3D CELL CULTURE TECHNOLOGY – A NEW INSIGHT INTO *IN VITRO* RESEARCH – A REVIEW*

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Abstract

Most *in vitro* cell-based research is based on two-dimensional (2D) systems where growth and development take place on a flat surface, which does not reflect the natural environment of the cells. The imperfection and limitations of culture in 2D systems eventually led to the creation of three-dimensional (3D) culture models that more closely reproduce the actual conditions of physiological cell growth. Since the inception of 3D culture technology, many culture models have been developed, such as technologies of multicellular spheroids, organoids, and organs on chips in the technology of scaffolding, hydrogels, bio-printing and liquid media. In this review we will focus on the advantages and disadvantages of the 2D vs. 3D cell cultures technologies. We will also try to sum up available 3D culture systems and materials for building 3D scaffolds.

Key words: cell culture models, 3D culture technology, 3D cell culture vs. 2D cell culture, hydrogels, scaffolds

Most *in vitro* cell-based research is based on two-dimensional (2D) systems where growth and development take place on a flat surface. 2D culture models do not reflect the natural environment of the cells. Observations carried out at the bottom of the artificial culture vessel are only able to a small extent to reproduce the actual conditions in a physiological niche. All components and the surroundings of other cells have a real impact on their functionality and phenotype. Traditional two-dimensional (2D) cell cultures are deprived of the appropriate environmental context and structural architecture, which may lead to functional changes in the microorganism that is the cell (Castiaux et al., 2019; Duval et al., 2017; Fang and Eglén, 2017; Kapalczyńska et al., 2018; Souza et al., 2018).

*Work financed from: Resources of the National Research Institute of Animal Production, no.: 04-19-05-00 and 01-19-09-11.

The extracellular matrix and adjacent cells create a unique tissue niche. This specific microenvironment is the essence of tissue function as it is involved in cell-to-cell and cell-to-tissue communication. The tissue environment is responsible for both the development and growth of the body tissue and is an important element influencing the intercellular interactions (Dong et al., 2018; Huang et al., 2019; Ryu et al., 2019). The polystyrene culture vessel is often additionally coated to promote cell adhesion. This arrangement reflects the disorganisation of the niche where cells should interact with each other and the ECM (Verjans et al., 2018). Normal epithelial cells maintained in 2D cultures often lose their differentiation, become highly plastic, and exhibit the features represented by neoplastic cells. In turn, neoplastic cells begin to differ from their counterparts isolated from solid tumours (Duval et al., 2017). The lack of an accurate mapping of the cell's living environment in 2D systems results in problems related to the predictability of results, which generates higher research costs and increases the failure rate in experiments. As a result, basing research solely on 2D models of melanoma cells leads to the exclusion of the expression of about 90% of genes in cells of 2D *in vitro* lines, compared to cells cultured *in vivo* as tumours or 3D spheroids implanted subcutaneously in a mouse. This is especially true of genes that are involved in biological processes such as tissue development, cell adhesion and extracellular matrix remodeling. In turn, the expression of genes involved in DNA repair does not differ significantly between the two types of culture (Fontoura et al., 2020; Horman et al., 2013). In particular, tumour cells cultured in a 3D system are prone to alter signaling pathways compared to the same cells cultured in a 2D system. One such example is HER2 amplified breast cancer cells in which the 3D culture conditions induced a change between activation of the PI3K-AKT and RAS-MAPK pathways (Horman et al., 2013). The imperfection and limitations of culture in 2D systems eventually led to the creation of three-dimensional (3D) culture models that more closely reproduce the actual conditions of physiological cell growth.

3D cell culture is an *in vitro* culture model that allows cells to grow and migrate in a space created on a support that mimics the cell microenvironment *in vivo* (Bodgi et al., 2019; Fontoura et al., 2020; He et al., 2016; Jensen and Teng, 2020). Given such important interactions as cell-cell and cell-ECM contact, 3D culture allows cells to recreate critical tissue characteristics including morphology, differentiation, polarity, proliferation rate, gene expression and genomic profiles, as well as maintaining cell heterogeneity, nutrient and oxygen gradients (Castiaux et al., 2019; He et al., 2016; Ravi et al., 2017; Souza et al., 2018; Verjans et al., 2018). Thus, 3D solutions not only reproduce biochemical conditions, but also biomechanical characteristics of conditions *in vivo* (Duval et al., 2017; Fang and Eglén, 2017). The distinct, unique molecular localisation patterns and composition of ECMs in 2D and 3D models trigger different signal transduction cascades and result in different cell behaviour (Cannon et al., 2017). The results of research using 3D systems show that extending the extracellular matrix (ECM) around cells from 2D to 3D can significantly affect proliferation, differentiation, mechanoreceptor responses and cell survival (Cannon et al., 2017; Duval et al., 2017). Here, we briefly described current solutions for 3D cell culture technology and arguments for the superiority of the 3D systems over 2D

solutions (Figure 1). We will also attempt to summarise available 3D culture systems and materials for building 3D scaffolds.

Figure 1. The essence of differences between 2D and 3D cell cultures. Well-designed 3D *in vitro* models (right panel) mimic the actual cell growth environment much more closely than 2D culture systems (left panel). Introduction of the extracellular matrix into the culture environment stimulates the cell growth characteristic of this multidimensional conditions observed *in vivo*. Graphic by Real Research

Comparison of 2D vs. 3D cell culture research results

The development and increasing advancement of 3D culture techniques has prompted researchers to compare the results obtained in 2D and 3D models in their research. Many studies showing the difference between the results obtained in 2D and 3D systems are described in the Duval et al. (2017) review. The experiments mentioned therein highlight the different effects of 2D and 3D cultivation methods on the proliferation, differentiation and expression levels of the cell's genes. It was emphasised, *inter alia*, that ESCs grown on (2D) slides coated with type I collagen showed a faster differentiation rate than those in 3D cultures (EB-embryoid bodies and GEL – collagen type I). It also pointed to studies that showed some features of cancer cells which are not properly modelled in 2D systems. In fact, in all observed tumour cell lines the proliferation rate was higher in 2D monolayers compared to 3D reconstituted basement membrane (rBM) cultures; however, in 3D cultures there was an increase in the expression of $\beta 4$ and $\beta 1$ integrins, which serve as markers of cell polarisation and differentiation (Duval et al., 2017).

The complexity of the experiments with the use of 3D systems also highlights the way of glucose uptake by various cell lines. It has also been proven that phenotypic expression is dependent on the culture method. The analysis of the valvular interstitial cells (VIC) microarrays revealed that the stiffness of the medium may influence the expression of the genes of the cell lines. Cells grown on rigid, 2D tissue culture polystyrene plate showed a greater change in gene expression than 2D or 3D cultures grown on less rigid materials such as hydrogels. The Young's modulus of the material on which VIC cells were grown influenced the expression of genes of

the cytoskeleton (e.g. ACTC1 or CNN1), matrix remodeling and contractility (e.g. ADAM 19), focal adhesions (e.g. VCL) or TGF β signaling. Moreover, it was proved that cells maintained in a monolayer expressed higher levels of cytoskeletal elements and ECM proteins than those cultured in 3D systems (Duval et al., 2017; Mabry et al., 2016).

Comparing the results obtained in 2D and 3D models, the focus was especially on the response to anticancer drugs. Previous research has already shown that drug response, gene expression, cell-to-cell communication and cell signaling overlap in 3D and *in vivo* tumour models, but are not reflected by 2D models (Cannon et al., 2017). Three-dimensional (3D) cancer models are used as preclinical systems to mimic physiological responses to drugs. In 3D cultures, spheroids showed relatively lower activities in the protein kinase B (AKT) signaling pathway, mammalian target of rapamycin (mTOR), and ribosomal protein S6 kinase beta-1 (S6K also known as RPS6KB1) compared to cells grown in two dimensions. The 3D models showed an enhanced anti-tumour response to inhibition of the AKT–mTOR–S6K pathway or inhibition of the mitogen activated protein kinase (MAPK) pathway compared to 2D models. Furthermore, inhibition of the AKT–mTOR–S6K pathway resulted in increased extracellular-signal regulated kinases (ERK) phosphorylation in 2D culture, while under these conditions ERK signaling was reduced in spheroids. Inhibition of mitogen-activated protein kinase (MEK1, also known as MAP2K1) led to a reduction in AKT–mTOR–S6K signaling in 3D but not in 2D culture. These results demonstrate clear changes in the signaling pathways in 3D cultures and during treatment compared to 2D cultures. Therefore, attempts to unravel signaling in spheroid-based models and to determine responses to pharmacological drugs in 3D systems may be more beneficial in the perspective of future anticancer therapies compared to 2D studies (Riedl et al., 2017).

One of the studies assessed the effectiveness of pharmaceuticals against melanoma cells of the B16F10 line and breast cancer cells of the 4T1 line. It was shown, *inter alia*, that the 3D cell culture models show a growth morphology more like that observed *in vivo* compared to 2D models. Administration of dacarbazine resulted in greater drug resistance of cells in 3D cultures than in 2D, and an approximately 30% increase in cell viability. Similar effects were seen with cisplatin. Gene expression analysis also confirmed the greater similarity between the 3D and *in vivo* models as compared to the 2D models (Fontoura et al., 2020). The increased similarity of the behaviour of cells in response to chemotherapeutic agents (paclitaxel and docetaxel) in 3D models with *in vivo* models was also demonstrated in the work of Souza et al. (2018). The research was conducted on 3 prostate cancer cell lines: PC-3, LNCaP and DU145 in 2D models and 3D magnetic bioprinting. The 3D cultures showed a lower rate of cell proliferation, increased resistance to both chemotherapeutic agents, and an altered gene expression profile compared to the 2D system (Souza et al., 2018).

Differences between the 2D and 3D culture systems were also shown during the response of bladder cancer cells lines: RT4, T24 and UM-UC-3 to radiation. The UM-UC-3 cells in the 3D model turned out to be the most resistant to irradiation with increasing doses (Bodgi et al., 2019). A different response to Herceptin was also

observed in the studies of dynamic metabolic differences of a pharmaceutical in 2D and 3D systems using two-photon microscopy of the intensity and lifetime of cellular autofluorescence, the so-called optical metabolic imaging (OMI). OMI uses the endogenous fluorescence of the metabolic coenzymes NADH (Nicotinamide Adenine Dinucleotide) and FAD (Flavin Adenine Dinucleotide), which makes it sensitive to changes in cellular metabolism caused by drug treatment. The results confirmed significant differences in response to the drug for 2D and 3D cultures for the BT474 and HR6 (BT474 cell line resistant to Herceptin) in combination therapies and in the number of cell sub-populations or the mean lifetime of NADH and FAD. Differences were also revealed in the number of cell subpopulations in 2D and 3D cultures and an increase in mean lifetime of NADH and FAD in 2D systems (Cannon et al., 2017).

The neoplastic niche of the tumour has an impact on targeted pharmacological therapies. The *in vitro* activity of anti-cancer drugs is highly dependent on the culture conditions (2D and 3D). Studies of BRAFV600E mutant cell lines for human papillary carcinoma (BCPAP) and anaplastic carcinoma (SW1736) of the thyroid gland revealed differences in response to serine/threonine-specific protein kinases (RAF) and MEK inhibitors depending on the culture model. In the case of the BCPAP line, both inhibitors inhibited growth and migration in the 2D system, while in the 3D system they decreased the viability of cancer cells. In contrast, in the SW1736 line the inhibitors did not show any effect on cell migration in the 2D, but limited invasiveness and cell growth in the 3D model (Ingesson-Carlsson et al., 2015). Lower sensitivity of 3D models to electrochemical therapy in combination with bleomycin was also confirmed in studies of human conjunctival melanoma in the HCjE-Gi line (Fiorentzis et al., 2019). The 3D models also appear to better illustrate the apoptotic processes induced by trastuzumab and the emerging drug resistance associated with the mutation between wild-type and mutant PIK3CA cell lines than 2D cultures. In 3D culture of wild-type PIK3CA, exposure to trastuzumab induced increased expression of poly (ADP-ribose) polymerase (PARP) compared to the 2D models of the mutant and wild-type PIK3CA lines. For the wild lineage, a greater decrease in the AKT protein phosphorylation ratio was also observed compared to the mutant line in the 3D culture, however these differences were not so noticeable in the 2D culture (Tatara et al., 2018).

The differences between the 2D and 3D cell culture models are not only observed in the response to drugs. In the experiment presented by Carter et al. (2019), the influence of the MSCs (mesenchymal stem cells) on the healing of corneal wounds was checked, depending on the *in vitro* culture model. The MSCs' secrets analysed with the Luminex immunoassay showed significant differences in composition depending on the model used. Hepatocyte growth factor (HGF) and intercellular adhesion molecule 1 (ICAM-1) concentrations were set at five times higher in 3D cultures than in 2D cultures. Corneal fibroblast (CFC) cell proliferation and wound ingrowth processes were more effective in the 3D model. Ultimately, it was shown that – due to the different secretome profiles – MSCs grown on electrospun fibres in the 3D model facilitate wound healing in CFCs and removed corneas much more effectively than MSCs grown in 2D models.

A higher sensitivity of the 3D model was also observed in the genotoxicity studies of heterocyclic aromatic amines and polycyclic aromatic hydrocarbons compared to the 2D model, carried out with the use of the HepG2 human hepatocellular carcinoma line. Genotoxicity of aromatic amines was confirmed only in the 3D model, while the genotoxicity of aromatic hydrocarbons was detected at a much lower concentration than in the 2D model. The 3D model also showed higher mRNA expression levels of albumin and some genes encoding phase I and II xenobiotic metabolic enzymes (Štampar et al., 2019). The toxicity of potential drugs is often investigated by assessing liver metabolism (Godoy et al., 2013; Hori and Kurosawa, 2018). Hori and Kurosawa developed an *in vitro* 3D cell culture method with a microstructured mesh sheet and applied it to culturing human hepatoma HepG2 cells. The MikroMesh is a sheet made up of openings larger than the size of a single cell. Cells seeded on such a sheet could form a 2D layer and a 3D layer on it, depending on the scaffold organisation such as pore size and the cell type. In the MikroMesh model (microstructured mesh sheet), an increase in the expression of liver marker genes in human hepatoma HepG2 cells was confirmed. Induction of cell polarity through the bile duct membranes was also observed during cultivation. The largest changes were noted for CYP1A1 (Cytochrome P450 Family 1 Subfamily A Member 1) and CYP1A2 (Cytochrome P450 Family 1 Subfamily A Member 2) mRNA – an 86- and 43-fold increase respectively – compared to the 2D controls (Hori and Kurosawa, 2018). In 3D cultures of human hepatocytes derived from pluripotent stem cells, polarised aggregates are formed with functional bile ducts, increased expression of albumin, urea and genes encoding phase I and II drug metabolism enzymes, and bile transporters (Ardalani et al., 2019; Godoy et al., 2013). In addition, an enhanced cytochrome P450-mediated metabolism was observed compared to 2D cultures (Ardalani et al., 2019).

Other studies have shown that extensively expanded MSCs can be activated by forming spheroids in 3D culture by the hanging drop method. MSC spheroids formed from cells from passage 3, 5 and 7 showed similar expression, but differed from the MSC in the 2D model. In addition, increased expression of significant anti-inflammatory/immunomodulatory factors cyclooxygenase-2, TNF-alpha-induced protein 6 and stanniocalcin 1 (STC1) was observed in all spheroid preparations (Bartosh and Ylostalo, 2019).

Zhang et al. (2018) confirmed that 3D cell culture models are also of great importance in the study of biological processes. Microarray analysis revealed 405 genes and 279 genes with doubled or more differentiated expression after 3 and 28 days, respectively, of culturing human pulp cells in a 3D model in comparison with a 2D monolayer. The spheroids (3D) showed increased expression of genes related to the extracellular matrix and decreased expression of cell growth genes. Additionally, RT-qPCR analysis showed higher levels of osteocalcin, dentin sialophosphoprotein and alkaline phosphatase expression in a 3D system of human pulp cells than in 2D.

Studies involving antigen-presenting dendritic cells (DCs) have shown that *in vitro* 2D and 3D microenvironment architecture and matrix density have a different effect on immune function and cell differentiation in regulating the DCs' immune response. DCs are the necessary element of an innate immune system by being capable of two main function in the organism: activating the immune response or inducing

and maintaining immune tolerance. DCs are differentiated from monocytes, transforming at first in the iDCs (immature DCs), which are responsible for searching the tissue in the case of looking for antigens. Upon recognition of the foreign body, they internalise it and mature into mDCs (matured DCs). The mDCs then move to the nearest lymph node and trigger an immune response by activating T cells. It was found in this study that many DC phenotype surface markers, functionality and the set of proinflammatory cytokines are differently regulated in different cell culture dimensionality. It was observed, *inter alia*, that the level of surface markers and secreted proinflammatory cytokines in DCs is higher in 2D cultures compared to the 3D model. Differences in antigen uptake by iDC and mDC-mediated T cell proliferation were also observed in the 3D model (Sapudom et al., 2020).

Depending on the culture systems, changes in the observed intrinsic apoptosis process were also visible. Recent studies have shown that the formation of dense spheroids may prevent apoptosis in certain breast cancer cell lines following exposure to anti-cancer drugs. In 2D cultures, access to nutrients was not affected by the cell gradient – as in 3D cultures – because necrotic cells detach from the substrate. Other studies also showed that the anti-tumour effect of LCFS (*Lactobacillus* cell-free supernatant) on cell viability was clearly enhanced in 3D cultured HCT-116 cells, which was associated with an increased level of split caspase 3 (Lee et al., 2019 b). In 3D cultures, the mRNA expression of the apoptotic marker gene was also enhanced compared to 2D systems. This result suggests an enhancement of internal apoptosis activation in HCT-116 cells by LCFS and its anti-tumour effect in 3D culture systems, so when testing the anti-tumour effects of probiotics, 3D systems show more advantages than 2D systems (Lee et al., 2019 b).

The differences between the 2D and 3D culturing systems are also visible at the level of migration. During fibroblast migration studies, the rate of cell migration was found to be about 1.3 times faster in the 3D culture compared to the 2D culture. Differences between 2D and 3D culture systems have also been demonstrated at the level of signaling cascades, where many more revealing mechanisms were observed in the 3D cultures. On this basis, it was suggested that the 3D scaffolds much better reflect the actual conditions for studying cellular migration, and therefore may become a key tool in the ongoing research on therapies against tumour metastasis and other diseases related to cell migration (Duval et al., 2017).

The consideration of multifactorial regulatory signals generated *in vivo* by the ECM in cell culture is another important aspect to be considered. In 2D culture approaches, *in vivo* ECM stimuli are mimicked by collagen or fibronectin, which provide the cells with binding substrates. Thus, the evaluation of the mechanical response of cells in 2D culture is much easier. It is therefore worth emphasising that, depending on the dimensionality of the system, both the chemical and mechanical microenvironment is different (Duval et al., 2017).

3D systems are increasingly considered in research projects. From 2008 to 2020, the number of publications using three-dimensional cultures increased almost eight fold (gathered from PubMed by searching for: 3D cell culture models). By 2014, 3D cell cultures accounted for just under 5% of the cell culture market, and this number is expected to grow rapidly.

3D cell culture models

Since the inception of 3D culture technology in the 1970s, many culture models have been developed that are used to recreate the morphological, functional and microenvironmental characteristics of tissues and organs. The technologies of multicellular spheroids, organoids and organs on chips in the technology of scaffolding, hydrogels, bio-printing and liquid media were developed (Fang and Eglén, 2017). All these technologies are divided into two groups of culture in the 3D system: 1) models without scaffolding and 2) models based on scaffolding (Fontoura et al., 2020; Jensen and Teng, 2020; Verjans et al., 2018).

When models without scaffolding in the 3D system are used, cells can be grown in a static or dynamic setup (Verjans et al., 2018). Static systems without scaffolding are microplates with hanging drops, magnetic levitation and microplates with a coating providing a very low level of adhesion (Jensen and Teng, 2020; Verjans et al., 2018). In dynamic systems, there is additionally an element of shaking, rotating, rocking or flowing the medium (Verjans et al., 2018). Models containing scaffolds include techniques based on hydrogel substrates, polymeric hard materials, hydrophilic glass fibres and organoids (Jensen and Teng, 2020). The scaffold provides a biologically active niche of intercellular interaction where cells can migrate and multiply in a medium similar to native ECM (Verjans et al., 2018). The most popular 3D cell culture models are spheroids, organoids and organs-on-chips.

Spheroids are spherical cell aggregates with poor tissue organisation, and spheroids do not need an external matrix to grow. They are characterised by self-organisation and high self-renewal capacity (Torras et al., 2018). Their undoubted advantage is the creation of geometric conditions for the growth of heterogeneous cell populations as a result of the reconstruction of oxygen and signaling gradients, as well as nutrients and metabolites observed *in vivo* (Fang and Eglén, 2017). Recalling the chaotic mass of the tumour with hypoxic and necrotic cores, they have become a popular model in oncological research (Torras et al., 2018). Despite ensuring cell-to-cell and cell-ECM communication mimicry, spheroids do not solve the problems that allow for quick screening, e.g. drugs. The formation of spheroids occurs in a stochastic manner. It is difficult to control both the size and proportion of specific cell populations, which results in the lack of standardisation of experimental samples. Spheroids, on the other hand, can be obtained by various 3D culture techniques, based on both scaffolds and those without scaffolds (Fang and Eglén, 2017). The construction of spheroids varies; to date, 3 types of aggregate structure have been identified: 1) loose aggregates, 2) compact cell aggregates and 3) compact spheroids (Kapałczyńska et al., 2018) (Figure 2).

Organoids are simplified models of organs and tissues that exhibit specific micro-anatomy. Organoids are highly organised 3D cultures that are formed from the stem or progenitor cells of the appropriate organs through spatial self-assembly similar to that observed *in vivo* (Alhaque et al., 2018; Corró et al., 2020; Fang and Eglén, 2017; Günther et al., 2019; Torras et al., 2018). Organoids, unlike spheroids, require an external matrix for hierarchical growth (Torras et al., 2018). In the development of organoid culture, co-cultures with a feeder-layer and cultures at the interface of various media, e.g. liquid-air, as well as the production of embryonic bodies in

a hanging drop or on low-adhesive plates are often used (Alhaque et al., 2018; Corrà et al., 2020; Fang and Eglén, 2017). Despite recreating the structure and functions of the proper organs, organoids are devoid of blood vessels and some niche cells present in the living organism. Moreover, they often mimic only the early stages of organ development (Alhaque et al., 2018; Fang and Eglén, 2017).

Figure 2. Different types of growth of aggregates in 3D models: 1) loose aggregates, 2) compact cell aggregates, 3) compact spheroids

Organs-on-chips, like organoids, are a miniature model of organs, but embedded on a microfluidic culture chip, allowing for the reproduction of both the geometry and architecture as well as the chemical and physical characteristics of a specific cell niche. The feature that distinguishes organs-on-chips from older 3D culture models is the ability to strictly control the conditions in the organ growth microenvironment. The model allows the reproduction of clinically relevant disease phenotypes and is suitable for cultivation in microplate formats, but not in high-throughput methods. However, organs-on-chips also lacks blood vessels and this should be taken into account when analysing the test results (Alhaque et al., 2018; Fang and Eglén, 2017).

An interesting method, used mainly in models of liver research, are precision cut liver slices (PCLS). PCLS is a method of alternative *in vitro* cultivation of hepatocytes, devoid of the step of cell separation and maintenance of the natural cellular environment with a complete metabolic program. PCLS enable the efficient creation of difficult hepatotoxicity models and the study of human innate immunity. In addition, PCLS can also be extremely useful in preclinical drug testing, such as fibrotic, cholestatic or steatotic. Despite the large scale of PCLS applications, the short-term life (only up to 4–6 days) remains a problem due to insufficient oxygenation and nutrition. Currently, in order to overcome the limitations of cell viability on sections, attempts are being made to use a different spectrum of slices thickness and to support the culture with the use of media enrichment factors (Othman et al., 2020). The maintenance of terminally differentiated cells, especially hepatocytes, *in vitro* is also facilitated by platforms for long-term primary cell cultures in combination with additional nutrients. The PHH (primary human hepatocytes) platform combined with a combination of five chemicals (5C): FSK (forskolin), SB43 (TGF- β inhibitor), DAPT (Notch inhibitor), IWP2 (Wnt inhibitor) and LDN193189 (BMP inhibi-

tor) enables extending the culture process to 4 weeks by modulating cell signaling pathways. The chemical approach used to maintain PHH functionality for a long time allowed not only maintaining the global transcriptional profile of differentiated hepatocytes, but also to survive HBV infection covering the entire viral life cycle and efficient spread of HBV *in vitro*, which proves that small molecule combinations can provide analogous regulatory functions in the case of stabilisation of the other cell types culture *in vitro*. In addition, the undoubted advantages of this platform are simplicity and ease of use in high-throughput screening tests (Xiang et al., 2019).

3D cell culture scaffolds materials and technology

Scaffold-free 3D cultures take advantage of the fact that the adhesion surfaces of cells are hindered or absent, thus promoting their aggregation into 3D structures (Verjans et al., 2018). Forming a 3D culture can be obtained through the technology of hanging drop on drop plates. Cells are seeded at the top of the hole-tipped plate and then a drop of cell suspension is formed below the slit of the upper well. The droplet movement is prevented by surface tension. This approach allows for easy control of aggregate size, but limits screening tests due to the need to transfer structures to an additional plate. One of the available solutions that fulfil this assumption is the use of low-adhesive tiles. The surface of such culture vessels is devoid of adhesive particles by coating the bottom with agar/agarose or poly (2-hydroxyethylmethacrylate) and has a well-defined bottom shape: rounded, V-shaped or conical. This approach is often used to create spheroids and facilitates high-throughput screening (Fang and Eglen, 2017; Verjans et al., 2018). For 3D cultivation under dynamic conditions, bioreactors are also used. The advantage here is the size of the culture and the homogeneity of the microenvironment. However, the disadvantages of some cells may be too high shear stress of the medium, which may damage the cells or block their proper tissue differentiation and the lack of standardisation of the formed structures (Fang and Eglen, 2017; He et al., 2016). The magnetic levitation method uses the charging of cells with nanoparticles with a magnetic charge, and then cultivation in a magnetic field forcing cells to aggregate at the liquid-air interface. The advantage of such culture is undoubtedly the quick time of forming the desired 3D structures (Jensen and Teng, 2020; Verjans et al., 2018). A completely different tissue engineering approach is the creation of multiple layers of superimposed cell sheets. This solution does not cause an inflammatory response and, therefore, has potential application in regenerative medicine (Duval et al., 2017).

The task of scaffold-based models is to create a substrate that mimics the properties of the natural ECM. The scaffold may have a hydrogel or solid material structure (i.e. glass fibres). Based on the source of origin, the polymers used for the production of scaffolds for 3D cell culture and applications in tissue engineering can be divided into natural and synthetic (Ahearne et al., 2005; Fontoura et al., 2020; He et al., 2016; Jensen and Teng, 2020; Wyleżół et al., 2016). Natural materials are extracted from plant or animal tissues (Godoy et al., 2013; He et al., 2016).

The most common natural polymers are: agarose, alginate, chitosan, collagen, fibrin, hyaluronic acid, gelatin, laminin, vitronectin and xyloglucane (Ahearne et al., 2005; Chaudhuri, 2017; Godoy et al., 2013; Verjans et al., 2018; Wyleżół et al.,

2016). Polyhydroxy butyrate (PHB) is especially popular – a natural polymer isolated for the first time from bacteria is characterised by high biocompatibility and is biodegradable (Fontoura et al., 2020). Natural polymers are characterised by high biocompatibility, low toxicity and allow for the development of a chronic inflammatory response, but they do not have a well-defined composition and therefore do not ensure repeatability. There is also a problem with availability of large quantities (Godoy et al., 2013; He et al., 2016). Such materials are also biodegradable and require pre-treatment, which makes routine *in vitro* use difficult and extends the experiment time (Godoy et al., 2013).

The most commonly used synthetic polymers are PAA (polyacrylic acid), PVA (polyvinyl alcohol), PEG (polyethylene glycol) and PLA (lactic acid) (Ahearne et al., 2005; Chaudhuri, 2017; Jensen and Teng, 2020; Verjans et al., 2018; Wyleżół et al., 2016). Synthetic polymers, despite the lack of biological activity, ensure high repeatability as well as perfectly defined composition and physical and mechanical properties (Godoy et al., 2013; He et al., 2016). They are also easier to use and shorter to prepare (Godoy et al., 2013). The disadvantages of natural and synthetic polymers can be mutually compensated for by creating polymer hybrids of various origins (Terrell et al., 2020). Polymer chains cross-linked in a hydrogel with increased water content (Terrell et al., 2020) are characterised by a high degree of tissue ECM mapping, while facilitating the exchange of nutrients, oxygen and metabolites (Chaudhuri, 2017; Duval et al., 2017; Jensen and Teng, 2020; Verjans et al., 2018).

Hydrogels based on natural polymers are highly bioactive but at the same time poorly characterised. The full composition of naturally derived hydrogels is not precisely defined, and this material has limitations in clinical applications, standardisation of research and predictability of cell behaviour. On the other hand, a well-defined composition is characterised by cross-linked hydrogels made of synthetic polymers. Their high repeatability is an indisputable advantage, but they are devoid of other active molecules desired by cells (Chaudhuri, 2017; Verjans et al., 2018). Artificial, synthetic polymers may also be characterised by higher hardness which, compared to the natural elasticities characteristic of the microenvironment of cell growth and development, is a limiting parameter, especially in the case of normal cells (Pogoda, 2015).

The stiffness of the growing medium significantly affects the biological processes. In the 3D model, cells are surrounded by a porous hydrogel in all dimensions. This environment is a natural mechanical barrier to the processes of shape change, migration and expansion of cells, and also affects the proliferation and differentiation (Chaudhuri, 2017). The modulus of elasticity of hydrogels tested by AFM (atomic force microscopy) are usually in the range of 10–100 kPa (Czerner et al., 2015). Hardness of 1% agarose gels and 2% alginate gels are similar and range between 14–15.5 kPa, additionally they are not protein products, which are sometimes crucial in cell culture (Ahearne et al., 2005; Xing et al., 2018). Among the protein products, on the other hand, we can mention collagen for which, however, the limiting step is hardness, which does not leave many manipulation possibilities. For polyacrylamide gels, the lowest tested Young's Modulus is, in turn, about 0.41 kPa (Lee et al., 2019 a; Pogoda, 2015), and for PPA the values are already from about 70 kPa upwards

(Muniz and Geuskens, 2001). The use of MBAA (N, N-methylene bisacrylamide) as a cross-linking agent, in turn, allowed obtaining elasticity modules at the level of 0.01–10 kPa (Gerlach and Arndt, 2013). Nevertheless, different tissues are characterised by different hardness, e.g. lymph nodes (0.12 kPa), brain (0.4 kPa), liver (0.64 kPa), lungs (5–6 kPa) or bone matrix (~106 kPa). Additionally, a modulating effect of cell environment stiffness on the rate of cell division was observed. For substrates with a stiffness range of 2.4–24 kPa, the rate of normal cell division is significantly slower than the rate of cancer cell division, but with the increase in environmental stiffness these differences become blurred (Pogoda, 2015).

Among the commercially available materials for 3D culture, the most popular is the Engelbreth-Holm Swarm (EHS) tumour extract hydrogel, commercially available as Matrigel® (Corning), Geltrex® (Invitrogen) and Cultrex® (Trevigen). The EHS hydrogel contains large amounts of basement membrane proteins, including: laminin, collagen IV, entactin, fibronectin and heparin sulphate proteoglycan, and tumour growth factors (Blondel and Lutolf, 2019; Godoy et al., 2013; Jensen and Teng, 2020). There are also other types of hydrogels on the market, including Extracel™ (Glycosan BioSystems) – collagen hydrogel, Algimatrix™ (Invitrogen) – based on alginate sponge and PuraMatrix™ – peptide hydrogel (BD Biosciences). Despite many advantages and a much better mapping of cell growth conditions *in vivo*, hydrogels pose problems related to weaker transport of nutrients and xenobiotics, and also hinder cell recovery (Godoy et al., 2013). One of the newest solution for hydrogel 3D cell culture is LifeGel (Real Research). LifeGel is a protein hydrogel composed of substrates found in the extracellular matrix. It ensures the elimination of all growth factors during the production process and other contaminants, thus ensuring that the cells in the culture are not in contact with neoplastic factors present, for example, in Matrigel. LifeGel technology enables the creation of hydrogels with a wide range of physical properties and eliminates most of the problems of commercially available hydrogels, including cell recovery from culture. Individual LifeGel modifications may differ from each other, among others, in flexibility, hardness, density or porosity. The stiffness of the culture surface may be from ~ 50 Pa to several dozen kPa. An additional advantage of LifeGel is the production repeatability, which until now was often a stage limiting the use of 3D models on an industrial scale.

Scaffolding is also created by electrospinning – a fibre production technique. In the electrospinning technique, polymer fibres are formed and then deposited on the substrate under the control of an electric field (Jensen and Teng, 2020). In this way, synthetic or natural polymers are used to produce membranes made of fibres of any diameter and porosity increasing the surface available to cells. Metals and ceramics can also be used for their production. Membranes with a more foam-like structure can in turn be produced by SCPL (solvent casting particle leaching). The polymer solution is combined with the porogen (usually salt) and dried and then leached to remove the used porogen (Fontoura et al., 2020).

The latest solution in the production of scaffolding is 3D bioprinting. This technology allows you to print an entire niche with the desired architecture layer by layer using cells, biocompatible materials and other auxiliary components. In simple terms, bioprinting resembles gluing 2D elements together, so you can create scaffolding.

folds themselves or models of entire tissues and organs. Bioprinting allows you to create models that meet the criteria of high-throughput screening tests, but does not fully reflect the development and functionality of the tissue (Fang and Eglén, 2017; Verjans et al., 2018). Bioprinting has opened new possibilities for 3D cultures; thanks to computer design, it is now possible to design your own culture solutions, including microfluidic devices (Jensen and Teng, 2020). Other available techniques for scaffolding prefabrication include stereolithography, polymer phase separation, freeze drying and gas foaming. The creation of scaffolding based on building blocks has also become a new trend. This method consists in forming basic units – the so-called microribbons – which are later cross-linked into larger structures under the control of porosity of appropriate sizes. The results obtained to date have confirmed the effectiveness of this method and a positive effect on the implantation and proliferation of cells and supporting the production of ECM (Duval et al., 2017).

Summary

The last 10 years of research has revealed a significant increase in the use of 3D cell cultures in the early stages of drug discovery. 3D cultures have enabled greater predictability of the efficacy and safety of pharmaceuticals and their possible side effects before the clinical trial phase, and eliminated species differences that made it difficult to translate results into humans in animal models (Alhaque et al., 2018; Fang and Eglén, 2017; Verjans et al., 2018). The microenvironment, and hence the culture model, can have a real impact on the behaviour of cells in many respects (Alhaque et al., 2018; Jensen and Teng, 2020; Kapałczyńska et al., 2018; Ravi et al., 2017; Verjans et al., 2018). The architectural and functional complexity of the tissues in a way necessitates the selection of an appropriate model of culture depending on the *in vivo* conditions in each microenvironment. The use of an appropriate cell culture method in a given research area may allow not only to better understand the biology of a given tissue, but also to optimise the technical approach and facilitate the development of new treatment strategies for many diseases, especially cancer, but also viral and neurodegenerative illnesses (Chaicharoenaudomrung et al., 2019; Kapałczyńska et al., 2018; Ravi et al., 2017). It is anticipated that in the future 3D culture models will enter the stages of most preclinical research, leading to a reduction in the use of laboratory animals in research not only for ethical, but also for practical and economic reasons (Chaicharoenaudomrung et al., 2019; Kapałczyńska et al., 2018).

3D cell cultures are still in the phase of intensive development and creation of the best possible mimicry of the cell niche *in vivo*. Despite better imitation of biomechanical conditions, 3D models do not reproduce all the physiological features of the native tissue, including of the vascular system, host immune response, intra-tissue tensions and full heterogeneity of the microenvironment, including metabolite exchange and transport. The architectural organisation of collagen, like orthogonally arranged collagen sheets and collagen fibril bundles is often very simplified and not

a perfect copy for a gradient of various factors (He et al., 2016). One of the major problems in tissue engineering is the lack of repeatability and standardisation of 3D models to ensure the predictability of results (Brevini et al., 2020). The problem of using 3D systems on a high-throughput scale is currently the greatest challenge facing scientists designing 3D systems.

3D models currently provide the best approach in developing new treatment strategies and application in screening necessary to advance personalised medicine (Kapalczyńska et al., 2018). Personalised medicine puts the patient in front of the disease by changing the approach from universal for all to care adapted to the disease and organism (Brittain et al., 2017). The evolving field of personalised medicine is particularly important in the prevention, diagnosis, prognosis and treatment of neoplastic diseases. A variety of individualised, molecularly targeted therapies with increased efficacy and/or reduced toxicity are incorporated into routine clinical practice (Goetz and Schork, 2019; Jackson and Chester, 2015), such as organoids and 3D bioprinting (Dutta et al., 2017; Jain et al., 2018; Trenfield et al., 2019). The development of such platforms could provide an alternative to preclinical studies in animal models to increase the success rate and improve the efficiency of drug screening while addressing the growing ethical concerns regarding the use of animal models. The further development of well-defined methods and materials for 3D culture as well as the optimisation of these solutions to standardised research protocols represents the future of their use in clinical applications (Alhaque et al., 2018).

In this work we have described the advantages and disadvantages of the 2D vs. 3D cell cultures technologies taking into account the latest available cells culturing in 3D systems. We have also summed up available 3D culture systems and materials for building 3D scaffolds. Especially the LifeGel product, which has been described for the first time, so the author could find the newest approaches in the field of 3D culture within this one paper. The market for available products for 3D culture is in a phase of rapid change and development, so we hope that this work will make it easier for modern researchers to find an appropriate solution to research challenges in the field of 3D culture and will take drug screening and toxicology to the next level. Especially in the case of research using laboratory and farm animals, work in the 3D system is not widespread, which could not only facilitate the research, but also reduce the number of animals in scientific experiments. The topic of three-dimensional cultures is very extensive, therefore this article is only a list of the most popular and newest solutions for cell and tissue cultures in 3D systems, according to the author. We understand that even more may have appeared during the preparation of this article. All the more, with this article we want to encourage researchers and scientists dealing with animal materials, i.e. mice, rats, pigs, cattle, goats and sheep, to work in the 3D culture system. We hope that our brief summary of the topic of 3D culture will make this topic more accessible and encourage research units to implement it in their experimental plans.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

This review is a summary of the cooperation between the National Research Institute of Animal Production in Kraków and the Real Research company. We would like to thank all the people involved in the research conducted during this period, the culmination of which was described in this paper. Especially our thanks go to Mr Marcin Krzykawski – the CEO of Real Research company.

References

- Ahearne M., Yang Y., El Haj A.J., Then K. Y., Liu K.-K. (2005). Characterizing the viscoelastic properties of thin hydrogel-based constructs for tissue engineering applications. *J. Royal Soc. Interface*, 2: 455–463.
- Alhaque S., Themis M., Rashidi H. (2018). Three-dimensional cell culture: From evolution to revolution. *Philos. Trans. R. Soc. Lond. B Biol. Sci.*, 373: 1750.
- Ardalani H., Sengupta S., Harms V., Vickerman V., Thomson J.A., Murphy W.L. (2019). 3-D culture and endothelial cells improve maturity of human pluripotent stem cell-derived hepatocytes. *Acta Biomater.*, 95: 371–381.
- Bartosh T.J., Ylostalo J.H. (2019). Efficacy of 3D culture priming is maintained in human mesenchymal stem cells after extensive expansion of the cells. *Cells*, 8: 9–13.
- Blondel D., Lutolf M.P. (2019). Bioinspired hydrogels for 3D organoid culture. *Chimia*, 73: 81–85.
- Bodgi L., Bahmad H.F., Araji T., Al Choboq J., Bou-Gharios J., Cheaito K., Zeidan Y.H., Eid T., Geara F., Abou-Kheir W. (2019). Assessing radiosensitivity of bladder cancer in vitro: A 2D vs. 3D approach. *Front. Oncol.*, 9: 1–11.
- Brevini T.A.L., Pennarossa G., Gandolfi F. (2020). A 3D approach to reproduction. *Theriogenology*, 150: 2–7.
- Brittain H.K., Scott R., Thomas E. (2017). The rise of the genome and personalised medicine. *Clin. Med.*, 17: 545–551.
- Cannon T.M., Shah A.T., Skala M.C. (2017). Autofluorescence imaging captures heterogeneous drug response differences between 2D and 3D breast cancer cultures. *Biomed. Opt. Exp.*, 8: 1911.
- Carter K., Lee H.J., Na K.S., Fernandes-Cunha G.M., Blanco I.J., Djalilian A., Myung D. (2019). Characterizing the impact of 2D and 3D culture conditions on the therapeutic effects of human mesenchymal stem cell secretome on corneal wound healing in vitro and ex vivo. *Acta Biomater.*, 99: 247–257.
- Castiaux A.D., Spence D.M., Martin R.S. (2019). Review of 3D cell culture with analysis in microfluidic systems. *Analyt. Met.*, 11: 4220–4232.
- Chaicharoenaudomrung N., Kunhorm P., Noisa P. (2019). Three-dimensional cell culture systems as an in vitro platform for cancer and stem cell modeling. *World J. Stem Cell.*, 11: 1065–1083.
- Chaudhuri O. (2017). Viscoelastic hydrogels for 3D cell culture. *Biomater. Sci.*, 5: 1480–1490.
- Corrò C., Novellasedmunt L., Li V.S.W. (2020). A brief history of organoids. *Am. J. Physiol. Cell Physiol.*, 319: C151–C165.
- Czerner M., Fellay L.S., Suárez M.P., Frontini P.M., Fasce L.A. (2015). Determination of elastic modulus of gelatin gels by indentation experiments. *Proc. Mat. Sci.*, 8: 287–296.
- Dong Y., Jin G., Hong Y., Zhu H., Lu T., Xu F., Bai D., Lin M. (2018). Engineering the cell microenvironment using novel photoresponsive hydrogels. *ACS Appl. Mat. Interfac.*, 10: 12374–12389.
- Dutta D., Heo I., Clevers H. (2017). Disease modeling in stem cell-derived 3d organoid systems. *Trends Mol. Med.*, 20: 1–18.
- Duval K., Grover H., Han L.-H., Mou Y., Pegoraro A.F., Fredberg J., Chen Z. (2017). Modeling physiological events in 2D vs. 3D cell culture. *Physiology*, 32: 266–277.

- Fang Y., Eglén R.M. (2017). Three-dimensional cell cultures in drug discovery and development. *SLAS Discov.*, 22: 456–472.
- Fiorentzis M., Katopodis P., Kalirai H., Seitz B., Viestenz A., Coupland S.E. (2019). Conjunctival melanoma and electrochemotherapy: preliminary results using 2D and 3D cell culture models in vitro. *Acta Ophthalmol.*, 97: e632–e640.
- Fontoura J.C., Viezzer C., dos Santos F.G., Ligabue R.A., Weinlich R., Puga R.D., Antonow D., Severino P., Bonorino C. (2020). Comparison of 2D and 3D cell culture models for cell growth, gene expression and drug resistance. *Mat. Sci. Eng. C*, 107: 110264.
- Gerlach G., Arndt K. (2013). Hydrogel sensors and actuators volume. Springer Ser. Chem. Sens. Biosens., 1–272.
- Godoy P., Hewitt N.J., Albrecht U., Andersen M.E., Ansari N., Bhattacharya S., Bode J.G., Bolleyn J., Borner C., Böttger J., Braeuning A., Budinsky R.A., Burkhardt B., Cameron N.R., Camussi G., Cho C.-S., Choi Y.-J., Craig Rowlands J., Dahmen U., Hengstler J.G. (2013). Recent advances in 2D and 3D in vitro systems using primary hepatocytes, alternative hepatocyte sources and non-parenchymal liver cells and their use in investigating mechanisms of hepatotoxicity, cell signaling and ADME. *Archiv. Toxicol.*, 87: 1315–1530.
- Goetz L.H., Schork N.J. (2019). Personalized medicine: motivation, challenges and progress. *Fertil. Steril.*, 109: 952–963.
- Günther C., Brevini T., Sampaziotis F., Neurath M.F. (2019). What gastroenterologists and hepatologists should know about organoids in 2019. *Digest. Liver Dis.*, 51: 753–760.
- He B., Chen G., Zeng Y. (2016). Three-dimensional cell culture models for investigating human viruses. *Viol. Sin.*, 31: 363–379.
- Hori T., Kurosawa O. (2018). A three-dimensional cell culture method with a micromesh sheet and its application to hepatic cells. *Tissue Eng. - Part C: Methods*, 24: 730–739.
- Horman S.R., To J., Orth A.P. (2013). An HTS-compatible 3D colony formation assay to identify tumor-specific chemotherapeutics. *J. Biomol. Screen.*, 18: 1298–1308.
- Huang G., Li F., Zhao X., Ma Y., Li Y., Lin M., Jin G., Jian Lu T.M., Genin G., Xu F. (2019). Functional and biomimetic materials for engineering of the three-dimensional cell microenvironment. *Chem. Rev.*, 117: 12764–12850.
- Ingeson-Carlsson C., Martinez-Monleon A., Nilsson M. (2015). Differential effects of MAPK pathway inhibitors on migration and invasiveness of BRAFV600E mutant thyroid cancer cells in 2D and 3D culture. *Exp. Cell Res.*, 338: 127–135.
- Jackson S.E., Chester J.D. (2015). Personalised cancer medicine. *Int. J. Cancer*, 137: 262–266.
- Jain V., Haider N., Jain K. (2018). 3D printing in personalized drug delivery. *Curr. Pharm. Des.*, 24: 5062–5071.
- Jensen C., Teng Y. (2020). Is it time to start transitioning from 2D to 3D cell culture? *Front. Mol. Biosci.*, 7: 1–15.
- Kapałczyńska M., Kolenda T., Przybyła W., Zajączkowska M., Teresiak A., Filaś V., Ibbs M., Bliźniak R., Łuczewski Ł., Lamperska K. (2018). 2D and 3D cell cultures – a comparison of different types of cancer cell cultures. *Arch. Med. Sci.*, 14: 910–919.
- Lee D., Zhang H., Ryu S. (2019 a). Elastic modulus measurement of hydrogels. *Cellulose-Based Superabsorbent Hydrogels*, pp. 865–884.
- Lee J.E., Lee J., Kim J.H., Cho N., Lee S.H., Park S.B., Koh B., Kang D., Kim S., Yoo H.M. (2019 b). Characterization of the anti-cancer activity of the probiotic bacterium *Lactobacillus fermentum* using 2D vs. 3D culture in colorectal cancer cells. *Biomolecules*, 9: 557.
- Mabry K.M., Payne S.Z., Anseth K.S. (2016). Microarray analyses to quantify advantages of 2D and 3D hydrogel culture systems in maintaining the native valvular interstitial cell phenotype. *Biomaterials*, 74: 31–41.
- Muniz E.C., Geuskens G. (2001). Compressive elastic modulus of polyacrylamide hydrogels and semi-IPNs with poly(N-isopropylacrylamide). *Macromolecules*, 34: 4480–4484.
- Othman A., Ehnert S., Dropmann A., Ruoff M., Nüssler A.K., Hammad S. (2020). Precision-cut liver slices as an alternative method for long-term hepatotoxicity studies. *Archiv. Toxicol.*, 94: 2889–2891.
- Pogoda K. (2015). Rola sił mechanicznych generowanych przez macierz zewnątrzkomórkową w roz-

- woju raka prostaty (in Polish). Docthoral Thesis, The Henryk Niewodniczański Institute of Nuclear Physics Polish Academy of Sciences, 1–123.
- Ravi M., Ramesh A., Pattabhi A. (2017). Contributions of 3D cell cultures for cancer research. *J. Cell. Physiol.*, 232: 2679–2697.
- Riedl A., Schleiderer M., Pudelko K., Stadler M., Walter S., Unterleuthner D., Unger C., Kramer N., Hengstschläger M., Kenner L., Pfeiffer D., Krupitza G., Dolznig H. (2017). Comparison of cancer cells in 2D vs 3D culture reveals differences in AKT-mTOR-S6K signaling and drug responses. *J. Cell Sci.*, 130: 203–218.
- Ryu N.E., Lee S.H., Park H. (2019). Spheroid culture system methods and applications for mesenchymal stem cells. *Cells*, 8: 1–13.
- Sapudom J., Alatoom A., Mohamed W.K.E., Garcia-Sabaté A., McBain I., Nasser R.A., Teo J.C.M. (2020). Dendritic cell immune potency on 2D and in 3D collagen matrices. *Biomater. Sci.* 8: 5106–5120.
- Souza A.G., Silva I.B.B., Campos-Fernandez E., Barcelos L.S., Souza J.B., Marangoni K., Goulart L.R., Alonso-Goulart V. (2018). Comparative assay of 2D and 3D cell culture models: proliferation, gene expression and anticancer drug response. *Curr. Pharmac. Design*, 24: 1689–1694.
- Štampar M., Tomc J., Filipič M., Žegura B. (2019). Development of in vitro 3D cell model from hepatocellular carcinoma (HepG2) cell line and its application for genotoxicity testing. *Archiv. Toxicol.*, 93: 3321–3333.
- Tatara T., Mukohara T., Tanaka R., Shimono Y., Funakoshi Y., Imamura Y., Toyoda M., Kiyota N., Hirai M., Kakeji Y., Minami H. (2018). 3D culture represents apoptosis induced by trastuzumab better than 2D monolayer culture. *Anticancer Res.*, 38: 2831–2839.
- Terrell J.A., Jones C.G., Kabandana G.K.M., Chen C. (2020). From cells-on-a-chip to organs-on-a-chip: scaffolding materials for 3D cell culture in microfluidics. *J. Mat. Chem. B*, 8: 6667–6685.
- Torras N., García-Díaz M., Fernández-Majada V., Martínez E. (2018). Mimicking epithelial tissues in three-dimensional cell culture models. *Front. Bioeng. Biotechnol.*, 6: 197.
- Trenfield S.J., Awad A., Madla C.M., Hatton G.B., Goyanes A., Gaisford S., Basit A.W., Trenfield S.J., Awad A., Madla C.M., Hatton G.B. (2019). Expert opinion on drug delivery shaping the future: recent advances of 3D printing in drug delivery and healthcare. *Exp. Opin. Drug Deliv.*, 16: 1–14.
- Verjans E.-T., Doijen J., Luyten W., Landuyt B., Schoofs L. (2018). Three-dimensional cell culture models for anticancer drug screening: Worth the effort? *J. Cell. Physiol.*, 233: 2993–3003.
- Wyleżół M., Ostrowska B., Wróbel E. (2016). Inżynieria biomedyczna. Metody przyrostowe w technice medycznej (in Polish). Politechnika Lubelska.
- Xiang C., Du Y., Meng G., Yi L.S., Sun S., Song N., Zhang X., Xiao Y., Jie Wan J., Yi Z., Liu Y., Xie B., Wu M., Shu J., Sun D., Jia J., Liang Z., Sun D., Huang Y., Deng H. (2019). Long-term functional maintenance of primary human hepatocytes in vitro. *Science*, 364: 399–402.
- Xing J., Luo Y., Zhan J., Kang Z. (2018). Global shape optimization of fixtures to suppress wrinkles in large-displacement membrane structures. *Int. J. Solids Struct.*, 144–145: 301–312.
- Zhang S., Buttler-Buecher P., Denecke B., Arana-Chavez V.E., Apel C. (2018). A comprehensive analysis of human dental pulp cell spheroids in a three-dimensional pellet culture system. *Archiv. Oral Biol.*, 91: 1–8.

Received: 19 XI 2020

Accepted: 20 V 2021