** Organoids and Their Origin, Culture Techniques, and Applications.

| Organoid | Origin | Culture Technique | Endpoints | Ref. |
|---|---|---|---|---|
| Thyroid | mESCs | EB differentiation in hanging drops | Functional thyroid organoid | 20 |
| Pancreas | Mouse embryo pancreas progenitor | Matrigel embedding | Epithelial derivatives including endocrine cells | 21 |
| Liver | mLGR5$^+$ SC | Matrigel embedding | Bile ducts and hepatocytes to model alpha-1 antitrypsin deficiency and Alagille syndrome | 22 |
| Liver | hPSCs | Co-culture with HUVECs and hMSCs on Matrigel after monolayer differentiation toward endoderm | Liver bud derivative | 23 |
| Stomach | Adult SC/gastric glands (m/h) | Matrigel embedding | Adult SC + all stomach epithelial derivatives, excluding parietal cells, to model *Helicobacter pylori* infection/gastric cancer | 24, 25 |
| Intestine | hESCs/PSCs | Spheroids embedded Matrigel after monolayer differentiation toward hindgut | Intestinal bud, epithelial and mesenchymal derivatives | 26 |
| Vascularized cardiac patch | hESCs | High FCS | Contractile muscle | 27 |
| Cerebral cortex | m/hESCs | EBs generated in low-adhesion U-shaped plates<br>Embedded in Matrigel and cultured in spinner flask | Cerebral cortex to model microcephaly | 28 |
| Thymus | Fibroblasts | Reprograming induced by FOXN1 | All types of thymic epithelial cells on transplantation | 29 |
| Kidney | hESCs/PSCs | Subculture in air-liquid interface after differentiation and dissociation | Nephrons associated with a collecting duct network surrounded by renal interstitium and endothelial cells | 30 |
| Kidney | hPSCs | Sandwiched between two layers of Matrigel, differentiation with GSK3β inhibitor | Proximal tubules, podocytes, and endothelium | 31 |
| Lung | mAdult SCs | Matrigel co-culture with lung endothelial cells | Epithelial derivatives + mesenchymal derivatives | 32 |
| Retina | hESCs | SFEBq in low-adhesion V-shaped plates with Matrigel embedding day 2, transfer to Petri dish day 12 | Epithelial + retinal derivatives | 33 |

EB, embryonic body; ESCs, embryonic stem cells; FCS, fetal calf serum; FOXN1, transcription factor forkhead box N1; HUVECs, human umbilical vein endothelial cells; LGR5, leucine-rich repeat containing G protein–coupled receptor 5; m/h, mouse or human; MSCs, human mesenchymal stem cells; PSCs, induced pluripotent stem cells; SCs, stem cells; SFEBq, serum-free floating culture of EB-like aggregates with quick reaggregation.

surface chemistries, and mechanical characteristics designed to mimic the microenvironment of specific tissues. Scaffolds can be classified into biological and polymeric scaffolds. Biological scaffolds mostly use naturally derived ECM such as Matrigel and collagen to promote appropriate cell attachment and reorganization into 3D structures. Compared to synthetic scaffolds, Matrigel can provide a more physiologically relevant microenvironment of soluble growth factors, hormones, and other molecules with which cells interact in an in vivo environment.[36] Matrigel has been widely used as the gold standard scaffold material to provide 3D cell cultures for a wide range of cell types. However, the disadvantages associated with Matrigel are commonly occurring lot-to-lot variability during manufacturing and complexity in composition, which are often ill-defined, making it difficult to determine exactly which signals are promoting cell function. Other natural gels such as fibrin, hyaluronic acid, chitosan, alginate, or silk fibrils have also been used for 3D cell culture; however, these natural gels have less versatility to promote 3D culture than Matrigel.

Polymeric scaffolds use synthetic hydrogels or other biocompatible polymeric materials to generate the physical supports for 3D cultures.[37,38] The hydrogels used for 3D culture include poly(ethylene glycol) (PEG), poly(vinyl alcohol), and poly(2-hydroxy ethyl methacrylate).[38] Furthermore, hydrogels can be made to be hydrolytically or enzymatically biodegradable by incorporating poly(lactic acid) units[39] or enzyme cleavable peptide sequences[40] into the polymer network backbone. The biodegradability is critical to applications in which cell utilization is a must, such as tissue engineering and regenerative medicine. Synthetic scaffolds have several clear advantages over Matrigel or other natural gels for 3D cultures. First, the use of synthetic materials can minimize the relatively poor reproducibility of biological ECMs between batches and the resulting lack of consistency between cultures, as they are often simply processed and manufactured. Second, these scaffolds allow for fine tuning of biochemical and mechanical properties, so it is possible to optimize both mechanical and chemical cues for 3D cell cultures. Third, these hydrogels possess high water content, enabling transport of oxygen, nutrients, waste, and soluble factors, all of which are important to cell functions.[41] However, these hydrogels do not contain the endogenous factors but act mainly as a template to regulate cell behavior. In addition, these hydrogels pose challenges related to oxygen availability, heterogeneities present in the synthetic cellular microenvironment, and uneven distribution of soluble growth factors within the matrix and complication in imaging and cell analysis.[38]

The scaffold characteristics, along with the material properties, can regulate cell adhesion, proliferation, activation, and differentiation.[42,43] For instance, naive mesenchymal stem cells (MSCs) were shown to specify lineage and commit to phenotypes with extreme sensitivity to substrate mechanical stiffness.[44] MSCs were neurogenic on soft matrices but myogenic on stiffer matrices that mimic muscle and osteogenic on comparatively rigid matrices that mimic collagenous bone. Upon treatment with soluble factors, MSCs were found to differentiate into the lineage specified by matrix elasticity.

Scaffolds can be made using a variety of techniques, such as 3D printing,[45] particulate leaching,[46] or electrospinning.[47] Alternative approaches include gas foaming, fiber meshes/fiber bonding, phase separation, melt molding, emulsion freeze drying, solution casting, or freeze drying (reviewed in ref. 48). The types of scaffolds obtained were dependent on the fabrication techniques. In general, particulate leaching or solvent casting can be used to produce porous scaffolds, whereas electrospinning is useful for fabricating fibrous scaffolds, and 3D printing can be used to produce scaffolds with defined shapes and geometries. All of these types of scaffolds have been realized for 3D culture. For instance, to overcome the progressive loss of functionality of MSC expansion in 2D monolayer culture, freshly isolated bone marrow nucleated cells were directly cultured within 3D porous hydroxyapatite ceramic scaffolds in a perfusion-based bioreactor system.[49] The stromal tissues obtained were enzymatically treated to yield CD45- MSCs, which gave rise to a 4.3-fold higher clonogenicity and the superior differentiation capacity toward all typical mesenchymal lineages, compared with the 2D expansion culture. Of note, cells grown on fibrous scaffolds are often not considered to truly represent 3D culture, as cells typically adhere and elongate along the fibers.[50] In addition, porous scaffolds have issues associated with their limited diffusion properties, which make it difficult to fabricate more complex tissues such as heart and liver.

## *Organs-on-Chips*

An organ-on-a-chip refers to an artificial, miniature model of a human organ on a microfluidic cell culture chip. The chip is made with great precision using microfabrication techniques such as soft lithography, photolithography, and contact printing.[51] The chip usually consists of a series of well-defined structures, patterns, or scaffolds. Therefore, the position, shape, function, and chemical and physical microenvironments of the cells in culture can be controlled with high spatiotemporal precision using microfluidics.[52] Organs-on-chips are designed to reconstitute the structural, microenvironmental, and functional complexity of living human organs. However, most organs-on-chips are often made to capture only the critical features of an organ type or a disease model due to practical reason, so researchers can reproduce clinically relevant disease phenotypes and pharmacological responses.[53,54] To date, a wide range of organs-on-chips have been reported, including skin,[55] lung,[56–58] vasculature,[59] heart,[60,61] muscle,[62] liver,[63–65] intestine,[66] and several others (see below for specific applications of some of these organ systems).

Organs-on-chips have been adapted to microplate formats, the de facto footprint used in drug discovery. For instance, a liver-on-a-chip that uses a bioreactor to foster maintenance of 3D tissue cultures under constant perfusion was developed in a multiwell plate format[67] and used for drug metabolism profiling and pharmacokinetic evaluation.[68] However, most organs-on-chips lack vasculature and also are difficult to adapt to HTS.

## *Three-Dimensional Bioprinting*

Three-dimensional bioprinting refers to the printing of cells, biocompatible materials, and supporting components into complex 3D living tissues with the desired cell/organoid architecture, topology, and functionality using additive manufacturing.[69] Three-dimensional bioprinting usually involves layer-by-layer positioning of biological materials,

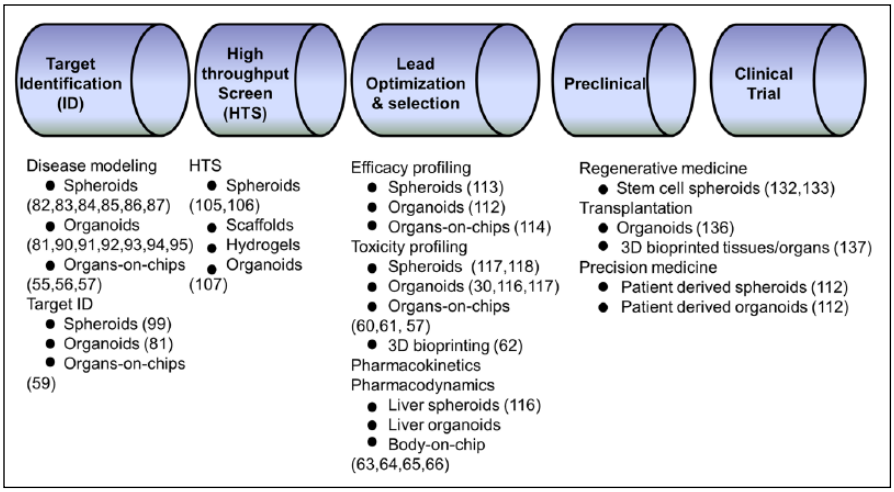


**Figure 2.** How different three-dimensional culture techniques have been implemented in different stages of drug discovery and development processes. Representative references for each application are cited in parentheses in the graph.

biochemicals, and living cells. There are three approaches used for bioprinting. The first one is biomimicry, which employs biologically inspired engineering to replicate the cellular and extracellular components of a tissue or organ (e.g., human ears).[70] The second approach is autonomous self-assembly, which relies on the cells as the primary driver of histogenesis to produce the desired biological microarchitecture and functional tissues.[71] The third approach is to fabricate and assemble mini-tissue building blocks, such as a kidney nephron, into the larger construct by rational design, self-assembly, or a combination of both.[72,73]

Three-dimensional bioprinting has been used to generate functional tissues, such as multilayered skin, bone, vascular grafts, tracheal splints, heart tissue, and cartilaginous structures, for transplantation applications.[74] Furthermore, 3D bioprinting has been used not only to create scaffolds for 3D cell cultures but also to directly produce 3D-bioprinted tissue models for drug screening and profiling.[75] Bioprinting has several advantages, such as custom-made microarchitecture, high-throughput capability, and co-culture ability. However, compared with other 3D cell cultures, 3D bioprinting faces many additional challenges associated with cell and material requirements as well as tissue maturation and functionality.[69]

## 3D Cell Cultures in Drug Discovery

Drug discovery is a long, complex process with growing difficulty. Three-dimensional cell cultures have been penetrating into the early drug discovery process, starting from disease modeling to target identification and validation, screening, lead selection, efficacy, and safety assessment (**Fig. 2**). This section discusses how to best implement different 3D cell culture technologies into different stages of drug discovery process.

### *Disease Modeling*

Drug discovery often starts with a disease or a clinical condition without suitable medical products available.[76] As growing efforts have been directed toward unmet therapeutic needs in recent years,[77] disease modeling has become increasingly important to the success of drug discovery programs. As they promise to bridge the gap between 2D culture and in vivo, a range of 3D cell cultures have been applied to understand the mechanisms of different diseases. In particular, 3D models have gained popularity in elucidating tumor biology, as standard 2D models are inadequate to address questions regarding indolent disease, metastatic colonization, dormancy, relapse, and the rapid evolution of drug resistance.[78]

Three-dimensional cultures on ECM gels have provided models to detect architecture transformation from preinvasive breast carcinoma to full malignancy induced by the progressive loss of tissue architecture and aberrant signaling[79] or from nonmalignant breast epithelial cells to malignant tumors induced by tuning stiffness of Matrigel/Collagen I gels used in 3D culture.[80] Recently, Drost et al.[81] investigated the phenotypes of sequential cancer mutations in cultured human intestinal stem cells by combining organoid culture and CRISPR/Cas9 gene editing. Here, normal human intestinal stem cells isolated from patients were genetically edited using CRISPR/Cas-9 for the four most commonly mutated colorectal cancer genes (APC, P53, KRAS, and SMAD4), followed by culturing on Matrigel or basal membrane extract–coated plates in medium containing the stem-cell-niche factors WNT, R-spondin, epidermal growth factor, and noggin. Results showed that the epithelial organoids obtained remained genetically and phenotypically stable for long periods of time, and xenotransplantation of quadruple mutant organoids into mice resulted in tumors with features of invasive carcinoma. Remarkably, the combined loss of APC and P53 was found to be sufficient for the appearance of extensive aneuploidy, a hallmark of tumor progression.

Spheroid cultures have become useful for modeling the tissue architecture, signaling, microenvironments, and invasion and immune behaviors of cancer, as well as for studying and expanding the cancer stem cells (CSCs).[82] Human

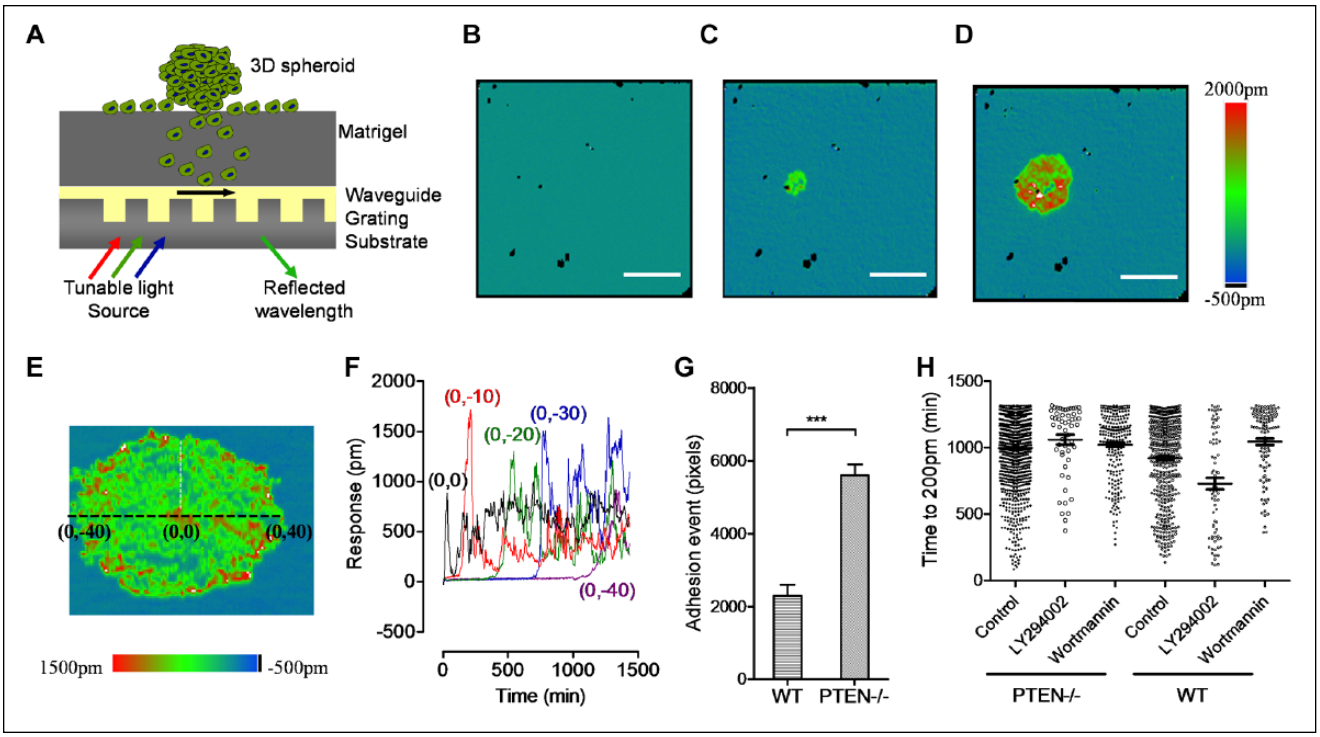


**Figure 3.** A label-free, single-cell, real-time assay to measure the invasion of cells in a single spheroid through a three-dimensional extracellular matrix (Matrigel). (**a**) Principle of the assay, which consists of four critical steps: coating the biosensor surface with Matrigel; adding medium to the well; transferring a spheroid from an ultralow attachment, round-bottomed microplate and placing it onto the top Matrigel surface; and monitoring the invasion of cells through the matrix and adhesion on the sensor surface in real time. (**b–d**) The time series dynamic mass redistribution (DMR) images before and after a single spheroid was placed onto the biosensor surface coated with 10 µL 0.1 mg/mL Matrigel: 0 min (**b**), 1 h (**c**), and 24 h (**d**). Spatial scale bar: 500 µm. Intensity scale bar: –500 pm to 2000 pm. (**e**) A DMR image taken 24 h after a spheroid was placed on the top Matrigel surface. Scale bar: 500 µm. (**f**) Representative pixelated real-time DMR signals for the black line indicated in (**e**). (**g**) The adhesion events versus cell types. (**h**) The adhesion time to reach 200 pm under different conditions. For (**e–h**), coating was 0.2 mg/mL Matrigel. Data represent mean ± SD for g ($n$ = 3). ***$p$ < 0.001. This figure is adapted from ref. 89 with permission.

cancer is known to harbor several heterogeneous subpopulations of CSCs that play distinct roles in tumor initiation, maintenance, and metastasis. For instance, in colon cancers, there were three types of CSCs isolated from patients: tumor-initiating cells that have limited or no self-renewal capacity but are contributed to tumor formation only in primary mice, self-renewal CSCs that allow long-term tumor growth, and rare delayed contributing CSCs that were exclusively active in secondary or tertiary mice.[83] Tumor invasion and metastasis is a multistep cascade process. It begins with local invasion of cancer cells through the ECM and stromal cell layers, then intravasation into the lumina of blood vessels. This is followed by transit through the lymphatic and hematogenous systems and arrest and extravasation out of the circulatory system, which leads to the formation and growth of micrometastatic lesions into macroscopic tumors at a distant site.[84] Spheroids of cancer cell lines have been used to investigate different aspects of the cancer invasion process, including the invasion of cells in a 3D spheroid into the surrounding 3D ECM structure[85,86] and endothelial cell–tumor cell interactions.[87] For instance, we had developed a label-free, real-time, single-cell, and quantitative assay to monitor the invasion of cells in a spheroid through a 3D Matrigel (**Fig. 3**). We found that epidermal growth factor accelerates the invasion of the colon cancer cell line HT-29, whereas vandetanib dose-dependently inhibits the invasion.[88] Vandetanib is a multitarget kinase inhibitor that has been clinically approved for the treatment of late-stage (metastatic) medullary thyroid cancer in adult patients who are ineligible for surgery and also has potential to treat non–small-cell lung cancer. Although the results obtained using the label-free assay are largely expected as vandetanib is known to inhibit vascular endothelial growth factor receptor, this assay enables real-time quantification of its effect on cancer invasion through the Matrigel, a capability that is otherwise difficult to obtain using conventional endpoint assays. We further found that PTEN knockout increased the invasion rate of HCT116 cells in spheroid through 3D Matrigel, and PI3K inhibitors LY294002 and wortmannin drastically reduced the invasiveness of the

cells.[89] This label-free imaging technique has revealed that besides the accelerated invasion kinetics, PTEN knockout expedites cell dissociation from the spheroidal structure and adhesion onto the surfaces. This study also indicates that the mechanisms governing cell invasion are sensitive to ECM matrix density, and the invasion inhibitory sensitivity of PI3K inhibitors is also sensitive to the PTEN expression level.

Organoid cultures have also been applied to model cancer, besides a great number of other diseases including developmental disorders, infectious diseases, and neuronal degeneration.[16] For example, several different intestinal organoids were obtained and used for modeling a range of diseases. Specifically, the human intestinal organoids derived from the ESC line WA09 were used to examine gastrointestinal infection with rotavirus.[90] The human intestinal organoids generated using intact crypts from human intestines were used to examine *Cryptosporidium parvum* infection.[91] The organoids obtained by culturing CD44+CD24+ cells enriched for colorectal CSCs in the HT29 and SW1222 cell lines were used to study colon CSC biology.[92] The intestinal organoids obtained using murine primary intestinal cells were used to study genetically reconstituted tumorigenesis (e.g., by knockdown adenomatous polyposis coli [APC]),[93] whereas the intestinal organoids cultured from patient biopsies were used to study genetic disorders.[94,95]

Many genetic disorders that have been difficult or impossible to model in animals can be modeled by using organoid cultures of patient iPSCs or, alternatively, through the introduction of patient mutations into human PSCs using genome-editing technologies, such as CRISPR/Cas9. For instance, the CRISPR-Cas9 genome-editing system was used recently to introduce multiple recurrent mutations in colon cancer patients into organoids derived from normal human intestinal epithelium.[96]

Organs-on-chips are also useful for cancer modeling. For instance, cultured human skin tissue has been successfully used as a surrogate for modeling melanoma cancer growth.[55] Here, when human melanoma cell lines were incorporated, the cultured skin tissue recapitulated natural features of melanocyte homeostasis and melanoma progression in human skin. They displayed the same characteristics reflecting the original tumor stage (vertical and radial growth phases and metastatic melanoma cells) in vivo. Organs-on-chips have also been used to model other diseases. For instance, a lung-on-a-chip was developed to mimic breathing by stretching and compressing an artificial alveolar-capillary barrier using a cyclic vacuum machine. This was used to model pathogen infection and inflammatory responses to air pollutants[56] or the development and progression of pulmonary edema induced by the toxicity of interleukin-2.[57] Recently, the airway-on-a-chip device lined by living human bronchiolar epithelium from normal or chronic obstructive pulmonary disease (COPD) patients was connected to an instrument that "breathes" whole cigarette smoke in and out of the chips to study smoke-induced pathophysiology in vitro.[58] This enables the detection of smoke-induced ciliary micropathologies, COPD-specific molecular signatures, and epithelial responses to smoke generated by electronic cigarettes.

## Target Identification and Validation

Target identification and validation is often the rate-limiting step in preclinical drug discovery.[97] Three-dimensional cultures have the potential to discover novel mechanisms and targets and to accelerate target identification and validation, given that the gene expression patterns found in 3D models are one step closer to in vivo, compared to 2D monolayer models.[98] For instance, gene expression analysis of mesothelioma cell lines cultured in spheroids had revealed the underlying causes of chemoresistance in malignant pleural mesothelioma.[99] Here, the spheroids were found to acquire increased chemoresistance compared with 2D monolayers. A total of 209 genes were differentially expressed in common by the three mesothelioma cell lines in spheroids, among which argininosuccinate synthase 1 (ASS1) was the only consistently up-regulated gene in both 3D spheroids and human tumors. siRNA knockdown of ASS1 significantly sensitized mesothelioma spheroids to the proapoptotic effects of bortezomib or cisplatin plus pemetrexed. These results suggest that ASS1 may be a druggable target to undermine mesothelioma multicellular resistance.

In another recent study, a microfluidic vasculature chip was developed to model intravascular steps in metastasis.[59] Here, the chip consisted of an upper intravascular compartment and lower stromal chambers, separated by a semiporous membrane lined with human microvascular endothelial cells. Upon stimulation of microvascular endothelium from the basal side, CXCL12 acted through the CXCR4 receptor on endothelium to promote adhesion of circulating breast cancer cells. This suggests that targeting CXCL12-CXCR4 signaling in endothelium may limit metastases in breast and other cancers.

## Screening for Hit Identification

Screening using cell-based assays has frequently been the starting point for identifying hit compounds in the early stage of drug discovery. In the past three decades or so, target-based HTS has been dominating in the hit identification process, given that HTS-compatible cellular assays have simplicity, relatively low cost, and high efficiency. However, in recent years, there has been a renaissance in phenotypic screening, driven by three factors. First, continuous improvement in the productivity of pharmaceutical R&D calls for innovative strategies for drug discovery. Second, although target-based screens are more effective

for discovering follow-on drugs for which molecular mode of action is known, phenotypic screens are more productive for discovering first-in-class drugs.[100] Third, advances in detection technologies have made it feasible to perform phenotypic screens with high throughput as well as more biologically relevant information relative to conventional molecular assays.[101–104]

Incorporating 3D cell cultures with HTS processes is still in infancy but shows promise in directly identifying clinically relevant compounds, enabling effective translational research. Unfortunately, not all 3D cell culture models are compatible with HTS or HCS in a routine and cost-effective manner. Among all 3D models under development, spheroids cultured in the low-adhesion plates have started gaining popularity in oncology drug screening because of their easy-to-use protocols, high-density microplate formats (e.g., 384-well and 1536-well), and compatibility with automation and multimode detection systems. For instance, using glucose-deprived multicellular tumor spheroids of colon cancer cell lines with inner hypoxia that were cultured in 384-well low-adhesion plates, Senkowski et al.[105] screened 1600 compounds with documented clinical history to identify five compounds that selectively target the hypoxic cell population. All five compounds inhibited mitochondrial respiration, suggesting that cancer cells in low-glucose concentrations depend on oxidative phosphorylation, instead of solely glycolysis. The antiprotozoal drug nitazoxanide was found to activate the AMPK pathway and down-regulate c-Myc, mTOR, and Wnt signaling at clinically relevant concentrations. Combining nitazoxanide with the cytotoxic drug irinotecan showed anticancer activity in vivo. Similar results were obtained from the HCS of 1120 compounds against spheroids of the human breast cancer cell line T47D.[106] At the 2016 SLAS annual conference, Dr. Timothy Spicer and his colleagues at The Scripps Research Institute presented results using Corning nonadherent 1536-well spheroid plates to screen the entire Scripps Drug Discovery Library of more than 650,000 compounds in less than 2 wk (personal communication).

Three-dimensional co-cultures of a cancer cell with another cell type (e.g., an immune or fibroblast cell line) have also been developed in high-throughput formats. For instance, using a multilayered organotypic culture containing primary human fibroblasts, mesothelial cells, and ECM, Kenny et al.[107] performed a screen of 2420 pharmacologically active compounds. This organotypic culture was used to reproduce the human ovarian cancer metastatic microenvironment. Subsequent validation in secondary in vitro and in vivo assays confirmed two active compounds, β-escin and tomatine, that prevented ovarian cancer adhesion, invasion, and metastasis, leading to the improved survival in mouse models. This study shows the power of complex 3D models to improve the disease relevance of assays used for drug screening.

## *Efficacy Profiling for Lead Identification*

Following hit identification is lead identification. Once identified in a screen, hits are first confirmed based on dose-response curves using the same assay for screening and orthogonal testing with different assay(s). Once confirmed, hits are further evaluated for synthetic tractability, freedom to operate, drug-likeness, and possible toxicity, metabolism, and stability-related risks. Medicinal chemistry optimization is the next step to generating lead candidate compounds with improved potency, reduced off-target activities, and desired physicochemical and metabolic properties. Critical to the entire process of lead identification is to have cost-effective in vitro models that can more reliably predict the efficacy, toxicity, and pharmacokinetics of drug compounds in humans. Three-dimensional cell culture models have a potential to play an important role in lead identification and to reduce the use of animal testing for preclinical studies.

Lacking in vivo efficacy is one of the key reasons why some late-stage clinical trials fail.[108] Three-dimensional cell culture models have been shown to in some cases more accurately evaluate drug efficacy than 2D models and may even enable personalized approaches to identify the mechanisms underlying disease and to screen and select the best drug(s) for the patients.[109–111] For instance, patient-derived spheroids have been developed as a predictive test to identify the most effective therapy for 120 patients with HER2-negative breast cancer of all stages.[112] Results showed that the tissue spheroid model reflected current guideline treatment recommendations for HER2-negative breast cancer. Tissue spheroid showed greater responses to anthracycline/docetaxel for hormone receptor–negative samples, a higher response to fluorouracil and anthracycline in high-grade tumors, and a higher treatment efficacy to anthracycline treatment combined with fluorouracil for smaller tumor size and negative lymph node status. Recently, Tong et al. applied spheroids of three ovarian cancer cell lines to investigate the differential oncolytic efficacy among three different viruses: myxoma, double-deleted vaccinia, and Maraba virus.[113] They found that the low-density lipoprotein receptor expression in ovarian cancer spheroids is reduced, which in turn affects the binding and entry of Maraba virus into cells.

Compared with spheroids, organs-on-chips provide a viable strategy to further increase the complexity and physiological relevance for reliable assessment of drug efficacy. For instance, Aref et al.[114] developed an organ-on-a-chip consisting of lung cancer spheroids in a 3D matrix gel adjacent to an endothelialized microchannel to recapitulate epithelial-mesenchymal transition during cancer progression. Results showed that for the A549 cell model, there are both qualitative and quantitative differences in drug response between 2D monolayer cells and 3D spheroids. For instance, for the TGF-βR inhibitor A83-01, the differences in

effective dose between 2D and 3D culture were more than three orders of magnitude (5 nM vs. 2.5 μM).

### Toxicity Profiling for Lead Selection

Drug-induced toxicities in liver, heart, kidney, and brain currently account for more than 70% of drug attrition and withdrawal from the market.[115] Adverse drug reactions are often due to off-target interactions or excessive binding of the drug molecule to toxicity-prone cells. Three-dimensional cell culture models are powerful in assessing drug-induced toxicity.

Organ buds of brain, liver, heart, and kidney can be used to assess drug toxicity.[16] Recently, a brain organoid was produced by combining human ESC-derived neural progenitor cells, endothelial cells, MSCs, and microglia/macrophage precursors on chemically defined polyethylene glycol hydrogels.[116] Machine learning was used to build a predictive model from changes in global gene expression when being exposed to 60 training compounds (34 toxic and 26 nontoxic chemicals). The model was then used to correctly classify 9 of 10 additional chemicals in a blinded trial. Human liver organoids obtained using HepaRG cell line, a terminally differentiated hepatic cell line derived from a human hepatic progenitor cell line, have already been shown to produce human-specific metabolites.[117] This is particularly useful because human liver often metabolizes drugs in a manner distinct from animal liver. Of note, these HepaRG 3D organotypic cultures are more sensitive to acetaminophen- or rosiglitazone-induced toxicity but less sensitive to troglitazone-induced toxicity than the 2D cultures. Kidney organ buds from human iPSC cells were found to differentially apoptose in response to cisplatin, a nephrotoxicant, showing such organoids represent powerful models of the human organ for drug-induced nephrotoxicity.[30]

Three-dimensional liver cell spheroid cultures are also valuable for investigating drug-induced liver injury, function, and diseases. An organotypic culture of the human hepatoma HepaRG cell line were obtained using hanging drop culture and was able to detect the potent toxicity of acetaminophen.[117] Human primary hepatocyte spheroids obtained using the low-adhesion plates were found to be phenotypically stable and retained morphology, viability, and hepatocyte-specific functions for at least 5 wk, enabling chronic toxicity assessment of drug molecules.[118] The chronic toxicity of fialuridine was detected after repeated dosing in this spheroid model; this type of toxicity was impossible to detect using 2D models. However, the primary hepatocyte spheroids also retain the interindividual variability, which may limit the ability of such models for large-scale screening. To this regard, unlimitedly renewable, primary-like hepatocytes, such as HepatoCells, HepRG, or iPS-derived cells, may be good alternatives for screening.

Organs-on-chips and other 3D cell culture models were also used to evaluate drug-induced toxicity.[104] Heart-on-a-chip devices were useful for assessing drug-induced cardiotoxicity.[60,61] The lung-on-chip model developed by Huh et al.[57] consisted of channels lined by closely apposed layers of human pulmonary epithelial and endothelial cells that experience air and fluid flow, enabling the detection of drug toxicity-induced pulmonary edema observed in human cancer patients treated with interleukin-2 at similar doses and over the same time frame.[57] This study also found that both angiopoietin-1 and GSK2193874 (a transient receptor potential vanilloid 4 ion channel inhibitor) were effective at preventing the drug toxicity-induced pulmonary edema. A 3D bioprinted, cell-based soft robotic device that was powered by the actuation of an engineered mammalian skeletal muscle strip was recently used to sense, process signals, and produce force.[62] The muscle strip was made by printing mouse skeletal muscle myoblast cell line C2C12 in the presence of hydrogels and other biological components. Skeletal muscle as a contractile power source is the primary generator of actuation in animals. This device can be used to assess drug-induced myopathy.

### Pharmacokinetics and Pharmacodynamics Profiling for Lead Selection

Inadequate pharmacokinetics and pharmacodynamics is also a key factor in why drugs fail. Three-dimensional cell culture models, in particular, liver spheroids, liver organoids, and body-on-chips, are useful to investigate the pharmacokinetic profiles of drug molecules. Liver spheroids and organoids have been used to study the metabolism of drug molecules.[116] Several versions of liver-on-a-chip systems were used to measure rates of metabolic drug clearance, which were compared with literature-reported values.[63–65] The gut-on-a-chip using the Caco-2 cell layer on a porous support to separate two chambers was used to reproduce characteristic absorptive properties and the barrier function of the human intestine, enabling drug absorption studies.[66] Integrating multiple organ types into one chip, termed as *body-on-a-chip*, can be powerful for comprehending the pharmacokinetics and pharmacodynamics of drug molecules.[119,120] However, developing screening-compatible body-on-a-chip remains a challenging task, in particular when one considers the known allometric scaling issue.[121]

## 3D Cultures in Cell Therapy and Tissue Engineering

Cell therapy and tissue engineering have started entering the market. They not only offer new hope for patients with injuries, end-stage organ failure, or other clinical issues but

also will eventually transform our lives. However, it is becoming clear that realizing the full potential of cell therapy and tissue engineering requires advances in cell culture technologies to meet the demand in quantity, quality, and process robustness for commercialization and clinical trials. Three-dimensional cell cultures offer not only a solution for cell scale-up production but also a new form of therapeutics for treating many different diseases.

## Stem Cell Spheroids for Regenerative Medicine

Stem cells are widely used as a cell source for regenerative medicine and cell therapy applications. However, conventional 2D culture techniques, in combination with the current best practice, may be ineffective to expand stem cells for clinical applications. This is reflected by the fact that 2D cultures are inadequate to reproduce the in vivo microenvironment of stem cells.[122] In addition, clinical observations show that the beneficial effects of stem cell–based therapeutics seen in initial small-scale clinical studies are often not validated by large, randomized clinical trials.[123,124] In fact, MSCs often decrease their replicative ability, colony-forming efficiency, and differentiation capabilities over time when culturing and passaging in 2D adherent monolayer.[125,126] In contrast, MSCs cultured in spheroids display a morphology that is significantly different from 2D culture.[127] The MSCs are spherical inside and elongated outside the spheroid, with an overall reduction of cytoskeletal molecules, ECM, and size (~75% reduction in individual cell volume),[128] indicating distinct differentiation preferences among different lineages.[129] Furthermore, compared with 2D culture, MSCs cultured in spheroids have different gene expression patterns, with up-regulation of many genes that are associated with hypoxia, angiogenesis, inflammation, stress response, and redox signaling.[130]

Spheroid cultures have been reported to improve the efficacy of MSC-based therapeutics. Compared with 2D cultures, MSC spheroid cultures were found to result in several additional beneficial effects, such as enhanced anti-inflammatory and tissue regenerative and reparative effects, as well as better posttransplant survival of MSCs.[130] Furthermore, compared with 2D cultured cells, spheroids of human adipose–derived MSCs produced higher levels of ECM proteins, exhibited stronger antiapoptotic and antioxidative capacities, and increased the paracrine secretion of cytokines.[131] When injected into the kidney of model rats with ischemia reperfusion-induced acute kidney injury, these MSC spheroids were more effective in protecting the kidney against apoptosis, reducing tissue damage, promoting vascularization, and ameliorating renal function compared with 2D cultured cells.

Spheroid cultures have been used to enrich patient-specific stem cells for disease treatment. For instance, Henry et al.[132] applied spheroid culture to enrich adult lung stem cells for use in treating idiopathic pulmonary fibrosis in mice. Here, in a suspension culture, the outgrowth cells from healthy lung tissue explants were self-aggregated into spheroids, which recapitulated the stem cell niche and acquired mature lung epithelial phenotypes. The mice that received these spheroids showed decreases in inflammation and fibrosis.

Spheroid cultures have also been used to scale up stem cell products for use in clinical trials. For instance, the manufacturing process of pancreatic endoderm cells (PEC-01) involves dynamic suspension spheroid culture and differentiation.[133] The PEC-01 is derived from CyT49 human ESCs and is the cellular component of the VC-01 combination product from ViaCytes for treating type 1 diabetes. PEC-01 matures after transplantation and functions to regulate blood glucose.

## Organoids for Transplantation

Organoids could provide a source of autologous tissue for transplantation, as organoid research advances rapidly. For instance, renal organoids derived from pluripotent stem cells were successfully transplanted under the renal capsules of adult mice.[134] Here, the organoid reconstituted the 3D structures of the kidney in vivo, including glomeruli with podocytes and renal tubules with proximal and distal regions and clear lumina. Furthermore, the glomeruli were efficiently vascularized upon transplantation, which is a promising step toward kidney replacement strategy.

Although early in development, organoid-based replacement may find applications in other diseases, such as retinal organoids obtained from human ESCs for treating certain types of retinal degeneration and blindness,[16] intestinal organoids for replacement of damaged colon after injury or following removal of diseased tissue,[135] and gene-corrected organoids for replacement of damaged organs with gene defect(s).[16] For instance, the intestinal organoids obtained from Lgr5+ adult colonic stem cells have been transplanted into superficially damaged mouse colon.[136] Results showed that the transplanted donor cells readily integrated into the mouse colon, covering the area that lacked epithelium as a result of the introduced damage in recipient mice. Long-term (>6 months) engraftment with transplantation of organoids derived from a single Lgr5+ colon stem cell was observed after extensive in vitro expansion. This study shows the feasibility of colon stem-cell therapy based on the in vitro expansion of a single adult colonic stem cell.

## 3D Bioprinted Tissues/Organs for Transplantation

Advances in tissue engineering, cell biology, and materials sciences have made 3D bioprinting possible to create functioning tissues or organ grafts with their natural

microenvironments and architectures from autologous cells for transplantation applications. Although printing an intact organ still remains elusive, 3D-bioprinted bladders, tracheal grafts, bone, and cartilage have proven to be functional after development and implantation in animals or humans.[74] These printed organs can be used as assist organs or viable replacements. For instance, Atala et al.[137] engineered a human bladder by isolating autologous bladder urothelial and muscle cells from the bladder biopsy, expanding the cells in vitro and seeding them to a biodegradable bladder-shaped scaffold made of collagen or a composite of collagen and polyglycolic acid. About 7 weeks after the biopsy, the autologous engineered bladder constructs were used for reconstruction and implanted either with or without an omental wrap. A clinical trial on seven patients in need of a cystoplasty showed that the engineered bladder tissues, created with autologous cells seeded on the collagen-polyglycolic acid scaffolds and wrapped in omentum after implantation, were safe and effective to use in patients. In another example, a microfluidic device with double-coaxial laminar flow was used to fabricate meter-long core-shell hydrogel microfibers encapsulating ECM proteins and primary pancreatic islet cells.[138] After transplanting through a microcatheter into the subrenal capsular space of diabetic mice, the microfibers containing the islet cells normalized blood glucose concentrations for about 2 wk.

More recent efforts were focused on the development of 3D-bioprinted tissues, such as livers and kidneys, with integrated vasculature.[139] Integral vascular structures are critical to the survival of the transplanted organs or tissues. Either autologous vascular conduits from deceased donor or synthetic vascular grafts have been used for anastomosing the new organ to the recipient when necessary; however, both come with disadvantages. Printing using spheroids of human umbilical vein smooth muscle cells and human skin fibroblasts, along with agarose rods, has resulted in single- and double-layered vascular tubes with small diameters.[140] Furthermore, printing branched vascular structures using human umbilical vein endothelial cells, 10T1/2 cells, human fibroblasts, or human embryonic kidney cells is also feasible.[141,142]

## Challenges, Limitations, and Future Perspectives of 3D Cell Cultures

Many challenges remain for the widespread adoption of 3D cell culture technologies in the drug discovery process. In fact, there are very limited 3D screens done with large compound libraries, although a multitude of 3D assays, mostly based on high-resolution fluorescence imaging techniques, have been validated for HTS/HCS in recent years.[143,144]

First, many 3D models such as organoids exhibit significantly more complex morphology and function than 2D cultured cells, thus leading to challenges in systematic assessment. Furthermore, current 3D cultures are diverse in terms of complexity, size, morphology, 3D architecture, and protocols for assaying. This leads to challenges in standardization with respect to culture and assay protocols, phenotypes, and output data for analysis. To this regard, the development of high-density microtiter plate–based spheroid-forming plates (e.g., 1536-well low-adhesion spheroid plates) represents an attractive solution to streamline 3D spheroid-based drug screening. The 1536-well spheroid-forming plates also make HTS economically affordable.

Second, lacking the understanding of the relevance of a 3D phenotype measured to the in vivo drug effects sought also possesses challenges for 3D screening, as typical 3D assay techniques measure a wide range of cellular phenotypic parameters (e.g., spheroid size or morphology, hypoxic core). As the mainstay in 3D assays, high-content imaging techniques could measure many different phenotypes. However, identifying a clinically relevant phenotype that is measurable in 3D models is critical to streamline and expedite the screening process. For instance, using spheroids of invasive human prostate cancer cell line PC3 cultured in the Matrigel matrix, Booij et al.[144] developed a phenotypic imaging assay to measure more than 800 phenotypic parameters. Multiparametric analysis identified several phenotypes that enable the discrimination of selective inhibitors for c-Met, or epidermal growth factor receptor, as well as putative biselective inhibitors of both receptor tyrosine kinases. However, this small-scale screen clearly highlights the complexity of identifying specific phenotypes for screening.

Third, assays using 3D cell models are far less developed with respect to imaging, analysis, quantification, and automation compared with established 2D methods. Confocal microscopy is the standard imaging tool for assessing cellular function within 3D cell models; however, it is certainly limited in throughput. Improvements in imaging modality, data acquisition throughput, and analysis tools are necessary for the wide adoption of 3D cell cultures for screening.

Fourth, the predictive values of 3D cell cultures for drug efficacy and toxicity need to be further determined and validated by using existing human data.[145] Although data had shown that the efficacy and toxicity of many drug molecules obtained using 3D models are different from 2D cultures, only a small set of these data confirmed that the efficacy and toxicity of drugs in 3D models are close to the clinical data.[23,112,114,117]

Fifth, regulatory authorities have yet to accept data obtained from 3D cell models, such as organoids or organs-on-chips, as a surrogate for preclinical animal testing. Partly related to this is that historically, the assessment of new technologies has been exceptionally slow (10 to 15 years)[145] but more importantly is that these models often do not capture the full complexity of human organ function, such as

lacking vascularization.[146] In addition, organoid technologies face a common issue related to maturation.[16]

Nonetheless, 3D cell cultures have a bright future in drug discovery and development. Three-dimensional cell cultures would have enormous potential to model development and disease, as advanced cell models under development may fully capture the in vivo functions of organs and tissues. Furthermore, the development of screening-compatible 3D cell cultures would transform the drug discovery process, as it becomes possible to obtain early the physiologically relevant efficacy and toxicity data. In addition, the optimization of 3D cell cultures for scaling-up cell production would improve quality, quantity, and efficacy, thus making cells as therapeutics a reality.

## Conclusion

A wide range of 3D cell culture technologies have been developed to address the need for continuously improving the productivity of pharmaceutical R&D. Three-dimensional cell cultures hold great potential as a tool for drug discovery—ranging from disease modeling to target identification to screening to lead identification—and as a new type of therapeutics/replacement therapy that may transform our lives. Future developments in screening readily available 3D cell models and assays, preclinically validated 3D cell models for animal replacement, and functional, safe, and transplantable 3D cell models will no doubt bring them closer to reaching these potentials.

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