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Applications of Cell Culture Studies in Pharmaceutical Technology

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ABSTRACT

There have been advances in the cell culture models for research and drug studies. The cytotoxicity and permeability of drug molecules and delivery systems are evaluated by cell culture models both in the pharmaceutical industry and in academia. Cell models serve as an important platform to investigate cytotoxicity and permeability studies by reducing the use of animal models. Since 3D cell models mimic in vivo cells better, it plays a significant role in the testing of drugs. This review article emphasizes an overview of cytotoxicity and permeability studies and 3D cell culture model used in pharmaceutical technology.

Key words: cell culture, cytotoxicity, permeability, pharmaceutical technology

INTRODUCTION

Studies conducted during the initial development of drugs such as toxicity, corrosion and drug activity were carried out on animals; however, in the past 10 to 20 years, alternatives have been sought due to the fact that animals do not effectively model human in vivo conditions and unexpected responses are observed in the studies. Cell culture studies made positive contributions to the initial development of drugs. Contrary to animal studies, the need for low drug and a short response time are the characteristics for in vitro cell culture methods[1]. In 2005, more than 100 million animals were used and 10 billion dollars were spent for animal toxicity experiments[2]. It is possible to reduce this cost and the amount of animal use for experiments with well-designed cell culture studies[3].

Among other health authorities, the FDA, has recommended the use of the human cell line to identify metabolic pathways for drugs and shared their applicability in in vitro tests in guidelines published in 2004[4]. In November 2013, the

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National Institutes of Health reported that more than 230 embryonic stem cell lines are appropriate for research[5].

The number of publications on cytotoxicity and permeability studies using cell cultures as seen on PUBMED clearly shows that these topics have been trending higher over the past several years (Figure 1).

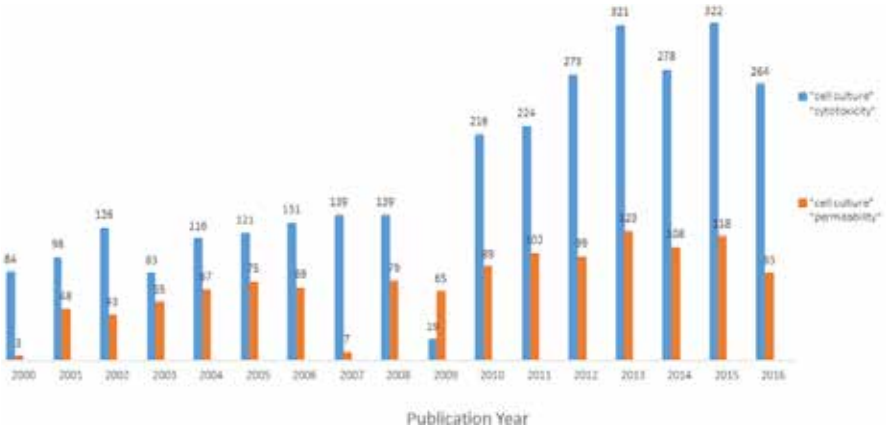

Figure 1. Numbers of publications in Pubmed using keywords 'cell culture', 'cytotoxicity' and 'permeability'.

CANCER CELL LINES AS MODEL FOR DRUG STUDIES

Cancer cell lines are used as a model for research and drug studies (Table 1). During the development of therapies, drugs are tested and developed by using cancer cell lines as an important model[6]. Drugs are tested on cancer cell lines by pharmaceutical companies[7].

Table 1. Most used cancer cell lines (modified from Ferreira et al., 2013[8]).

Cancer cell line	Species	Disease
HeLa	*Homo sapiens*	Cervix adenocarcinoma
Caco-2	*Homo sapiens*	Colorectal adenocarcinoma
MCF-7	*Homo sapiens*	Breast adenocarcinoma
A549	*Homo sapiens*	Human lung carcinoma
U87MG	*Homo sapiens*	Glioblastoma-astrocytoma
HT-29	*Homo sapiens*	Colon adenocarcinoma
HEP-G2	*Homo sapiens*	Hepatocellular carcinoma
K-562	*Homo sapiens*	Chronic myeloid leukaemia
Cos7	*Cercopithecus aethiops*	SV40 transformed - kidney
PC3	*Homo sapiens*	Prostate adenocarcinoma
A375	*Homo sapiens*	Malignant melanoma
HEK 293	*Homo sapiens*	Human Embryonic Kidney 293 cells
CHO	*Chinese hamster*	Chinese hamster ovary cell line

The use of cancer cell lines for drug studies has advantages and disadvantages. Advantages of cancer cell lines are: They can be easily handled and manipulated. They have high homogeneity. Cancer cell lines have similarity with the initial tumour and it makes them advantageous to test cancer drugs on cancer cell lines. For experiments, they are unlimited auto-replication source and are easily substituted. The experiment results of the cancer cell lines for drug studies are reproducible. They also have disadvantages, such as they can be cross-contaminated with Hela cells. During studies, they can lose homogeneity and genomic stability, and they are also susceptible to contamination with bacteria and mycoplasma. And also, another difficulty is that the growth of long-term cancer cell lines is challenging[8].

Drug Screening in Cancer Cell Lines

Drug development begins with drug testing in cancer cell lines. Afterwards, drugs can be tried in in vivo clinical trials. Researchers have evaluated cytotoxicity of drugs on cancer cell lines for many years and data from these experiments have been proven to have clinical predictive value[9,10]. Diverse responses to drugs are displayed by different cancer cell lines[9]. Cell line panels are also useful for drug tests. The first cancer cell line is the panel NCI-60 which utilizes 60 cancer cell lines. This cancer cell line panel was developed to reduce animal experiments for testing of the drugs[11]. The mechanism, physiological processes and treatments of diseases can be explored by the help of the release of molecules from drug delivery carriers, drug diffusion tests and drug toxicity tests. Drug efficacy tests and drug toxicology tests are valuable as they present an alternative to animal experiments[6,12]. REACH is a regulation of European Union with four phases named registration, evaluation, authorization and restriction of chemicals and indicates that animal testing on chemicals should be avoided. 7th Amendment to the Cosmetics Directive of European Union declares that finished cosmetic products and ingredients should be tested on alternative non-animal tests. This regulation of European Union has made cell-based experiments even more important[13].

In Vitro Systems for Toxicity Testing

Cell culture conditions are improved to mimic more closely an in vivo growth environment. These improvements are co-culture with normal cells such as myofibroblasts and immune cells and three-dimensional (3D) matrices. Levels of specific growth factors and additives can be controlled by microfluidic perfusion systems[14].

Toxicity testing of new drugs will eventually be done in animal models to under-

stand overall toxicity. If toxicity testing of new drugs is done in appropriate in vitro cell lines, limited animal model toxicity tests are needed. Toxicity tests on cell lines can be evaluated by testing the drug on a variety of cell types for which cell lines are available. These toxicity tests on cell lines can be an indication for the drug treatments for the cancer type which was studied[14,15].

Although a suitable source of in vitro normal cell cultures were needed, only cancer cell lines were a suitable source for these tests. Normal cells from human-induced pluripotent stem cells (hiPSC) and improved epithelial cell culture conditions can be used nowadays to broaden in vitro toxicity testing at the normal cellular level[14,15]. hiPSC-derived cardiomyocytes, endothelial cells, hepatocytes, and neuronal cells are commercially available. In the future, liver and heart cells will be available through these techniques to be tested and toxicity testing in whole animals may be reduced[14].

Cell Viability Assays

Cell viability assays are widely used for in vitro drug and formulation toxicology studies. There are alternative assays for cell viability[16,17,18]. Commonly used assays are for cytotoxicity or cell viability detection: the MTT assay, the LDH assay, the neutral red, XTT assay and AlamarBlue assay. Activity of lactate dehydrogenase in the extracellular medium is measured by the LDH assay. Cell death is indicated by intracellular LDH release into the culture medium[19]. The neutral red assay also indicates cell viability. The neutral red is taken into the cell by living cells and sequestrated in the lysosomes of cells[20,21]. The MTT assay is a cell viability assay which determines cytotoxicity, and the validity of this assay was determined in cell lines[22]. MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) is a tetrazolium salt. The tetrazolium ring of tetrazolium salt within the mitochondria is cleaved by succinate dehydrogenase, which results in its conversion to an insoluble purple formazan. The insoluble purple formazan accumulates in healthy cells due to the impermeability of the purple formazan to the cell membranes[23]. MTT or XTT are tetrazolium salt reduced to a colored formazan according to viable cell number[16,17]. Tetrazolium salts (MTT or XTT) are reduced by generation of NADH and NADPH. These colored formazans are measured in an automated colorimeter. MTT assay has an extra solubilization step for formazans which has to be dissolved in dimethylsulfoxide (DMSO) before colorimetric measurement. XTT tetrazolium assay was developed to eliminate this solubilization step and viable cells directly metabolize XTT reagent to a water soluble formazan[16,24]. Optical density in the culture wells can be directly read by calorimetry. Use of Alamar Blue as a fluorescent dye for cell viability tests started in 1993[25]. Alamar Blue is a non-fluorescent, non-toxic blue dye which is

reduced to a pink fluorescent dye as a result of cell viability[26].

Cell-based in vitro models have been used to study drug permeability through buccal (TR146 cell culture), intestinal (Caco-2 cell, TC7, MDCK, LLC-PK1), nasal (cultured nasal cells), pulmonary (Calu-3), ocular (corneal epithelial cells), rectal, vaginal (cervical cell lines) routes[27]. Among these, intestinal permeability is the most studied because the oral route of drug administriation is the most common[28].

Intestinal Permeability by Cell Culture

Genomics, proteomics, robotics and in silico chemistry are used to reduce costs in the drug discovery cycle. Understanding biopharmaceutical properties such as solubility, metabolic stability and intestinal permeability is an important task in the industry[29]. The physico-chemical properties of the active drug substance and its product, the physiological functions of body tissues and organs, and the complex process of drug absorption are influenced by physical and biochemical properties of the epithelial barrier[30]. While permeability of drug compounds through the intestinal membrane is a complex process[29]. The mechanism of drug transport in cell cultures is by passive transcellular and paracellular transport and active-carrier mediate transport[31]. The intestinal permeability of a drug can be evaluated by many techniques[29]. These methods are: 1) in vitro tissue methods (using diffusion chamber); 2) in vitro cell methods- (Caco-2; MDCK); 3) in vitro artificial membranes (parallel artificial membrane permeability assay (PAMPA) or immobilized artificial membrane (IAM) columns); 4) in vivo methods (whole animal pharmacokinetic studies; 5) in situ methods (single-pass perfusion); 6) computational approaches. These methods for the permeability assessment of drugs can be used individually or in combination. Cell culture models are preferred for the permeability assays due to predictability and throughput [29]. Recent studies for cell culture models for pharmaceutical technology are shown in Table 2.

Human cell culture models for the toxicity test of aerosolized nanoparticles are Alveolar epithelial cells (A549) and Airway epithelial cells (Calu-3, 16HBE140-, BEAS-2B)[32]. As nanosized ZnO are used in sunscreens, the effect of the cytotoxicity of ZnO is important. Two commercially available ZnO powders' cytotoxicity was tested in human colon-derived RKO cells[33]. Albanese and Chan, 2011 produced transferin-coated gold particles by Frens method and they tested the effect of aggregation of particles on three different cell lines (HeLA, A549, MDA-MD-435) due to the fact that aggregation of particles can affect toxicity. They concluded that uptake of particles are more affected than toxicity[34]. In another study, the effects of different sized silver nanoparticles on cytotoxicity were eval-

Table 2. Examples of recent studies for pharmaceutical technology

Studies	**Cell line used toxicity testing /permeability test**
In vitro cell exposure studies for the assessment of nanoparticle toxicity in the lung—A dialog between aerosol science and biology[32]	co-cultures of two different epithelial cell lines, A549 and 16HBE14o- epithelia cell lines
ZnO particulate matter requires cell contact for toxicity in human colon cancer cells[33]	RKO colon cancer cells
Effect of gold nanoparticle aggregation on cell uptake and toxicity[34]	HeLa and MDAMB-435 cells
Impact of silver nanoparticles on human cells: effect of particle size[35]	A549, SGC-7901, HepG2 and MCF-7 cells
Cellular uptake and toxicity of gold nanoparticles in prostate cancer cells: a comparative study of rods and spheres[36]	PC-3 cells
Cytotoxicity and oxidative stress induced by different metallic nanoparticles on human kidney cells[37]	IP15 (glomerular mesangial) and HK-2 (epithelial proximal) cell lines
Thermoreversible Pluronic® F127-based hydrogel containing liposomes for the controlled delivery of paclitaxel: in vitro drug release, cell cytotoxicity, and uptake studies[38]	KB cancer cells
Cytotoxic effects of iron oxide nanoparticles and implications for safety in cell labelling[39]	C17.2 neural progenitor cells, PC12 rat pheochromocytoma cells and human blood outgrowth endothelial cells
Cytotoxicity induced by engineered silver nanocrystallites is dependent on surface coatings and cell types[40]	Mouse macrophage (RAW-264.7) and lung epithelial (C-10) cell lines
Vitamin E TPGS coated liposomes enhanced cellular uptake and cytotoxicity of docetaxel in brain cancer cells[41]	C6 glioma cells
Curcumin loaded poly (2-hydroxyethyl methacrylate) nanoparticles from gelled ionic liquid–In vitro cytotoxicity and anti-cancer activity in SKOV-3 cells[42]	SKOV-3 ovarian cancer cell lines
Toxicity of copper oxide nanoparticles in lung epithelial cells exposed at the air-liquid interface compared with in vivo assessment.[43]	HBEC or A549 cells.
Cytotoxicity assessment of lipid-based self-emulsifying drug delivery system with Caco-2 cell model: Cremophor EL as the surfactant[44]	Caco-2 cells
Impact of lipid-based drug delivery systems on the transport and uptake of insulin across Caco-2 cell monolayers[45]	Caco-2 cells
Cellular uptake and transcytosis of lipid-based nanoparticles across the intestinal barrier: relevance for oral drug delivery[46]	Caco-2 cells
Regional Morphology and Transport of PAMAM Dendrimers Across Isolated Rat Intestinal Tissue[47]	Caco-2 cells

uated on four human cell models (A549, SGC-7901, HepG2 and MCF-7). The experiments with 5 nm, 20 nm and 50 nm silver nanoparticles showed that the smallest - (5 nm) is the most toxic nanoparticle among them. They concluded that the reason may be that when nanoparticles are smaller, they enter cells more easily than larger ones[35]. Properties of gold nanoparticles make them an important tool for cancer therapy, gene delivery and cancer detection. Gold nanoparticles of various types (plain spherical, PEGylated spherical and PEGylated rods) were compared with each other to evaluate cytotoxicity on a human prostate cancer cell line (PC-3 cells). The results showed that the cytotoxicities of these gold nanoparticles were not different from one another[36].

Metallic nanoparticles are used in medical treatments but can have a toxic effect on the kidneys. Pujalté et al., 2011 tested nanoparticles (TiO_2, ZnO and CdS) which were produced for industry on human renal culture cells. While TiO_2 nanoparticles showed no cytotoxicity, ZnO nanoparticles showed dose-dependent cytotoxicity and CdS nanoparticles are the most toxic[37].

In another study, Paclitaxel loaded liposomes were incorporated into a thermoreversible hydrogel called Pluronic F127 in order to improve the solubility of paclitaxel and increase drug loading. Human oral cancer KB cell lines were incubated with PTX formulation loaded with liposomal 18% F127 gel, Taxol or liposome. Blank liposomal F127 gel was found to be safer than pure liposome[38].

Iron oxide nanoparticles are used for cell labelling in biomedical research. 4 different types of iron oxide nanoparticles were produced and their toxicity was tested on human blood outgrowth endothelial cells, C17.2 neural progenitor cells, and PC12 rat pheochromocytoma cells. Non-toxic concentration was determined for these nanoparticles to be used for the MR visualization[39].

Silver nanoparticles were used for their antimicrobial properties and biomedical applications such as wound dressings. Different silver nanoparticles were produced. Toxicity measurements were performed on lung epithelial (C-10) cell lines and mouse macrophage (RAW-264.7). In this study, they concluded that surface charge and coating materials used in the synthesis, particle aggregation, and the cell-type used for the tests affect the cytotoxicity results. Based on cytotoxicity results, macrophage cells were found to be more sensitive than lung epithelial cells[40].

In one study, liposomes coated with a PEGylated vitamin E (TPGS) with docetaxel were developed for treatment of brain tumours. Cytotoxicity of the liposomes were tested on C6 glioma cells. TPGS coated liposomes have higher cytotoxicity than PEG coated liposomes [41].

The anticancer activity of Curcumin loaded poly (2-hydroxyethyl methacrylate) nanoparticles was tested on ovarian cancer cells (SKOV-3) and the results showed that Curcumin loaded poly (2-hydroxyethyl methacrylate) nanoparticles exhibited a better level of tumor cells regression activity than free curcumin[42].

In another study, an evaluation of the toxicity of copper oxide nanoparticles was performed on lung adenocarcinoma cells (A549 cells) and human bronchial epithelial cells (HBEC) using an *in vitro* air–liquid interface (ALI) exposure system. Exposure of CuONP significantly reduced cell viability in a dose-dependent manner. CuONP were more toxic on A549 cells than HBEC[43].

Lipid-based self-emulsifying drug delivery systems are used for solubilizing poorly soluble drugs. When excipients and formulations are toxic, they damage cell monolayers and this artificially increases drug permeation. Understanding their toxicity is important for the correct interpretation of results. Bu et al., 2016 showed that using Cremophor EL as the surfactant did not damage the Caco-2 cell layer and did not induce toxicity in the lipid-based self-emulsifying drug delivery system[44].

In one study, Self-(nano)-emulsifying drug delivery systems (SNEDDSs) containing insulin were produced to transport insulin across the intestinal membrane. Size of SNEDDS were between 35-50 nm. They demonstrated that two SNEDDS formulations increased the permability of insulin in Caco-2 cell monolayers[45]. In another study, permeability of nanostructured lipid carriers and solid lipid nanoparticles were compared and validated by Caco-2 cell monolayers. Permeability results of nanostructured lipid carriers were higher than solid lipid carriers[46].

Intestinal permeability of Polyamidoamine (PAMAM) dendrimers were compared to Caco-2 monolayers and isolated rat intestinal regional mucosae. TEER values of Caco-2 monolayers and isolated rat intestinal regional mucosae matched each other[47].

Cell Culture Model for Drug Permeability Studies

Absorption of drugs mostly occurs in the small intestinal region of the gastrointestinal tract. The small intestine selectively absorbs major nutrients, digests foreign substances and is a barrier to digestive enzymes. The surface of the small intestinal region increases the potential surface area available for digestion and absorption[29]. Models of human intestinal epithelium have been developed. This culture model is an ideal system to test the intestinal permeability of drug candidates. The Caco-2 cell is the model cell line which has been studied the most, characterized and is most useful for drug permeability studies[48,49,50] (Figure 2).

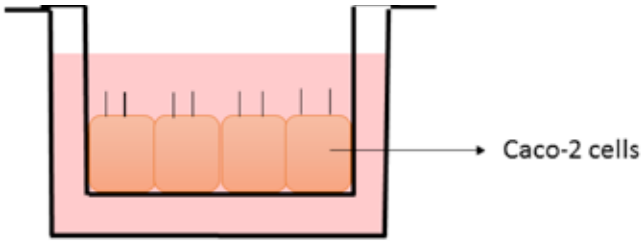

Figure 2. Caco-2 permeability assay.

Caco-2 is a human colon adenocarcinoma cell line[48]. It has well-established tight junctions and undergoes spontaneous enterocytic differentiation. Caco-2 is also used to predict the oral absorption of drugs in humans due to similarity of the Caco-2 cell line's permeation characteristics of drugs with permeation characteristics of human intestinal mucosa. Use of Caco-2 cells as a screening tool is becoming more widespread in the pharmaceutical industry. Lewis lung carcinoma-porcine kidney 1 (LLC-PK1) cells and Madin–Darby canine kidney (MDCK) are other cell line models used in permeability studies. Also, the 2/4/A1 line, transfected cells and Caco-2 cell clones are modified cell models which can be used for permeability studies. MDCK cell line is obtained from dog kidney cells. MDCK cell line is used as a model for intestinal permeability and use of this cell line as a model was first discussed in 1989[51]. Since then, MDCK cells were used for the permeability studies of early drug discovery compounds. Differentiation into columnar epithelial cells, formation of tight junctions and epithelial cell characteristics are common properties of Caco-2 and MDCK cells[52]. Permeation of passively absorbed drugs in Caco-2 cells and MDCK cells were correlated with each other.While Caco-2 cells grow in three weeks, MDCK cells grow in three days and it makes MDCK cells advantageous for the shorter cultivation period. As cell contamination and labour are disadvantage of longer cell culture time of Caco-2, shorter cultivation time of MDCK cells becomes important. The disadvantages of MDCK cells versus Caco-2 are: permeability values of drugs may be different for transporter-mediated uptake and/or efflux compounds due to species difference[53]. LLC-PK1 cells are also alternative cell line to Caco-2 for permeability studies and this porcine cell line can be utilized for the passive absorption of drugs[54]. The 2/4/A1 line is obtained from fetal rat intestine and passive paracellular permeability of 2/4/A1 line is similar to human small intestine. In vitro permeability models were improved by these modified cell lines for carrier mediated transport[55,56].

Challenges Associated with Cell Culture Models

The use of cell culture models in permeability studies presents some issues; these are: Important transporters for drugs are expressed in Caco-2 cells[57]; however, expression of transporters in Caco-2 is lower than human small and large intes-

tine[58]. Lower expression of these transporters may yield to less correlated results between Caco-2 cells and human intestine. Gene expression profiles of solute carrier transporters (SLC), efflux transporters (ABC) and cytochrome P-450 enzymes are different among Caco-2, MDCK and human intestine[60]. These differences may affect the permeability of compounds which are specific to transporters showing different expression profiles[57]. TEER values and permeability values of Caco-2 from different laboratories can be different due to varying culture conditions[60,61,62]. Although the transepithelial electrical resistance (TEER) of small intestine is estimated to be in the range of 25–40 Ω cm^2, TEER of Caco-2 cells are 234 Ω cm^2. Pore sizes of intestinal epithelium and Caco-2 cells are 5 Å and 6 Å, repectively[63]. When Caco-2 and MDCK cell lines were transfected with influx transporters, proper permeability results similar to human intestine were achieved. Cytochrome P450 (CYP3A4) is oxidative CYP enzyme in intestine and it is less expressed in Caco-2 cells compared to human intestine[64]. Caco-2 cells can have different permeability values compared to human intestine due to cacophilicity, which means a drug reversibly binds to Caco-2 and it results in underestimation of permeability values. Although pH of human intestine varies from acidic to slightly basic pH[65], Caco-2 grows in fixed pH conditions. After cell-based permeability studies, sample analysis is mostly done using LC-MS tools. However, high content of salt in the transport buffer affects LC-MS by interfering with ionization[29].

3D CELL CULTURE MODELS

Cell-based screening has been revolutionized by 3D cell culture technologies. While in 2D cell culture cells were grown on flat surfaces, in 3D cell culture cells were grown with the help of attachment surfaces such as extracted extracellular matrix (ECM)[66]. The ECM is the complex mixture of proteins and sugars beyond the membrane of the cell[67]. Collagen, laminin and glycosaminoglycans, such as chondroitin sulfate and heparan sulfate are widely used components of ECM[68,69]. The basement membrane is a specialization of the ECM required for adhesion of the epithelial cell layer and responsible for a wide range of epithelial cell phenomena including cell identity, wound healing and migration[69]. ECM is not just a random mix of secreted components, but a specific composition of biochemicals and defined geometrical structure, which stimulates specific cell responses, such as differentiation[70]. Filter well inserts, sponges and gels and microcarriers are types of ECM. Filter well inserts are the first technology used for ECM[66]. 2D cell culture tests, animal model tests and clinical trials are the processes for drug discovery. Drugs may fail during phase III due to the toxicity of the drugs or efficacy of the drugs[71,72]. Drug test failures on 2D cell cultures led to the development of 3D cell cultures as an improved model for testing. Results of the drugs' responses

are different between 2D and 3D cultures due to differences between 2D culture and human intestine. Understanding the toxicity of the new drug before animal tests is important in minimizing costs during research and development[73,74]. Cellular responses, spatial organization of cell surface receptors, gene expression and cellular behaviour in 3D culture cells can differ from 2D cell culture and it can be concluded that 3D-cultured cells reflects in vivo cellular responses better than 2D cell culture[75]. 3D cell cultures are grown using a scaffold or in a scaffold-free manner[76]. In 2D cell culture, cells form a monolayer while they are growing on a flat surface; attached cells proliferate. When they die, they detach from the surface and these dead cells are removed during the medium change[77]. 2D cells are flatter than in vivo cells. This dissimilar morphology affects the characteristics of the cells and they do not properly mimic the behaviours of the cells in the body. However, 2D cell culture is still the commonly used in vitro test in drug screening[76].

In 3D cell culture, cell–cell interactions and cell–ECM interactions can provide the in vivo environment easily. 3D cell culture contains cells that are in various stages. Viable cells are at the outer part of the cluster, the core part contains cells at hypoxic state due to deficiency of medium[78]. The relative proliferation between 3D and 2D-cultured cells showed different trends and this proliferation rate difference also depends on cell line and matrix[79,80,81,82]. 3D cell culture has different gene, protein, and cell receptor expression compared to 2D cell culture[75]. Susewind et al., 2016 developed a 3D intestinal model by embedding human macrophages (THP-1) and human dendritic cells (MUTZ-3) in a collagen cell and seeding Caco-2 cells on top of them. Non-inflamed and inflamed co-cultures were used to understand inflammation effects and cytotoxicity of nanoparticles. Comparison of Caco-2 monocultures and 3D co-cultures showed that cytotoxicity and interleukin release, which is an important biomarker for inflammation, was higher in 3D co-cultures[83]. Gomez-Roman et al., 2017 tested temozolomide, bevacizumab and erlotinib on 2D and 3D Glioblastoma cultures. They proved that these three drugs affect 2D and 3D cultures and 3D model responses were similar to clinical trials[84]. In a recent study by Ribas et al., 2016, the authors created a vascular microenvironment of the heart for drug development[85]. In another recent study by Marsono et al., 2016, human iPSC-derived cardiomyocytes were used to stimulate 3D construction of myocardium[86].

CONCLUSION AND FUTURE PROSPECTS

Cell culture is increasingly used in pharmaceutical research and regulators support the use of cell culture during the drug development stage. This review summarizes basic techniques of human cell line studies in the pharmaceutical tech-

nology field. Cell-based experiments are highly predictive for preclinical drug toxicity and permeability assessments. It is assumed that, in the future, animal and human clinical trials will be greatly reduced by 3D cell culture experiments.

AUTHOR CONTRIBUTIONS

These authors contributed equally.

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