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Chapter 2

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

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Additional information is available at the end of the chapter

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

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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.

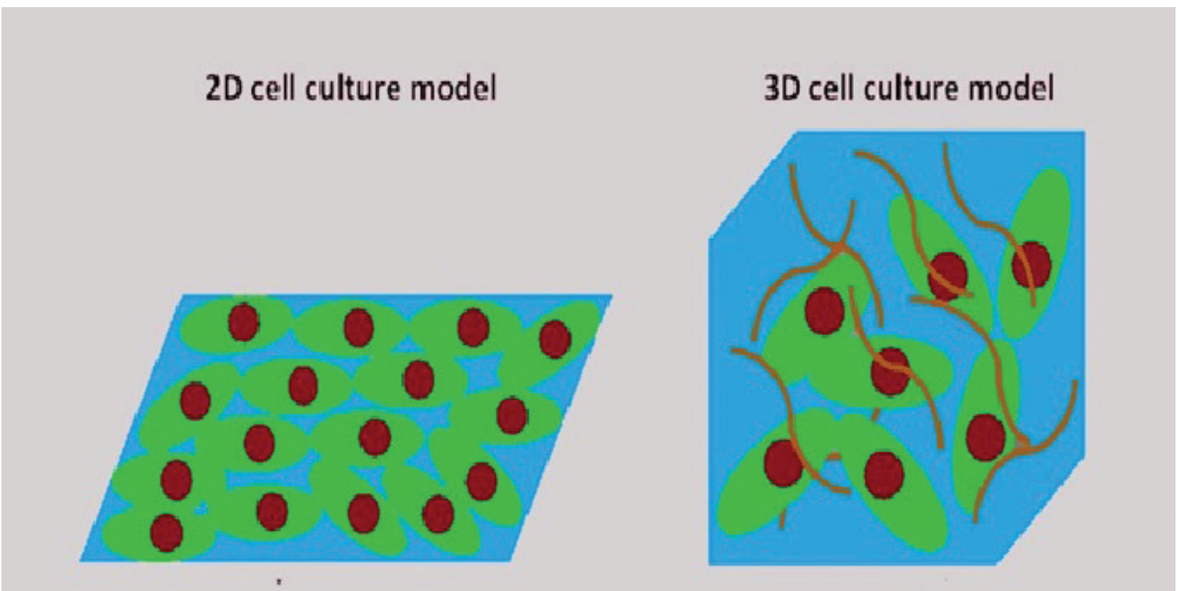


Figure 1. Simplified sketch of 2D and 3D cell culture.

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% CO_2 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% CO_2.

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 l 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,

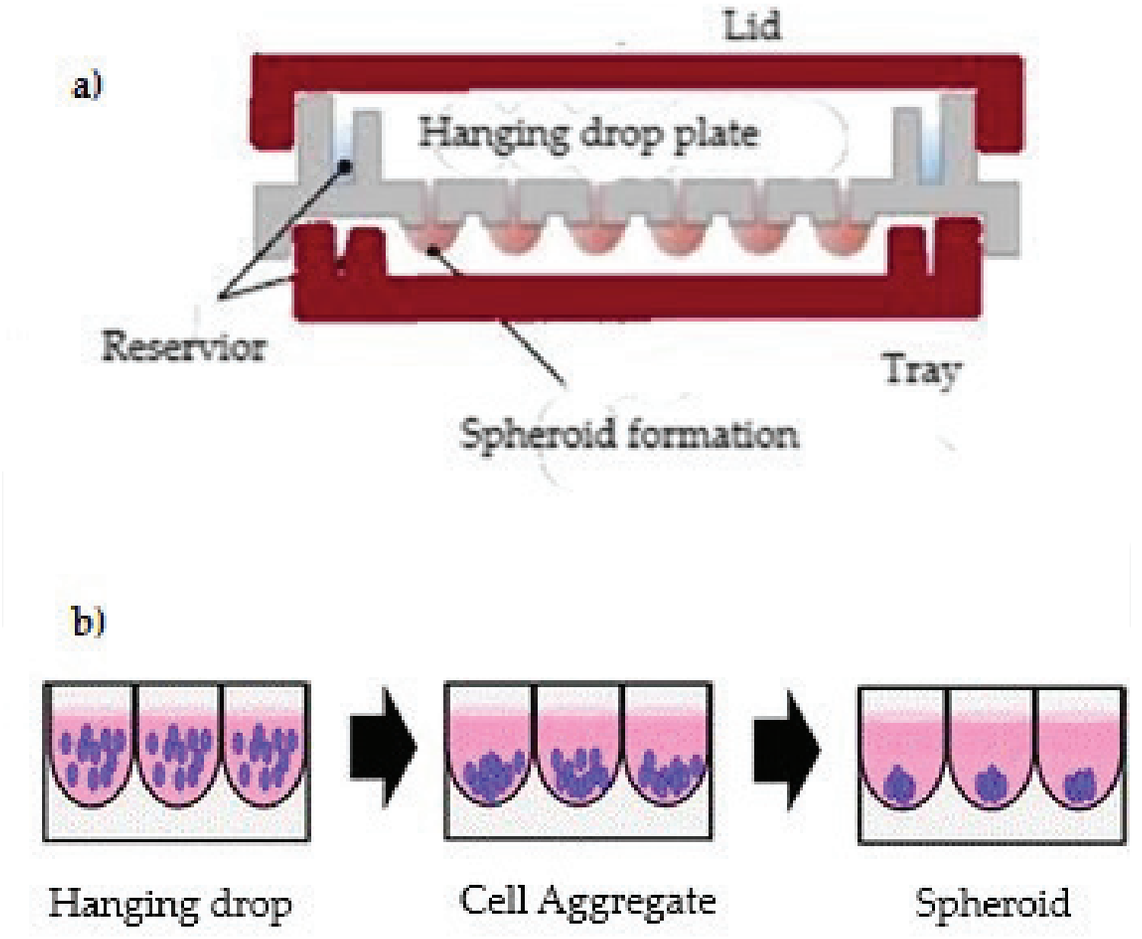


Figure 2. (a) A schematic of the hanging drop plate and (b) Schematic of spheroid formation techniques for hanging drop spheroids.

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], gastrointestinal 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 trail 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 *in vivo*	[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 *in vivo* tissues, especially in tumors	[115, 127]
Cell proliferation	Generally, cells proliferate at a fast rate than *in vivo*	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 *in vivo* models	Protein and gene expression profiles more similar to *in vivo* 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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