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Two-Dimensional (2D) and Three-Dimensional (3D) Cell Culturing in Drug Discovery

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<http://dx.doi.org/10.5772/intechopen.81552>

Abstract

Cell culture is an indispensable *in vitro* tool used to improve our perception and understanding of cell biology, the development of tissue engineering, tissue morphology, mechanisms of diseases and drug action. Efficient cell culturing techniques both *in vitro* and *in vivo* allow researchers to design and develop new drugs in preclinical studies. Two-dimensional (2D) cell cultures have been used since 1900s and are still a dominant method in many biological studies. However, 2D cell cultures poorly imitate the conditions *in vivo*. Recently three-dimensional (3D) cell cultures have received remarkable attention in studies such as drug discovery and development. Optimization of cell culture conditions is very critical in ensuring powerful experimental reproducibility, which may help to find new therapies for cancer and other diseases. In this chapter, we discuss the 2D and 3D cell culture technologies and their role in drug discovery.

Keywords: 2D cell culture, 3D cell culture, drug discovery, cell-based assays

1. Introduction

The discovery and development of new drugs is a very lengthy and costly process. The cost of developing a new drug and bringing it to the market is between \$800 million and \$2 billion, and can take up to 15 years. In part, termination of the development process is due to failure at late preclinical stages of development at great expenditure [1]. The drug discovery and development process for new drugs consists of four phases; drug discovery, preclinical development, clinical development and regulatory approval. Most drugs

fail at phase II and phase III clinical stages due to poor efficacy and safety issues [2]. The high attenuation rates in drug discovery suggest that the main reasons for drug failure are inappropriate preclinical testing methods and *in vitro* models, which do not sufficiently produce information needed for prediction of drug efficacy and safety issues [3]. Hence, one of the main areas expected to improve the success rate of drug development process could be the use of new technologies in preclinical testing and *in vitro* models, in order to get better accurate data.

Cell-based assays are crucial in the drug discovery and development process. Mammalian cell culture provides a defined platform for investigating cell and tissue physiology and pathophysiology outside of the organism. For over a century, traditional 2D cell culture was used in drug discovery. In 2D cell culture, cells are grown on flat dishes optimized for cell attachment and growth (**Figure 1**). Nowadays, 2D cell culture models are still used to test cellular drug responses to drug candidates. Although 2D cell culture is generally accepted and has increased understanding of drug mechanisms of action, there are limitations associated with it. The main limitation is that the cells grown as a monolayer on flat petri plates or flasks. This is a stiff platform, offering unnatural growth kinetics and cell attachments. Therefore, natural microenvironments of the cells are not fully represented [4]. Recently, significant work by researchers produced improvements in the form of better *in vitro* cell culture models that resemble *in vivo* conditions. Three-dimensional cell cultures are such products and better mimic tissue physiology in multicellular organisms (**Figure 1**) [5].

While traditional monolayer cultures still are predominant in cellular assays used for high-throughput screening (HTS), 3D cell cultures techniques for applications in drug discovery are making rapid progress [6, 7]. In this chapter, we provide an overview of 2D and 3D cell culture techniques, and their role in the discovery of new drugs.

Figure 1. Simplified sketch of 2D and 3D cell culture.

2. Cell culture system

Cell culture involves the dispersal of cells in an artificial environment that is composed of an appropriate surface, nutrient supply, and optimal conditions of humidity, temperature and gaseous atmosphere [6]. Usually cells are grown for days or weeks in a sterile 37°C humidified incubator with 5% CO₂ until a sufficient number of cells are reached. This system allows the study of cellular response to different environmental cues such as physiological stimulants or agonists/antagonists, potential drugs or pathogens.

2.1. Two-dimensional (2D) cell culture system

Two-dimensional culture conditions vary widely for each cell type. Appropriate cell culture medium suitable for the growth of particular cells has to be used. Various laboratories use different recipes of cell culture media prepared in the laboratory or commercially produced. The commercially produced cell culture medium is obtained sterile and ready to use in liquid or powder form and is usually dissolved in sterile water. Most laboratories obtain commercial components, which are mixed in the lab to make a complete culture medium for optimal cell growth. In addition, the culture media are usually supplemented with antibiotics and/or fungicides to inhibit contamination (**Table 1**).

Many continuous mammalian cell lines can be maintained on a relatively simple medium such as MEM supplemented with serum and antibiotics. However, most laboratories use DMEM as mammalian cells can be easily grown in DMEM supplemented with serum as well as antibiotics. When working with specialized cell types, a specialized cell culture medium may be required to maintain the growth of cells such as RPMI-1640 medium that is mostly used to grow cells in suspension such as HL-60 (promyelocytic leukemia) with varying serum amounts.

2.1.1. Sub-culturing cells

As cells reach confluency, they must be sub-cultured or passaged. The first step in sub-culturing adherent cells is to detach them from the cell culture plate or flask. This is done by subjecting them to trypsin-EDTA or by physically scraping them off the plate using a sterile cell scraper. One must take care because some mechanical and chemical methods have the

Adherent cells		Non-adherent cell lines	
	Cancer cell lines	Non-cancerous cell lines	
Cell culture medium	89% DMEM or MEM with high glucose, L-glutamine + 10% FBS + 1% penicillin/streptomycin	89% DMEM or MEM with medium/low glucose, L-glutamine + 10% FBS + 1% penicillin/streptomycin	89% RPMI-1640 + 10% FBS + 1% penicillin/streptomycin

DMEM, Dulbecco's Modified Eagles Medium; MEM, minimum essential medium; RPMI, Roswell Park Memorial Institute; FBS, fetal bovine serum.

Table 1. Common 2D cell culture media recipes.

potential to damage the cellular structure and possibly kill cells. Once detached, pre-warmed medium is added to stop the activity of trypsin-EDTA or to dilute the cell suspension. Varying amounts of the cell suspension are then transferred into fresh culture vessels and the appropriated amount of pre-warmed medium added and further incubated in 37°C incubator with humidified atmosphere of 5% CO₂.

2.1.2. Two-dimensional cell cultures in drug discovery and development

Many types of *in vitro* assays are performed in Drug Discovery and Development Research (DDDR), however, use of cell cultures receives extensive use. For example, determination of drug absorption, distribution, metabolism, excretion and toxicity (ADMETox) or drug pharmacokinetics is initially assessed in *in vitro* experiments involving cell cultures. Various cell lines in 2D cultures are used to determine different aspects of ADMETox. For instance, the Human colon carcinoma cells (Caco-2) are commonly used to determine absorption of drug candidates. Cultured Caco-2 cells form tight junctions in a monolayer and mimic intestinal epithelium. Additionally, Caco-2 cells express proteins that are involved in drug transport making them a good model for testing drug absorption [8]. Another cell line commonly used to test absorption is the Madin-Darby canine kidney (MDCK-MDR1) cell line, which mimics efflux activity of P-glycoprotein and allows faster performance of transport assays [9]. Hepatic metabolism plays a critical role in the removal of xenobiotics. Hepatocytes are usually the best model to study drug metabolism [10]. Although immortalized hepatocyte cell lines such as HepG2 and HepaRG are used to test drug metabolism and excretion, freshly isolated hepatocytes are the best model as they exhibit complete expression of metabolic enzymes [10, 11].

Although 2D cell cultures are used widely in DDDR and play a big role in preclinical drug testing, data generated from their use often do not translate to what occurs *in vivo*. Nowadays, 3D cell cultures and co-cultures receive more attention as they exhibit protein expression patterns and intracellular junctions that are similar to *in vivo* states compared to classic monolayer cultures.

3. Three-dimensional cell culture system

Three-dimensional cell culture was developed to improve the structure of cells and physiological equivalence of *in vitro* experiments performed. It refers to the culture of living cells inside micro assembled devices with a 3D structure mimicking tissue and organ specific microarchitecture [12]. In 3D cell culturing, growth of cells in their 3D physical shape allows better cell-to-cell contact and intercellular signaling networks [13]. The 3D environment also facilitates developmental processes allowing cells to differentiate into more complex structures [14].

3.1. Three-dimensional cell culture techniques

Three-dimensional cell culture techniques are classified as Scaffold-based or non-scaffold-based techniques. Researchers are required to select the most appropriate model for their cell-based assay.

3.1.1. Scaffold-based cell culture

Scaffold-based culture technologies give physical support to basic mechanical structures to extra-cellular matrix (ECM)-like matrices, on which cells can aggregate, proliferate and migrate [15]. In scaffold-based techniques, cells are implanted into the matrix and the chemical and physical properties of the scaffold material mold the characteristics of cell. The ultimate aim of a scaffold is to produce characteristics for the native cell function within the ECM. The 3D scaffold is usually biocompatible and it characterizes the shape and function of the assimilated cell structure [16]. The design of scaffold is based on the tissue of interest and the bigger or complex the scaffold is; the more difficult or harder the extraction of cells for analysis becomes [17]. Regardless of the tissue type, there are important factors to consider when designing the scaffold as described in **Table 2**.

Scaffolds are manufactured from natural and synthetic materials by a plethora of fabrication techniques. The main natural materials used for scaffold synthesis are different components of the ECM including fibrin, collagen and hyaluronic acid [22–24]. In addition, natural derived materials such as silk and gelatin may also be used [25]. Synthetic materials used for scaffold synthesis include polymers, titanium, bioactive glasses and peptides [26–28]. Polymers have been widely used as biomaterials for the fabrication of scaffolds, due to their unique properties such as high porosity, small pore size, high surface to volume ratio, biodegradation and mechanical properties [29, 30]. Scaffolds are designed to support cell adhesion, cell-biomaterial interactions, adequate transport of gases and nutrients for cell growth and survival and to avoid toxicity [31]. The fabrication technique for scaffold synthesis depends on the size and surface properties of the material and recommended role of the scaffold. The relevant fabrication techniques for a particular target tissue must be identified to facilitate proper cell distribution and guide their growth into 3D space. The various techniques for scaffolds fabrication are given in **Table 3**.

Scaffold-based 3D culture can be broadly divided into two approaches—hydrogels and solid-state scaffolds.

Property	Purpose	References
Biocompatibility	Ability to provide normal cellular function	[18]
Bioactivity	Ability to activate fast tissue attachment to the implant surface	[18]
Biodegradability	Allow cells to produce their own ECM	[19]
Mechanical response	Scaffold should be strong enough to allow surgical handling during implantation and must have enough mechanical integrity for the completion of the remodeling process	[20]
Scaffold architecture	Porous interconnected structure provide cellular penetration and adequate diffusion of nutrients to cells and mean pore size should large enough to allow cells to migrate into the structure	[21]

Table 2. Scaffold requirements.

Scaffold fabrication techniques	Advantages	References
Solvent casting/particulate leaching	Easy method, pore size can be controlled, desired crystallinity, highly porous structure	[32]
Melt molding	Able to construct scaffolds of any shape by changing the mold geometry, free of organic solvents, controlled pore size and porosity	[33]
Gas foaming	Controlled porosity and pore size, free of strong organic solvents	[34, 35]
Fiber bonding	Large surface area for cell attachment, interconnected fiber structure and high porosity	[36]
Freeze drying	High porosity and interconnectivity, controlled pore size, leaching step not required, work at low temperature	[37, 38]
Electrospinning	Controlled over porosity and pore size, produces ultra-thin fibers with special orientation and large surface area	[39, 40]
Fiber mesh	Variable pore size, large surface area for cell attachment	[41, 42]
Porogen leaching	High porosity, controlled pore size and geometry, bigger pore size and increased pore interconnectivity	[43, 44]
Micro molding	It is biologically degradable, mechanical and physical complexity	[45]

Table 3. The different scaffold fabrication techniques and their advantages.

3.1.1.1. *Hydrogel scaffolds*

Hydrogels are water swollen polymeric materials formed by chemical reactions of monomers that generate main-chain free radicals that make cross-link junctions or by hydrogen bonding [46]. Hydrogels are one of the most used scaffolds because they mimic the ECM to a certain extent [17]. Hydrogels are highly hydrated hydrophilic polymer networks with pores and void space between the polymers [47]. The hydrophilic structure facilitates absorption and retention of large quantities of water. It is regarded as a powerful method when applied for biomedical purposes [48]. Because hydrogels have properties such as soft and rubbery consistence, low surface tension and high water content, they are more suitable substitutes for natural tissues [49]. Sources of hydrogels can be natural, synthetic or a mixture of both (hybrid) materials, offering a broad spectrum of chemical and mechanical properties. The natural materials used for hydrogels are collagen, gelatin, alginate, fibrin, hyaluronic acid, agarose, chitosan and laminin [50–53]. Natural hydrogels confer adhesive properties, high cell viability, controlled proliferation and differentiation. Collagen is the most widely used natural polymer for hydrogel preparation and it is the main component of tissues such as ligament, bone, cartilage skin and tendon [54, 55].

Synthetic hydrogels can mimic biological properties of ECM and are ideal material to use for 3D scaffolds. They have well defined chemical, physical and mechanical properties to achieve stiffness and porosity [56]. The main synthetic materials used to formulate hydrogels are polyacrylic acid, polyethylene glycol (PEG), polyvinyl alcohol, polyglycolic acid (PGA) and poly (2-hydroxy ethyl methacrylate [57–60]. Synthetic hydrogels are the most used hydrogels because of their longer service life, high gel strength and water absorption capacity [61]. PEG and its derivatives are used mainly for synthetic hydrogels [62].

3.1.1.2. Solid state scaffolds

Culturing cells into a solid scaffold provides 3D space and helps generate natural 3D tissue-like structures. Solid scaffolds for 3D culture can be designed with different materials such as ceramics, metals, glass and polymers. Polymers are mainly used to construct solid scaffolds of different sizes, varying shapes, porosity, stiffness and permeability [63]. The main advantage of solid scaffolds is their ability to create organized positioning of cells *in vitro* in a controllable and reproducible manner [64]. The cell adhesion, growth and behavior in solid scaffold significantly depends on factors such as scale and topography of the internal structure, material used for its construction, the surface chemical properties, permeability and mechanical properties [65]. Solid scaffolds are commercially available, and are distributed sterile and ready to use. One of the main solid scaffolds is described below. An example is the porous scaffold. Porous scaffold creates a 3D microenvironment for cells to enter and maintain their natural 3D structure. It has a homogenous interconnected pore network, allowing cells to interact effectively to create tissue like structures and provides improved nutrient supply to the center of the device [64]. Sponge or foam porous scaffold have been especially used for bone regrowth and organ vascularization. Porous scaffold can be synthesized with specific porosity, pore size, crystallinity and surface area to volume ratio [66]. Synthetic biodegradable polymers such as polylactic-co-glycolic acid (PLGA), polyether ester (PEE), poly-L-lactic acid (PLLA) and PGA are the main materials used for porous scaffolding [67].

3.1.2. Scaffold-free 3D cultures

3.1.2.1. Scaffold-free 3D spheroid cultures

Scaffold-free-based 3D systems facilitate the development of multi-cellular aggregates, commonly known as spheroids, and can be generated from wide range of cell types [68]. Common examples of spheroids comprise tumor spheroids, embryonic bodies, mammospheres, neurospheres and hepatospheres. A cellular spheroid 3D model has a variety of properties such as (i) naturally mimicking/imitating various aspects of solid tissues; (ii) establishing geometry and ideal physiological cell-to-cell interactions; (iii) cells form their own ECM components and better cell-ECM interactions; (iv) excellent gradient for efficient diffusion growth factors as well as the (v) removal of metabolic waste [69]. The size of the spheroid can be based on the primary number cells seeded and it can increase in size where until they show oxygen and nutrient gradients similar to target tissue [70]. Spheroids are either self-assembling or are forced to grow as cell clusters [71]. Spheroids can be easily analyzed by imaging using light fluorescence, and confocal microscopy and that is an added advantage of spheroids compared to other 3D models. There are different approaches for facilitating spheroid cultures as described below.

Hanging drop method co-culture used to generate tissue-like cellular aggregates for molecular and biochemical analysis in a physiological suitable model. The hanging drop method was first developed in 1994 and became the basis of the non-scaffold method for the formation of multicellular spheroids. In hanging drop method, cells are cultured in a drop of media suspended on the lid of a cell culture dish, which is carefully inverted and placed on top of the

dish containing media to maintain a humid atmosphere. Suspended cells then come together and form 3D spheroids at the apex of the droplet of media [72, 73]. This method has many advantages such as cost effectiveness, controlled spheroid size, and various cell types can be co-cultured and produced into spheroids [74, 75]. Moreover, it has been reported that 3D cell culture generated with hanging drop method have 100% reproducibility [69]. Due to limited volume of droplets generated with this technique, it is difficult to maintain spheroids and change the medium. Presently, there are many commercial devices for hanging drop culture (Figure 2).

The use of low adhesion plates helps to promote self-aggregation of cells into spheroids [76]. Low adhesion plates have been developed as the commercial product of the liquid overlay technique, which is a low cost highly reproducible culture method that easily promotes 3D aggregates or spheroids [77]. Low adhesion plates are spheroid microplates with round, V-shaped bottoms and very low attachment surfaces to generate self-aggregation and spheroid formation. Plates are designed with hydrophilic or hydrophobic coating, which reduces cell from attaching to the surface. The main advantage of low adhesion plates is the potential to produce one spheroid per well making it appropriate for medium-throughput screening,

Figure 2. (a) A schematic of the hanging drop plate and (b) Schematic of spheroid formation techniques for hanging drop spheroids.

as well as creating defined geometry suitable for multicellular culture [78]. These plates have initial higher volume capacity than hanging droplets and there is no need to manipulate the spheroids.

Spheroids can also be cultured by using bioreactors under specific dynamic conditions [79]. The dynamic conditions are generated by stirring or rotating using spinner flask or NASA (National Aeronautics and Space Administration) rotating wall vessel, respectively [80]. The rotating wall vessel produces larger sized spheroids than spinner flask [81]. Bioreactors provide greater spheroid production control and reproducibility [82]. However, production of spheroids through this method requires expensive instruments and high quality cell culture medium.

3.1.2.2. Scaffold-free organoid cultures

Organoids are *in vitro* derived 3D cell aggregates that are capable of self-renewal, self-organization, and exhibit organ functionality [83]. Organoids are produced either from stem cells or primary tissues by providing suitable physical (support for cell attachment and survival) and biochemical (modulate signaling pathways) cues [84]. Organoids are classified into tissue organoids and stem cell organoids, based on how the organ buds are created [85]. Distinctive examples of tissue organoids culture are intestine, prostate, mammary and salivary glands. Stem cell organoids are created from either embryonic stem cells or primary stem cells (neonatal tissue) or induced pluripotent cells. Presently, different *in vitro* organoids have been set to simulate numerous tissues such as functional organoids for pancreas [86], liver [85], intestine [87], kidney [88], lung [89], retina [90], stomach [91] and thyroid [92]. Organoids mimic some of the structure and function of real organs [83]. Several approaches have been used to obtain organoids. The first approach is to culture cells as a monolayer on an ECM coated surface; organoids are then produced after the cells differentiate. The second is a mechanically supported cell culture to provide further differentiation of primary tissues. The third approach is to produce embryoid bodies through hang drop culture or on the low adhesion plates [93]. The main disadvantages of organoids are the lack of vasculature, lack of key cell types found *in vivo* and some organoids only replicate early stages of organ development [83].

3.2. Three-dimensional cell culture in drug discovery and development

Cell-based assays are the major tool used to evaluate the potency of a new compound in drug discovery. Three dimensional cell culture technologies have been used in different stages of drug discovery including diseases modeling, target identification and validation, screening, target selection, potency profiling and toxicity assessment. **Table 4** indicates the 3D models used in different stages of drug discovery. Three-dimensional culture models behave similarly to the cells *in vivo*, and are therefore used in the early stage of the drug discovery process, especially in cytotoxicity tests [94] such as MTT, Flow Cytometry and so on. The most effective cell-based assays with 3D cultures are cell viability, proliferation, signaling and migration [95]. It is now broadly accepted that cells act differently in 3D environments compared to 2D ones, especially when it comes to drug discovery—many prospective cancer therapeutics look favorable in the 2D cell culture dish, but fall painfully later on in clinical development.

Drug discovery stages	3D model	References
Disease modeling	Spheroids	[104, 105]
	Organoids	[106, 107]
Target identification	Spheroids	[108]
	Organoids	
Screening	Spheroids	[109–111]
Efficacy profiling	Spheroids	[112]
Toxicity profiling	Spheroids	[114, 115]
	Organoids	[113, 114]

Table 4. Three-dimensional culture techniques used in different stages of drug discovery.

Three-dimensional cell cultures promise to bridge the gap between traditional 2D cell culture and *in vivo* animal models. Studies have shown that cellular response to drug treatment in 3D cell culture are more similar to what occurs *in vivo* compared to 2D cell culture [96–98]. In addition, a number of studies show that cells cultured in 3D models are more resistant to anticancer drugs than those in 2D cultures [99, 100]. For example, the cell viability of ovarian cancer cells in 3D spheroid cell cultures after paclitaxel treatment was reduced by 40%, while the same treatment led to 80% reduced cell viability in 2D cell cultures [101]. The stronger drug resistance in 3D culture can be attributed to different factors including, phenotype and genotype changes [100], signals from cellular interactions between cells and ECM [102], activation of genes involved in cell survival and drug sensitivity due to limited diffusion through the spheroid [103].

Spheroid 3D cell cultures have been used for modeling the microenvironments, signaling, invasion and immune characteristics of cancer, also for studying cancer stem cells [104]. Studies have shown that cancer cell line spheroids have been used to analyze different characteristics of the cancer invasion process such as endothelial cell to tumor cell contact [116] and invasion of cells in a spheroid into the nearby 3D ECM structure [117]. Additionally, organoid cell cultures have been used to model number of diseases infectious diseases, neurodevelopmental and neuronal degeneration disorders [83]. For example, intestinal organoids were used to investigate genetically reconstituted tumorigenesis [118], gastro-intestinal infection with rotavirus [119], *Cryptosporidium parvum* infection [106], and colon cancer stem cell biology [107]. A large number of genetic disorders that have not been possible to model in animals can be modeled using organoid 3D cultures. For example, intestinal organoids derived from patient biopsies have been used to understand onset and progression of genetic disorders [120, 121]. Organoid 3D culture model is also a powerful tool for modeling neurodevelopmental disorders such as microencephaly, caused by Zika virus infection at early stages of brain development. Moreover, brain organoid model of neural stem cells was used to understand implications of Zika virus infection during neurogenesis [122]. These are some examples of uses of 3D cell cultures as models to study disease.

Gene expression patterns seen in 3D systems are more similar to *in vivo* conditions compared to 2D cell culture systems [123]. For instance, analysis of gene expression in mesothelioma cell lines cultured in spheroids shows the basic cause of chemoresistance in malignant mesothelioma [108]. In addition, cancer cell lines grown in 2D and 3D models show different gene expression levels of various genes responsible for proliferation, chemo sensitivity, angiogenesis and invasion [63]. Ovarian cells grown in 3D system shown higher level of gene expression of the cell receptors integrins compared to 2D cell culture [99]. Moreover, 3D cell cultures are cost effective and time saving for drug screening because they decrease drug trial time whilst generating accurate representation of *in vivo* conditions [6]. Screening using cell-based assays has been the initial point for identifying the potential compounds in the early stage of drug discovery. Most 3D cell culture models, together with HTS and HCS (high-content screening) processes shows promise in identifying clinically relevant compounds.

Characteristics	2D cell culture	3D cell culture	References
Morphology	Cells grow on a flat surface and have flat or stretched shape	Cells grow naturally into 3D aggregates/ spheroids in a 3D environment and natural shape retained	[126]
Cell shape	Single layer	Multiple layers	[6]
Cell to cell contact	Limited cell to cell contact, only on edges	Physiologic cell to cell contact similar to <i>in vivo</i>	[127]
Distribution of medium	Cells receive an equal amount of nutrients and growth factors from the medium during growth.	Cells do not receive an equal medium during growth. The core cell receive less growth factors and nutrients from the medium and tend to be in a hypoxic state, which is very similar to <i>in vivo</i> tissues, especially in tumors	[115, 127]
Cell proliferation	Generally, cells proliferate at a fast rate than <i>in vivo</i>	Cells proliferate faster or slower depending on the type of cell or 3D system used	[128–130]
Protein/gene expression	Protein and gene expression profiles differ compared with <i>in vivo</i> models	Protein and gene expression profiles more similar to <i>in vivo</i> models	[131]
Cell differentiation	Moderately differentiated	Properly differentiated	[132]
Response to stimuli	Poor response to mechanical stimuli of cells	Good response to mechanical stimuli of cells	[133]
Viability	Sensitive to cytotoxin	Greater viability and less susceptible to external factors	[134]
Drug sensitivity	Cells are more sensitive to drugs and drug show high efficacy	Cells are more resistant to drugs and drug show low potency	[135]
Cell Stiffness	High stiffness	Low stiffness	[105]
Sub-culturing time	Allows cell to be grown in culture for up to 1 week	Allows cells to be grown in culture for almost 4 weeks	[136]

Table 5. Characteristics of 3D cell culture versus 2D cell culture.

Three-dimensional cell culture models have been shown to be more accurate in assessing drug screening, selection and efficacy than 2D models of the diseases [115, 124]. For instance, spheroids obtained from patients were used to identify an effective therapy for 120 patients with HER2-negative breast cancer of all stages. The results indicated that spheroid 3D culture models display present guideline treatment recommendation for breast cancer [113]. In addition, 3D cell culture models are very powerful in analyzing drug induced toxicity. Organ buds of heart, liver, brain and kidney can be used to identify drug toxicity [83]. For instance, liver cell spheroid 3D culture used for investigating drug induced liver injury, function and diseases. Spheroids generated from human primary hepatocyte found to be phenotypically stable and retained morphology and viability for almost 5 weeks, providing toxicity analysis of drug molecules [115]. Liver spheroids and organoids also have been used to understand the metabolism of drug molecules.

However, many challenges remain in 3D cell culture technologies in the drug discovery process. Three-dimensional culture are different in terms of size, morphology, complexity and protocol for assaying compared to 2D cell culture, which can lead to challenges in systematic assessment, culture and assay protocol standardization. It also has complexity of identifying specific phenotypes for drug screening [125]. Moreover, some 3D models have limited permeability, which can impact cell viability and functions thus making it difficult to have accurate automated system for HTS. A summary of the differences between 2D and 3D cell cultures is given in **Table 5**.

4. Conclusion

Two-dimensional and 3D cell culture models have been widely used for improving the productivity of pharmaceutical research and development. It is evident that 3D culture systems hold great potential as a tool for drug discovery compared to 2D cell culture. This is due to the improved cell-cell and cell-ECM interactions, cell populations and structures that similar to *in vivo*. However, there are still hurdles to overcome before 3D systems can be widely used in industry. More studies are needed to promise reproducibility, high throughput analysis and compatibility to demonstrate standardized and validated 3D culture models. In future, development of screening compatible 3D models would help to identify early physiological relevant efficacy and toxicity data in drug discovery.

List of abbreviations

ADMETox	absorption, distribution, metabolism, excretion and toxicity
CaCo-2	human colon carcinoma
CO ₂	carbon dioxide
DDDR	drug discovery and development research

DMEM	Dulbecco's Modified Eagle Medium
ECM	extracellular matrix
EDTA	ethylenediaminetetraacetic acid
HCS	high-content screening
HEP-G2	liver hepatocellular carcinoma
HER-2	human epidermal growth factor receptor 2
HTS	high-throughput screening
MDCK-MDR1	Madin-Darby canine kidney cells
MEM	minimum essential medium
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
PEE	polyether ester
PEG	polyethylene glycol
PGA	polyglycolic acid
PLGA	polylactic-co-glycolic acid
PLLA	poly-L-lactic acid
RMPI	Roswell Park Memorial Institute medium
2D	two dimensional
3D	three dimensional

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References

- [1] Kola I, Landis J. Can the pharmaceutical industry reduce attrition rates? *Nature Reviews Drug Discovery*. 2004;**3**:711-715
- [2] Arrowsmith J, Miller P. Trial watch: Phase II and phase III attrition rates 2011-2012. *Nature Reviews. Drug Discovery*. 2013;**12**:569

- [3] Kim JB. Three-dimensional tissue culture models in cancer biology. *Seminars in Cancer Biology*. 2005;**15**:365-377
- [4] Cukierman E, Pankov R, Stevens DR, et al. Taking cell-matrix adhesions to the third dimension. *Science*. 2001;**294**:1708-1712
- [5] Pampaloni F, Reynaud EG, Stelzer EH. The third dimension bridges the gap between cell culture and live tissue. *Nature Reviews. Molecular Cell Biology*. 2007;**8**:839-845
- [6] Edmondson R, Broglie JJ, Adcock AF, Yang L. Three-dimensional cell culture systems and their applications in drug discovery and cell-based biosensors. *Assay and Drug Development Technologies*. 2014;**12**:207-218
- [7] Montanez-Sauri SI, Beebe DJ, Sung KE. Microscale screening systems for 3D cellular microenvironments: Platforms, advances, and challenges. *Cellular and Molecular Life Sciences*. 2015;**72**:237-249
- [8] Cai Y, Xu C, Chen P, Hu J, Hu R, Huang M, et al. Development, validation, and applications of a novel 7-day Caco-2 culture system. *Journal of Pharmacological and Toxicological Methods*. 2014;**70**(2):175-181
- [9] Jin X, Loung LT, Reese N, Gaona H, Collazo-Velez V, Vuong C, et al. Comparison of MDCK-MDR1 and Caco-2 cell based permeability assays for anti-malarial drug screening and drug investigations. *Journal of Pharmacological and Toxicological Methods*. 2014;**70**(2):188-194
- [10] Berry MN, Grivell AR, Grivell MB, Phillips JW. Isolated hepatocytes-past present and future. *Cell Biology and Toxicology*. 1997;**13**:223-233
- [11] Schaeffner I, Petters J, Aurich H, Froberg P, Christ B. A microtiterplate-based screening assay to assess diverse effects on cytochrome P450 enzyme activities in primary rat hepatocytes by various compounds. *Assay and Drug Development Technologies*. 2005;**3**:27-38
- [12] Dongeum H, Geraldine AH, Donald EI. From three dimensional cell culture to organ on chips. *Trends in Cell Biology*. 2011;**21**(12):745-754
- [13] Abbott. Cell culture biology's new dimensions. *Nature*. 2003;**424**:870-872
- [14] Cukierman E, Pankov R, Yamada KM. Cell interactions with three-dimensional matrices. *Current Opinion in Cell Biology*. 2002;**14**:633-639
- [15] Freed GV, Biron RJ, et al. Biodegradable polymer scaffolds for tissue engineering. *Biotechnology*. 1994;**12**(7):689-693
- [16] Place E, George J, Williams C, Stevens M. Synthetic polymer scaffolds for tissue engineering. *Chemical Society Reviews*. 2009;**38**:1139-1151
- [17] 3D cell culture 101: An introduction to 3D cell culture to tools and techniques 3 d biomatrix-white-paper-3d-cell-culture-101. *Science*. 2016
- [18] O'Brien FJ. Biomaterials & scaffolds for tissue engineering. *Materials Today*. 2011;**14**:88-95

- [19] Mikos AG, McIntire LV, Anderson JM, Babensee JE. Host response to tissue engineered devices. *Advanced Drug Delivery Reviews*. 1998;**33**(1-2):111-139
- [20] Dietmar WH. Scaffolds in tissue engineering bone and cartilage. *Biomaterials*. 2000;**21**: 2529-2543
- [21] Henry CK, Bruce KM, Clive DM, et al. Engineering thick tissues—The vascularisation problem. *European Cells and Materials*. 2007;**14**:1-19
- [22] Baharvand H, Hashemi SM, Ashtian SK, et al. Differentiation of human embryonic stem cells into hepatocytes in 2D and 3D culture systems in vitro. *The International Journal of Developmental Biology*. 2006;**50**:645-652
- [23] Willerth SM, Arendas KJ, Gottlieb DI, et al. Optimization of fibrin scaffolds for differentiation of murine embryonic stem cells into neural lineage cells. *Biomaterials*. 2006;**27**:5990-6003
- [24] Gerecht S, Burdick JA, Ferreira LS, et al. Hyaluronic acid hydrogel for controlled self-renewal and differentiation of human embryonic stem cells. *Proceedings of the National Academy of Sciences*. 2007;**104**:11298-11303
- [25] Awad HA, Wickham MQ, Leddy HA, et al. Chondrogenic differentiation of adipose-derived adult stem cells in agarose, alginate, and gelatin scaffolds. *Biomaterials*. 2004;**25**:3211-3222
- [26] Gunatillake PA, Adhikari R. Biodegradable synthetic polymers for tissue engineering. *European Cells & Materials*. 2003;**5**:1-16
- [27] Van den Dolder J, Spauwen PHM, Jansen JA. Evaluation of various seeding techniques for culturing osteogenic cells on titanium fiber mesh. *Tissue Engineering*. 2003;**9**:315-325
- [28] Lu HH, El-Amin SF, Scott KD, et al. Three-dimensional, bioactive, biodegradable, polymer-bioactive glass composite scaffolds with improved mechanical properties support collagen synthesis and mineralization of human osteoblast-like cells in vitro. *Journal of Biomedical Materials Research. Part A*. 2003;**64A**:465-474
- [29] Ji K, Ghosh XZ, et al. Electrospun three-dimensional hyaluronic acid nanofibrous scaffolds. *Biomaterials*. 2006;**27**:3782-3792
- [30] Piskin E. Biodegradable polymers as biomaterials. *Journal of Biomaterials Science Polymer Edition*. 1994;**6**:775-795
- [31] Langer R, Tirrell. Designing a material and biological and medicine. *Nature*. 2004;**428**(6982): 487-792
- [32] Xiang Z, Liao R, Kelly MS, Spector M. Collagen–GAG scaffolds grafted onto myocardial infarcts in a rat model: A delivery vehicle for mesenchymal stem cells. *Journal of Tissue Engineering*. 2006;**12**:2467-2478
- [33] Thompson RC, Wake MC, Yaszemski, Mikos AG. Biodegradable polymer scaffolds to regenerate organs. *Advances in Polymer Science*. 1995;**122**:245-274
- [34] Ikada Y. Scope of tissue engineering. In: *Tissue Engineering: Fundamental and Applications*. Elsevier/Science Direct. 2006. eBook ISBN: 9780080464008

- [35] Mooney DJ, Baldwin DF, Suh NP, Vacanti JP, Langer R. Novel approach to fabricate porous sponges of poly(D,L-lactic-co-glycolic acid) without the use of organic solvents. *Biomaterials*. 1996;**17**(14):1417-1422
- [36] Moroni L, Hamann D, Paoluzzi L, Pieper J, de Wijn JR, van Blitterswijk CA. Regenerating articular tissue by converging technologies. *PLoS One*. 2008;**3**(8):e3032
- [37] Schoof H, Apel J, Heschel I, Rau G. Control of pore structure and size in freeze-dried collagen sponges. *Journal of Biomedical Materials Research*. 2001;**58**:352-357
- [38] Mandal BB, Kundu SC. Cell proliferation and migration in silk fibroin 3D scaffolds. *Biomaterials*. 2009;**30**:2956-2965
- [39] Liang D, Hsiao BS, Chu B. Functional electrospun nanofibrous scaffolds for biomedical applications. *Advanced Drug Delivery Reviews*. 2007;**59**:1392-1412
- [40] Li WJ, Tuan RS. Fabrication and application of nanofibrous scaffolds in tissue engineering. *Current Protocols in Cell Biology*. 2009;**25**:Unit 25.2
- [41] Chen G, Ushida T, Tateishi T. Development of biodegradable porous scaffolds for tissue engineering. *Materials Science and Engineering C*. 2002;**17**:63-69
- [42] Martins AM, Pham QP, Malafaya PB, et al. The role of lipase and alphaamylase in the degradation of starch/poly(varepsilon-caprolactone) fiber meshes and the osteogenic differentiation of cultured marrow stromal cells. *Tissue Engineering. Part A*. 2009;**15**(2):295-305
- [43] Mikos AG, Sarakinos G, Leite SM, Vacanti JP, Langer R. Laminated three-dimensional biodegradable foams for use in tissue engineering. *Biomaterials*. 1993;**14**:323-330
- [44] Mano JF, Silva GA, Azevedo HS, et al. Natural origin biodegradable systems in tissue engineering and regenerative medicine: Present status and some moving trends. *Journal of the Royal Society Interface*. 2007;**4**:999-1030
- [45] Fukuda J, Khademhosseini Y, et al. Micromolding of photocrosslinkable chitosan hydrogel for spheroid microarray and co-culture. *Biomaterials*. 2006;**79**:522-532
- [46] Yan C, Pochan DJ. Rheological properties of peptide-based hydrogels for biomedical and other applications. *Chemical Society Reviews*. 2010;**39**:3528-3540
- [47] Daniele MA, Adams AA, Naciri J, North SH, Ligler FS. Interpenetrating networks based on gelatin methacrylamide and PEG formed using concurrent thiol click chemistries for hydrogel tissue engineering scaffolds. *Biomaterials*. 2014;**35**:1845-1856
- [48] Wang T, Jiao Y, Chai Q, Yu X. Gold nanoparticles: Synthesis and biological applications. *Nano LIFE*. 2015;**5**:1542007
- [49] Hamidi M, Azadi A, Rafiei P. Hydrogel nanoparticles in drug delivery. *Advanced Drug Delivery Reviews*. 2008;**60**:1638-1649
- [50] Huh D, Hamilton GA, Ingber DE. From 3D cell culture to organs-on-chips. *Trends in Cell Biology*. 2011;**21**(12):745-754

- [51] Ehrbar M, Djonov V, Schnell C, et al. Cell-demanded liberation of VEGF121 from fibrin implants induces local and controlled blood vessel growth. *Circulation Research*. 2004;**94**: 1124-1132
- [52] Ahmed T, Dare E, Hincke M. Fibrin: A versatile scaffold for tissue engineering applications. *Tissue Engineering. Part B, Reviews*. 2008;**14**:199-215
- [53] Allison D, Grande-Allen K. Review. Hyaluronan: A powerful tissue engineering tool. *Tissue Engineering*. 2006;**12**:2131-2140
- [54] Glowacki J, Mizuno S. Collagen scaffolds for tissue engineering. *Biopolymers*. 2008;**89**: 338-344
- [55] Pathak A, Kumar S. Biophysical regulation of tumor cell invasion: Moving beyond matrix stiffness. *Integrative Biology*. 2011;**3**:267-278
- [56] Zhang YS, Khademhosseini A. Advances in engineering hydrogels. *Science*. 2017;**356**: eaaf3627
- [57] Sawhney AS, Pathak CP, Hubbell JA. Bioerodible hydrogels based on photopolymerized poly(ethylene glycol)-co-poly(α -hydroxy acid) diacrylate macromers. *Macromolecules*. 1993;**26**(4):581-587
- [58] Martens P, Anseth KS. Characterization of hydrogels formed from acrylate modified poly(vinyl alcohol) macromers. *Polymer*. 2000;**41**(21):7715-7722
- [59] Chirila TV, Constable IJ, Crawford GJ, et al. Poly(2-hydroxyethyl methacrylate) sponges as implant materials: In vivo and in vitro evaluation of cellular invasion. *Biomaterials*. 1993;**14**(1):26-38
- [60] Raeber GP, Lutolf MP, Hubbell JA. Molecularly engineered PEG hydrogels: A novel model system for proteolytically mediated cell migration. *Biophysical Journal*. 2005;**89**: 1374-1388
- [61] Hoffman AS. Hydrogels for biomedical applications. *Advanced Drug Delivery Reviews*. 2012;**64**:18-23
- [62] Caiazzo M, Okawa Y, Ranga A, et al. Defined three-dimensional microenvironments boost induction of pluripotency. *Nature Materials*. 2016;**15**:344-352
- [63] Gurski L, Petrelli N, Jia X, Farach-Carson M. Three-dimensional matrices for anti-cancer drug testing and development. *Oncology Issues*. 2010;**25**:20-25
- [64] Knight E, Przyborski S. Advances in 3D cell culture technologies enabling tissue-like structures to be created in vitro. *Journal of Anatomy*. 2015;**227**(6):746-756
- [65] Haycock JW. 3D cell culture: A review of current approaches and techniques. *Methods in Molecular Biology*. 2011;**695**:1-15
- [66] Ourimechi EM, Vergnaud JM. Process of drug transfer with three different polymeric systems with transdermal drug delivery. *Computational and Theoretical Polymer Science*. 2000;**10**:391-401

- [67] Hou Q, Grijpma DW, Feijen J. Porous polymeric structures of tissue engineering prepared by a coagulation, compression, moulding and salt leaching technique. 2003;**24**:1937-1947
- [68] Sutherland RM. Cell and environment interactions in tumor microregions: The multicell spheroid model. *Science*. 1988;**240**:177-184
- [69] Breslin S, O'Driscoll L. Three-dimensional cell culture: The missing link in drug discovery. *Drug Discovery Today*. 2013;**18**:240-249
- [70] Ekert JE, Johnson K, Strake B, et al. Three-dimensional lung tumor microenvironment modulates therapeutic compound responsiveness in vitro—Implication for drug development. *PLoS One*. 2014;**9**:e92248
- [71] Yamada KM, Cukierman E. Modeling of tissue morphogenesis and cancer in 3D. *Cell*. 2007;**130**:601-610
- [72] Keller GM. In-vitro differentiation of embryonic stem-cells. *Current Opinion in Cell Biology*. 1995;**7**:862-869
- [73] Rimann M, Graf-Hausner U. Synthetic 3D multicellular systems for drug development. *Current Opinion in Biotechnology*. 2012;**23**:803-809
- [74] Pham P. Breast cancer stem cell culture and proliferation. In: *Breast Cancer Stem Cells & Therapy Resistance*. Cham: Springer International Publishing; 2015. pp. 41-55
- [75] Hsiao AY, Tung YC, Qu X, Patel LR, Pieta KJ, Takayama S. 384 hanging drop arrays give excellent Z-factors and allow versatile formation of co-culture spheroids. *Biotechnology and Bioengineering*. 2012;**109**:1293-1304
- [76] Vinci M, Gowan S, Boxall F, et al. Advances in establishment and analysis of three-dimensional tumor spheroid-based functional assays for target validation and drug evaluation. *BMC Biology*. 2012;**10**:29
- [77] Carlsson J, Yuhas JM. Liquid-overlay culture of cellular spheroids. Recent results in cancer research. *Cancer*. 1984;**95**:1-23
- [78] Thoma DS, Buranawat B, Hammerle CHF, Held U, Jung RE. Efficacy of soft tissue augmentation around dental implants and in partially edentulous areas: A systematic review. *Journal of Clinical Periodontology*. 2014;**41**:S77-S91
- [79] Yu X, Chen X, Chai Q, Ayres N. Synthesis of polymer organogelators using hydrogen bonding as physical cross-links. *Colloid & Polymer Science*. 2016;**294**:59-68
- [80] Shin CS, Han BKB, Park K, Panitch A. 3D cancer tumor models for evaluating chemotherapeutic efficacy. *Biomaterials for Cancer Therapeutics*. 2013:445-460
- [81] Lelkes PI, Galvan DL, Hayman GT, Goodwin TJ, et al. Simulated microgravity conditions enhance differentiation of cultured PC 12 cells towards the neuroendocrine phenotype. *In Vitro Cellular & Developmental Biology*. 1998;**34**(4):316-325
- [82] Ou KL, Hosseinkhani H. Development of 3D in vitro technology for medical applications. *International Journal of Molecular Sciences*. 2014;**15**:17938-17962

- [83] Lancaster MA, Knoblauch JA. Organogenesis in a dish: Modeling development and disease using organoid technologies. *Science*. 2014;**345**:124-125
- [84] Clevers H. Modeling development and diseases with organoids. *Cell*. 2016;**165**:1586-1597
- [85] Huch M, Dorrell C, Boj SF, et al. In vitro expansion of single Lgr5+ liver stem cells induced by Wnt-driven regeneration. *Nature*. 2013;**494**:247-250
- [86] Greggio C, De Franceschi F, Figueiredo-Larsen M, et al. Artificial three-dimensional niches deconstruct pancreas development in vitro. *Development*. 2013;**140**:4452-4462
- [87] Spence JR, Mayhew CN, Rankin SA, et al. Directed differentiation of human pluripotent stem cells into intestinal tissue in vitro. *Nature*. 2011;**470**:105-109
- [88] Takasato M, Er PX, Chiu HS, et al. Kidney organoids from human iPS cells contain multiple lineages and model human nephrogenesis. *Nature*. 2015;**526**:564-568
- [89] Lee JH, Bhang DH, Beede A, et al. Lung stem cell differentiation in mice directed by endothelial cells via a BMP4-NFATc1-thrombospondin-1 axis. *Cell*. 2014;**156**:440-455
- [90] Nakano T, Ando S, Takata, et al. Self-formation of optic cups and storable stratified neural retina from human ESCs. *Cell Stem Cell*. 2012;**10**:771-785
- [91] Barker N, Huch M, Kujala P, et al. Lgr5(+ve) stem cells drive self-renewal in the stomach and build long-lived gastric units in vitro. *Cell*. 2010;**6**:25-36
- [92] Antonica F, Kasprzyk DF, Opitz R, et al. Generation of functional thyroid from embryonic stem cells. *Nature*. 2012;**491**:66-71
- [93] Turner DA, Baillie-Johnson P, Arias AZ. Organoids and the genetically encoded self-assembly of embryonic stem cells. *BioEssays*. 2015;**38**:181-191
- [94] Karmen B, Marija T, Ivo Z, Dubravko J. Three-dimensional cell cultures as a new tool in drug discovery. *Periodicum Biologorum*. 2016;**118**:59-65
- [95] Comley J. 3D cell culture: Easier said than done! *Drug discovery world*, summer. 2010;**11**: 25-41
- [96] Lee J, Cuddihy MJ, Kotov NA. Three-dimensional cell culture matrices: State of the art. *Tissue Engineering. Part B, Reviews*. 2008;**14**:61-86
- [97] Zietarska M, Maugeri CM, Filali-Mouhim A, et al. Molecular description of a 3D in vitro model for the study of epithelial ovarian cancer (EOC). *Molecular Carcinogenesis*. 2007;**46**:872-885
- [98] Shield K, Ackland ML, Ahmed N, Rice GE. Multicellular spheroids in ovarian cancer metastases: Biology and pathology. *Gynecologic Oncology*. 2009;**113**:143-148
- [99] Loessner D, Stok KS, Lutolf MP, Huttmacher DW, Clements JA, Rizzi SC. Bioengineered 3D platform to explore cell-ECM interactions and drug resistance of epithelial ovarian cancer cells. *Biomaterials*. 2010;**31**:8494-8506

- [100] Karlsson H, Fryknäs M, Larsson R, et al. Loss of cancer drug activity in colon cancer HCT-116 cells during spheroid formation in a new 3-D spheroid cell culture system. *Experimental Cell Research*. 2012;**318**:1577-1585
- [101] Nguyen TA, Yin TI, Reyes D, Urban GA. Microfluidic chip with integrated electrical cell-impedance sensing for monitoring single cancer cell migration in three-dimensional matrixes. *Analytical Chemistry*. 2013;**85**:11068-11076
- [102] Walker DM, Boey G, McDonald LA. The pathology of oral cancer. *Pathology*. 2003;**35**:376-383
- [103] Trédan O, Galmarini CM, Patel K, Tannock IF. Drug resistance and the solid tumor microenvironment. *Journal of the National Cancer Institute*. 2007;**99**(19):1441-1454
- [104] Weiswald LB, Bellet D, Dangles-Marie V. Spherical cancer models in tumor biology. *Neoplasia*. 2015;**17**:1-15
- [105] Dieter SM, Ball CR, Hoffmann CM, et al. Distinct types of tumor-initiating cells form human colon cancer tumors and metastases. *Cell Stem Cell*. 2011;**9**:357-365
- [106] Castellanos-Gonzalez A, Cabada MM, Nichols J, et al. Human primary intestinal epithelial cells as an improved in vitro model for *Cryptosporidium parvum* infection. *Infection and Immunity*. 2013;**81**:1996-2001
- [107] Yeung TM, Gandhi SC, Wilding JL, et al. Cancer stem cells from colorectal cancer derived cell lines. *Proceedings of the National Academy of Sciences of the United States of America*. 2010;**107**:3722-3727
- [108] Barbone D, Van Dam L, Follo C, et al. Analysis of gene expression in 3D spheroids highlights a survival role for ASS1 in mesothelioma. *PLoS One*. 2016;**11**:e0150044
- [109] Drost J, van Jaarsveld RH, Ponsioen B, et al. Sequential cancer mutations in cultured human intestinal stem cells. *Nature*. 2015;**521**:43-47
- [110] Senkowski W, Zhang X, Olofsson MH, et al. Three-dimensional cell culture-based screening identifies the anthelmintic drug nitazoxanide as a candidate for treatment of colorectal Cancer. *Molecular Cancer Therapeutics*. 2015;**14**:1504-1516
- [111] Kenny HA, Lal-Nag M, White EA, et al. Quantitative high throughput screening using a primary human three-dimensional organotypic culture predicts In vivo efficacy. *Nature Communications*. 2015;**6**:6220
- [112] Tong JG, Valdes YR, Barrett JW, et al. Evidence for differential viral oncolytic efficacy in an In vitro model of epithelial ovarian cancer metastasis. *Molecular Therapy Oncolytics*. 2015;**2**:15013
- [113] Halfter K, Hoffmann O, Ditsch N, et al. Testing chemotherapy efficacy in HER2 negative breast cancer using patient-derived spheroids. *Journal of Translational Medicine*. 2016;**14**:112
- [114] Gunness P, Mueller D, Shevchenko V, et al. 3D organotypic cultures of human HepaRG cells: A tool for in vitro toxicity studies. *Toxicological Sciences*. 2013;**133**:67-78

- [115] Bell CC, Hendriks DF, Moro SM, et al. Characterization of primary human hepatocyte spheroids as a model system for drug-induced liver injury, liver function and disease. *Scientific Reports*. 2016;**6**:25187
- [116] Ghosh S, Joshi MB, Ivanov D, et al. Use of multicellular tumor spheroids to dissect endothelial cell–tumor cell interactions: A role for T-cadherin in tumor angiogenesis. *FEBS Letters*. 2007;**581**:4523-4528
- [117] Blacher S, Erpicum C, Lenoir R, et al. Cell invasion in the spheroid sprouting assay: A spatial organisation analysis adaptable to cell behaviour. *PLoS One*. 2004;**9**(5):e97019
- [118] Onuma K, Ochiai M, Orihashi K, et al. Genetic reconstitution of tumorigenesis in primary intestinal cells. *Proceedings of the National Academy of Sciences of the United States of America*. 2013;**110**:11127-11132
- [119] Finkbeinera SR, Zenga XL, Utamaa B, Atmara RB, Shroyerc NF, Mary K, et al. Stem cell-derived human intestinal organoids as an infection model for rotaviruses. *MBio*. 2012;**3**(4):e00159-e00112
- [120] Dekkers JF, Wiegerinck CL, de Jonge HR, et al. A functional CFTR assay using primary cystic fibrosis intestinal organoids. *Nature Medicine*. 2013;**19**:939-945
- [121] Bigorgne AE, Farin HF, Lemoine R, et al. TTC7A mutations disrupt intestinal epithelial apicobasal polarity. *The Journal of Clinical Investigation*. 2013;**124**:328-337
- [122] Garcez PP, Loiola EC, Madeiro da Costa R, et al. Zika virus impairs growth in human neurospheres and brain organoids. *Science*. 2016;**352**:3
- [123] Ghosh S, Spagnoli GC, Martin I, et al. Three dimensional culture of melanoma cells profoundly affects gene expression profile: A high density oligonucleotide array study. *Journal of Cellular Physiology*. 2005;**204**:52-531
- [124] Hickman JA, Graeser R, de Hoogt R, et al. IMI PREDECT consortium. Three-dimensional models of cancer for pharmacology and cancer cell biology: Capturing tumor complexity in vitro/ex vivo. *Biotechnology Journal*. 2014;**9**:1115-1128
- [125] Booi TH, Klop MJ, Yan K, et al. Development of a 3D tissue culture-based high-content screening platform that uses phenotypic profiling to discriminate selective inhibitors of receptor tyrosine kinases. *Journal of Biomolecular Screening*. 2016;**21**:912-922
- [126] Huang H, Ding Y, Sun XS, Nguyen TA. Peptide hydrogelation and cell encapsulation for 3D culture of MCF-7 breast cancer cells. *PLoS One*. 2013;**8**:59482
- [127] Li Z, Cui Z, et al. Three-dimensional perfused cell culture. *Biotechnology Advances*. 2014;**32**:243-254
- [128] Chitcholtan K, Sykes P, Evans J. The resistance of intracellular mediators to doxorubicin and cisplatin are distinct in 3D and 2D endometrial cancer. *Journal of Translational Medicine*. 2012;**10**:1-16
- [129] Fallica B, Mafia JS, Villa S, Makin G, Zaman M. Alteration of cellular behavior and response to PI3K pathway inhibition by culture in 3D collagen gels. *PLoS One*. 2012;**7**:48024

- [130] Luca AC, Mersch S, Deenen R, et al. Impact of the 3D microenvironment on phenotype, gene expression, and EGFR inhibition of colorectal cancer cell lines. *PLoS One*. 2013;**8**:e59689
- [131] Price KJ, Tsykin A, Giles KM, et al. Matrigel basement membrane matrix influences expression of microRNAs in cancer cell lines. *Biochemical and Biophysical Research Communications*. 2012;**427**:343-348
- [132] Chitcholtan K, Asselin E, Parent S, Sykes PH, Evans JJ. Differences in growth properties of endometrial cancer in three dimensional (3D) culture and 2D cell monolayer. *Experimental Cell Research*. 2013;**319**:75-78
- [133] Li Y, Huang G, Li M, et al. An approach to quantifying 3D responses of cells to extreme strain. *Scientific Reports*. 2016;**6**:19550
- [134] Elkayam T, Amitay-Shaprut S, Dvir-Ginzberg M, Harel T, Cohen S. Enhancing the drug metabolism activities of C3A-a human hepatocyte cell line--by tissue engineering within alginate scaffolds. *Tissue Engineering*. 2006;**12**:1357-1368
- [135] Bokhari M, Carnachan RJ, Cameron NR, Przyborsk SA. Culture of HepG2 liver cells on three dimensional polystyrene scaffolds enhances cell structure and function during toxicological challenge. *Journal of Anatomy*. 2007;**211**:567-576
- [136] Baker BM, Chen CS. Deconstructing the third dimension—How 3D culture microenvironments alter cellular cues. *Journal of Cell Science*. 2012;**125**:3015-3024