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Research Article

3D organotypic cell structures for drug development and Microorganism-Host interaction research

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Abstract

Introduction: The article describes a new method of tissue engineering, which is based on the use of three-dimensional multicellular constructs consisting of stem cells that mimic the native tissue *in vivo* – organoids.

3D cell cultures: The currently existing model systems of three-dimensional cultures are described.

Characteristics of organoids and strategies for their culturing: The main approaches to the fabrication of 3D cell constructs using pluripotent (embryonic and induced) stem cells or adult stem cells are described.

Brain organoids (Cerebral organoids): Organoids of the brain, which are used to study the development of the human brain, are characterized, with the description of biology of generating region-specific cerebral organoids.

Lung organoids: Approaches to the generation of lung organoids are described, by means of pluripotent stem cells and lung tissue cell lines.

Liver organoids: The features of differentiation of stem cells into hepatocyte-like cells and the creation of 3D hepatic organoids are characterized.

Intestinal organoids: The formation of small intestine organoids from stem cells is described.

Osteochondral organoids: Fabrication of osteochondral organoids is characterised.

Use of organoids as test systems for drugs screening: The information on drug screening using organoids is provided.

Using organoids to model infectious diseases and study adaptive responses of microorganisms when interacting with the host: The use of organoids for modeling infectious diseases and studying the adaptive responses of microorganisms when interacting with the host organism is described.

Conclusion: The creation of three-dimensional cell structures that reproduce the structural and functional characteristics of tissue *in vivo*, makes it possible to study the biology of the body's development, the features of intercellular interactions, screening drugs and co-cultivating with viruses, bacteria and parasites.

Keywords

drug screening, microorganism-host interactions, organoids, 3D organotypic cell structures, tissue engineering.

Introduction

A new tissue engineering technique proposed by scientists is based on using three-dimensional (3D) multicellular constructs consisting of stem cells which mimic tissue *in vivo* – organoids (Lancaster and Knoblich 2014b; Yin et al. 2016). Due to the incredible potential of 3D cell systems applying to study human biology and disease, organoids were picked by the Nature Methods as a Method of the Year in 2017 (Method of the year 2017: Organoids, 2018), and they still remain an advanced method in stem cells research (Devarasetty et al. 2018; Marsoner et al. 2018), which is confirmed by an annual growth in the number of publications (Fig. 1).

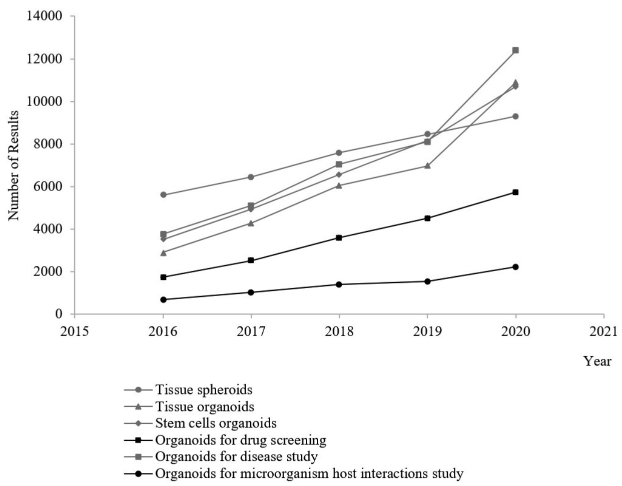

Figure 1. The number of search results in the Google Scholar system.

Organoids are a new “model organism” to study complex disease phenotypes and genetic diversity among individuals using autologous tissues from patients (Lehmann et al. 2019). The use of bioengineered three-dimensional tissue and tumor organoids becomes today the gold standard of organ and tissue replication *ex vivo* (Lancaster and Knoblich 2014b; Mills and Estes 2016; Skardal et al. 2016a; Devarasetty et al. 2018). Organoids make it possible to carry out research in 3D environment in comparison with traditional 2D cell cultures, which possibly could be limiting when trying to reproduce physiological interactions at the tissue level (Pampaloni et al. 2007).

Cells, as a part of a tissue, communicate with the neighbouring cells and extracellular matrix (ECM) via biochemical and mechanical signals. Cell-cell and cell-extracellular matrix interactions create a tissue specific 3D communication network which provides homeostasis. The key events in a cell cycle, such as adhesion, migration, proliferation and apoptosis, are regulated by the principles of tissue organization determined by tissue morphofunctional features. Thus, 3D cell cultures which replicate physiological reactions between cells and extracellular matrix are able to mimic specificity of different real tissues better than common two-dimensional cell cultures (Pampaloni et al. 2007).

Today there is evidence that organoids can be widely applied both in research and in pharmacological screening of advanced therapeutic drugs as pre-clinical diagnostic models, which makes them essential instruments in experimental medicine (Devarasetty et al. 2018).

3D cell cultures

Currently there are several 3D culture model systems. Spheroids are clusters of cells grown in suspension, which can be formed either from cell lines (homotypic spheroids) or from dissociated tumor tissues (heterotypic spheroids) (Ishiguro et al. 2017; Es et al. 2018). Spheroids can be obtained from cells of the same type or as a result of co-cultivation (mono- and multicellular spheroids, respectively) (Pampaloni et al. 2007). Such structures usually lack any organization and consist of undifferentiated cells, which is the reason to use these models to study stem cells (Baker 2018).

Spheroids can be easily obtained by cell aggregation using hanging drop technique, or when culturing in non-adhesive round-bottom plates (Loessner et al. 2010) and when cultivating cells in rotating-wall vessels (bioreactors). The main advantage of spheroids is that cell aggregation occurs without scaffolds. The cell spheroid system is often the choice for therapeutic biomedical research. They are applied in biotechnology and can be used for high-throughput screens in pharmacological studies. Simple spherical geometry makes it relatively easy to simulate dynamic processes, such as growth and invasion of solid tumors (Pampaloni et al. 2007).

Polarized epithelial tissue models are obtained by culturing non-transformed immortalized epithelial cell lines in three-dimensional conditions, e.g. growing MDCK cells or mammary epithelial MCF-10A cells as polarized monolayers on the surfaces of microporous membranes or as hollow spherical monolayers in ECM gels. Tissue analogues of such suborganic structures are common to most epithelial organs and are known as acini in mammals’ tissues. More complicated epithelial constructs, such as skin, are formed from 3D cultures on the basis of membrane inserts or using microscale materials, such as supporting fibre meshes (Pampaloni et al. 2007). At the same time, large cell aggregates require careful control of gas exchange and diffusion of soluble nutrients and chemical substances (Pampaloni et al. 2007).

Tissues can be manufactured using hydrogels, which inoculate cells during matrix formation, or using scaffolds, onto which cells are directly seeded (Loessner et al. 2010; Devarasetty et al. 2018). Engineering of 3D cell constructs based on biomaterials has advantages over the scaffold free approach, since biomaterials used as scaffolds allow better control of organoids and their microenvironment, including stiffness, ECM components and spatial organization of different cell types (Yamada et al. 2012). Biomaterials used to manufacture organoids are characterized by different porosity, levels of stiffness, cellular-adhesive motifs and viscosity, any of which can effect cellular morpho-physiological properties and tissue functioning on the whole (Skardal et al. 2012; Beck et al. 2013). Biomaterials used for manufacturing organoids mostly include collagen, hyaluronic acid, gelatine, and chitosan (Devarasetty et al. 2018). Hydrogels used as biomaterials can be used in bioproduction approaches, such as bioprinting, and they are focused on improving design and production when fabricating organoids and organs. In addition, there are hybrid approaches, such as the inclusion of aggregated tissue spheroids in hydrogels to form larger multicolonies and highly functional tissue construct models (Devarasetty et al. 2018).

Among strategies to form organoid platforms are the use of microfluidic technology which makes it possible to combine tissue organoids of different origin to generate a full body-on-a-chip. Using this system-biology-based approach, significant advances can be made in the drug development and personalized regenerative medicine (Sung et al. 2013; Devarasetty et al. 2018).

Organ-on-a-chip systems are miniature microfluidic 3D models of human tissues and “organs” designed to reproduce the most important biological and physiological parameters of their *in vivo* analogs. A number of organoid models can be combined using microfluidics, taking into account the organization of structures *in vivo*, thus providing the ability to analyze interorgan interactions (Fukuda et al. 2006; Loessner et al. 2010; Skardal et al. 2012; Yamada et al. 2012; Messner et al. 2013; Sung et al. 2013; Bhise et al. 2014; Friedman et al. 2015; Purwada et al. 2015; Peng et al. 2016; Skardal et al. 2016b; Devarasetty et al. 2017; Mazzocchi et al. 2017; Purwada and Singh 2017; Zhang et al. 2017). Furthermore, considerable attention in this area is paid to the construction of biomimetic models of organs (Moraes et al. 2012; Bhatia and Ingber 2014; Esch et al. 2015). It is increasingly recognized that the inclusion of biosensing will enable *in situ* monitoring of the status of these miniature organs (Wikswo et al. 2013; Zhang and Khademhosseini 2015).

Characteristics of organoids and strategies for their culturing

Taking into account a large number of fabrication techniques, the term “organoid” is used to define organ-specific 3D cultures (Yamada et al. 2012).

Organoids can be characterized as 3D constructs containing tissue-specific cells that perform the function of restoring the cellular microenvironment and include structures of the extracellular matrix or biomaterials (Lancaster and Knoblich 2014b). Each organ-specific 3D construct – organoid, as a rule, includes many types of cells, and the ratio of cell types is optimized in order to induce the organoid function, which can be investigated using organ-specific biomarkers or other techniques (Skardal et al. 2016a; Devarasetty et al. 2018).

Pluripotent (embryonic or induced) stem cells or adult stem cells obtained from various sources can be used to establish organoids (Lancaster and Knoblich 2014b; de Souza 2018). Under conditions of growth in a three-dimensional environment, stem cells of different origins (hESCs/hiPSCs/hAdSCs) get self-organized into organoids due to the proliferation and differentiation of various types of cells, which suggests that they are identical to their analog *in vivo* (Drost and Clevers 2017) if at least some of the functions of the organ are replicated (Huch and Koo 2015). Some methods of establishing organoids from pluripotent stem cells imply the communication of minimal information to the cells for differentiation (apart from providing them with the necessary conditions for growth and nutrients), which contributes to the implementation of an internal program of self-organization and, probably, mediates the launch of random processes necessary for tissue formation (de Souza 2018) or, on the contrary, organoids are cultivated in a solid matrix in order to reproduce the natural structural characteristics and cellular composition of native tissues (Baker 2018).

Two main approaches to the formation of organoids from stem cells were first described in 2009.

Sato et al. characterized the conditions for unlimited 3D expansion of organotypic structures *in vitro* from one Lgr5 + ISCs obtained from intestinal crypts (Sato et al. 2009). These organoids contained all types of intestinal epithelial cells and were structured into proliferating crypts and differentiated villi, thus preserving the architecture of the intestine *in vivo* (Sato et al. 2009). Additionally to Wnt/R-spondin, the researchers included epidermal growth factor (EGF) in the cocktail, noggin (which inhibits BMP) and an artificial extracellular matrix enriched with laminin (provided by Matrigel) for the successful propagation of organoids derived from mouse ISCs (Dignass and Sturm 2001; Haramis et al. 2004; Drost and Clevers 2017). At the same time, Ootani et al. showed another culture of ISCs activated via Wnt (Ootani et al. 2009). In contrast to the experiments by Sato et al., the supply of growth factors to the medium in this culture was provided by the underlying stromal component, rather than by the inclusion of specific growth factors into the medium, and the organoids were cultured in collagen at the air-liquid interface (Drost and Clevers 2017).

It is important to note that both studies “restored” the stem cell niche *in vitro* either by artificially adding niche factors (Noggin, EGF, Wnt, R-spondin) (Sato et al. 2009) or by including the mesenchymal component into the culture (Ootani et al. 2009; Drost and Clevers 2017).

The Sasai lab started using pluripotent stem cells to create various components of the central nervous system. They differentiated human pluripotent stem cells into self-organizing three-dimensional aggregates, which were named SFEBq (serum-free floating culture of embryoid body-like aggregate with quick reaggregation) which contained apically-basally polarized cortical tissue (Eiraku et al. 2008; Eiraku et al. 2011; Marsoner et al. 2018).

The technology for organoids establishing developed from cultures of embryoid bodies, which are 3D aggregates of stem cells, self-organizing to form various tissues *in vitro* (Huch and Koo 2015).

Organoid establishing process involves the isolation of tissue of interest from the body and its careful dissociation to release a population of progenitor cells, either tissue-specific adult stem cells or pluripotent embryonic stem cells, usually with no isolation and purification of stem cells required (Sato et al. 2011). Induced pluripotent cells can be used as an alternative. Usually, cells are immersed in a 3D Matrigel matrix, which contains laminin, entactin, proteoglycans, and collagen IV (Es et al. 2018). Cells are fed with enriched media if they originate from mesodermal tissues (for instance, intestines and liver) or with depleted media if they come from neuroectodermal tissues (for example, brain and retina). In both cases, the medium should contain tissue-specific niche growth factors that provide controlled differentiation and tissue organization (Lancaster and Knoblich 2014b). For intestinal tissues, these are epidermal growth factor, morphogenetic proteins Noggin and WNT3, and the WNT signal amplifier R-spondin (Dutta et al. 2017).

For nerve tissues, pluripotent stem cells must first undergo neuronal induction and then it becomes possible to form organoids of specific brain regions using appropriate factors (Yin et al. 2016).

It is known that organoids obtained from adult stem cells can keep native organ characteristics and genetic stability for a long time (Behjati et al. 2014; Huch et al. 2015; Drost and Clevers 2017), which has been proven by multiple passaging (Sato et al. 2011; Huch et al. 2015) and complete genome sequencing of some organoids at the beginning and at the end of culturing (Behjati et al. 2014; Huch et al. 2015; Drost and Clevers 2017).

Since the first organoids were obtained from adult stem cells – Lgr5 + stem cells, which were used to establish the so-called "mini-intestine", phenocopying the epithelium of intestinal crypts and villi (Sato et al. 2009), organoids have been established from tissues of various organs and structures of the body (Clevers 2016; Rios and Clevers 2018) – mouse and human liver, pancreas, stomach, fallopian tubes, prostate, taste buds, salivary gland, kidneys, breast, lungs and brain (Barker et al. 2010; Huch et al. 2013a; Huch et al. 2013b; Chua et al. 2014; Karthaus et al. 2014; Ren et al. 2014; Boj et al. 2015; Bartfeld et al. 2015; Kessler et al. 2015; Huch et al. 2015; Maimets et al. 2016; Drost and Clevers 2017; Ng-Blichfeldt et al. 2019).

It has already become apparent that the study of organoids is the most developing field in cell biology and tissue engineering (Marsoner et al. 2018).

There are several characteristics of organoids that determine their value as test systems (models). They can be obtained from both healthy and damaged tissues of animals and humans, making it possible to simulate tissue homeostasis, recovery, and various pathological processes. Organoids can be cultured for an indefinitely long period of time in a medium with a given composition, remaining stable and preserving tissue features, making it possible to accurately scale the experimental material corresponding to the source tissue. And finally, organoids are suitable for the implementation of almost all cellular biological and molecular technologies that are used on cell lines and can be transplanted into the body of animals for the study of human diseases *in vivo* (Rios and Clevers 2018). It should be noted that the technology for the establishing of organoids is universal. For their cultivation, both small fragments of tissue obtained by biopsy and samples obtained by surgical excision can be used (Drost and Clevers 2017). In addition, organoids provide a unique opportunity to model human organogenesis, which is not available in traditional experimental models (Qian et al. 2016).

Brain organoids (Cerebral organoids)

The complexity of the human brain structure complicates the study of its disorders using model organisms, emphasizing the need to create an *in vitro* model of human brain development (Lancaster et al. 2013; Lancaster and Knoblich 2014a). Brain organoids represent a completely new platform for the study of human brain development (Qian et al. 2016; Quadrato et al. 2016). Generation of region-specific brain organoids (Muguruma et al. 2015; Sakaguchi et al. 2015; Jo et al. 2016; Qian et al. 2016) further facilitates the modeling of specific areas of the brain. Recently, tangential migration of cortical interneurons has been replicated *in vitro* by fusion of organoids that reproduce the cortex (hCO) and MGE or subpallium (hMGEO) of the brain to allow functional integration (Bagley et al. 2017; Birey et al. 2017; Xiang et al. 2017).

It was revealed that the developing neocortex is organized into separate compartments of proliferative progenitors, the ventricular zone (VZ) and subventricular zone (SVZ), which give rise to the outer neuronal layers in the cortical plate (CP). VZ and SVZ contain different types of neuronal progenitors: apical radial glial cells (aRG) in VZ and basal radial glial cells (bRG), intermediate progenitors (IPs) and transint amplifying cells in SVZ. The key factor contributing to the growth of the human neocortex is the expansion of SVZ progenitors (Watanabe et al. 2017).

The development of the mammalian brain begins with the expansion of the neuroepithelium, followed by the generation of radial glial stem cells (RGs) (Lancaster et al. 2013).

Lancaster et al. characterized the formation of neuroectoderm from embryoid bodies. The neuroectodermal tissue was then maintained in 3D culture and placed in Matrigel drops to provide a scaffold for more complex tissue growth. Matrigel drops were then transferred to a bioreactor to enhance nutrient absorption. At the initial stages (15–20 days), cerebral organoids formed a neuroepithelium surrounding a fluid-filled cavity resembling a ventricle with a characteristic apical localization of neural specific N-cadherin (NCAD). After being cultured for 2 months in the bioreactor, organoids became the formations consisting of heterogeneous tissues, showing the structures resembling cortex, choroid plexus, retina, and meninges. Cerebral organoids of similar morphology and composition can be established from both embryonic stem cells (ESCs) and induced human pluripotent stem cells (Lancaster et al. 2013).

Several protocols have been described for the cultivation of cerebral organoids derived from hPSCs that precede the emergence of basal progenitors. Watanabe et al. showed that enhanced stimulation of the STAT3 pathway increased the production of basal progenitors, improved the formation and separation of neural layers, and promoted astrogliogenesis (Watanabe et al. 2017).

Most cortical region-specific organoids are formed under the influence of small pathway modulators, including SMAD signaling inhibitors, required to prevent mesoderm and endoderm differentiation (Eiraku et al. 2008; Kadoshima et al. 2013; Mariani et al. 2015; Paşca et al. 2015; Qian et al. 2016; Iefremova et al. 2017; Xiang et al. 2017).

Some studies examined the migration of neuronal cells, which reproduce the migration of cortical inhibitory interneurons originating from the ventral part of the forebrain by fusion of organoids originating from the dorsal and ventral regions of the brain (Bagley et al. 2017). It was also shown that microglia, originating from the mesoderm and being one of the key players in neuronal development, can also develop within the cerebral organoid model (Ormel et al. 2018).

Li et al. showed that the PTEN-AKT signaling pathway is involved in the formation of human cerebral organoids that are enlarged and exhibit surface folding. At the same time, removal of PTEN is accompanied by an increase in neuronal progenitors proliferation and the formation of larger and folded cerebral organoids (Li et al. 2017).

There is evidence of growth factors being involved in the regulation of expansion and folding of the cortex, including PDGF-D (platelet-derived growth factor D) (Li et al. 2017).

Xiang et al. developed a technque for differentiating hESCs into thalamic brain organoids (hThOs), manufactured a 3D model to reproduce reciprocal thalamic-cortical projections (TC projections) between the thalamus and cortex by fusion of hThOs and hCOs, resulting in the formation of united thalamo-cortical organoids (hThCOs) (Xiang et al. 2019).

Lung organoids

Human lung organelles (HLOs) are composed of basal cells, ciliated epithelial cells, goblet cells, and CC10-secreting club cells (Sachs et al. 2019).

The method of lung lineages formation due to stimulation of differentiation of hPSCs, both hESCs and iPSCs, is described (Kadzik and Morrisey 2012; Longmire et al. 2012; Mou et al. 2012; Wong et al. 2012; Ghaedi et al. 2013; Huang et al. 2014; Dye et al. 2015); however, most studies were performed on 2D cultures. Dye et al. established a three-dimensional model of the human lung by stimulating human stem cells differentiation, influencing several signaling pathways that control organ formation during embryonic development. At first, stem cells form endoderm, from which lungs, liver and some other internal organs are formed during embryogenesis. Then, in endodermal cultures derived from hPSCs, WNT and FGF signaling pathways were activated, and simultaneously BMP/TGFβ signaling pathways were inhibited, which prevented commitment along the intestinal line, and instead led to the formation of SOX2+ anterior foregut and to the rapid formation of SOX2+ anterior foregut 3D spheroidal structures. In order to further restrict the differentiation of the foregut spheroids in the direction of the pulmonary line by using HH^{Hi} conditions during the generation of foregut spheroids, the expression of NKX2.1 increased in cells that formed spheroids, and then the spheroids were placed for further growth in a medium containing FGF10, which enabled them to grow into organoids. Organoids persisted in culture for over 100 days and developed into well-organized proximal-like epithelial structures of the airways, which included many cell types found in the proximal lung epithelium, basal cells, ciliated cells, and rare club cells. Moreover, the proximal airway structures were often surrounded by smooth muscle actin positive mesenchymal tissue. Organoids also contained distal-like epithelial cells that expressed progenitor markers, SFTPC/SOX9 and HOPX/SOX9, corresponding to early bipotent alveolar progenitor cells found in mice (Desai et al. 2014; Treutlein et al. 2014; Dye et al. 2015). Obviously, the proximal and distal regions of lungs contain different types of epithelial cells which realise various functions. For instance, basal, secretory and ciliated cells in the airways and type-2 alveolar epithelial cells (AEC2s), which secrete surfactant and other proteins, and sensitive type-1 alveolar epithelial cells (AEC1s), which form a large surface in the alveoli, which serves for gas exchange with the surrounding capillaries. In addition, the outer mesothelial layer and immune cells are important populations of lung tissue cells (Tan and Krasnow 2016; Barkauskas et al. 2017).

During organoids establishing from basal cells obtained from the trachea or large airways, a medium containing EGF, CFE (up to 20%) is used for cultivation; human fibroblast cell lines, such as MRC5 cells, can be added there. Under the standard conditions, organoids contain

TRP63+ KRT5+ basal cells, functional ciliated cells, and secretory goblet cells (MUC5AC+, MUC5B+) (Danahay et al. 2015; Butler et al. 2016; Hild and Jaffe 2016).

It is possible to use organoids formed from human basal cells for screening cytokines and other proteins that could influence the ratio of ciliated cells to secretory cells, which is impaired, for example, in chronic asthma (Barkauskas et al. 2017). Epithelial cells and mesenchymal stem cells are required to form alveolospheres using AE-C2s (Barkauskas et al. 2017).

TGF-β activation is known to impair the ability of fibroblasts to support the formation of organoids from lung epithelial progenitor cells (Ng-Blichfeldt et al. 2019). TGF-β is a pleiotropic cytokine that exhibits a variety of transcriptional effects by interacting with type I and type II TGF-β receptors and subsequent phosphorylation and nuclear translocation of Smad2/3. Exposure to TGF-β may also interact with the activation of proreductive signaling pathways, including the Wnt/β-catenin signaling pathway (Ng-Blichfeldt et al. 2019).

Liver organoids

To replicate liver development *in vitro*, several scientific groups successfully differentiated human iPSCs into hepatocyte-like cells using a sequential differentiation protocol based on several chemical inhibitors (Palakkan et al. 2017; Prior et al. 2019). A different approach was chosen in the Suzuki and Hui laboratories, in which they triggered forced expression of the first hepatic transcription factors (*HNF4a*, *FOXA1*,*2*,*3*) (Sekiya and Suzuki 2011) or (*HNF4a*, *GATA4 u HNF1B*) (Huang et al. 2011) in order to induce direct differentiation of iPSCs into hepatocyte-like cells *in vitro*. However, these approaches were implemented under the 2D cell culture conditions. The first endeavors to form 3D hepatic organoids were made using cultures of the embryonic liver bud by Takebe et al.: during the experiment, hepatocytes obtained from iPSCs were cultured with umbilical cord mesenchymal stem cells (Takebe et al. 2013). Since then, the scheme developed by Takebe et al. has been modified so that hepatic endoderm, mesenchymal and endothelial progenitors are derived from iPSCs (Takebe et al. 2017). In addition, human iPSCs can be differentiated by targeting specific signaling pathways into cholangiocyte organoids (Sampaziotis et al. 2015). It is also emphasized that hepatobiliary structures containing hepatocytes and cholangiocytes were obtained from iPSCs (Vyas et al. 2018; Wu et al. 2019). However, a limitation to the use of iPSCs organoids in clinical practice is their genomic instability resulting from exposure to reprogramming factors (Tapia and Schöler 2016).

Intestinal organoids

Organoids can be obtained from two sources of stem cells: organ-specific adult stem cells (ASCs) and pluripotent stem cells (PSCs), both induced (iPSCs) and embryonic (ESCs) (Rahmani et al. 2019). The use of these approaches makes it possible to obtain 3D structures which would have microarchitecture of villi and crypts of the small intestine, capable of self-renewal and self-organization over a long period. Wnt-3a, Epidermal growth factor, Noggin and R-spondin are the key components, the presence of which in the culture medium is mandatory; a medium containing a complex of these factors is called WENR medium. The spatio-temporal incorporation of these growth factors into the culture medium regulates the triggering of stem cell niche signaling pathways, including Wnt, bone morphogenetic protein (BMP), and Notch, which induce ISCs to self-renew, proliferate, and differentiate. It was also shown that the inclusion in the ENR medium of such additional combinations of components as CHIR99021 and valproic acid, or LDN-193189, and CHIR99021 has a synergistic effect that contributes to the maintenance of Lgr5+ ISCs in a self-renewing and undifferentiated state, which leads to the enrichment of the culture of ISCs. Whereas a differentiated phenotype can be obtained using ENR media supplemented with the following pairs of molecules: DAPT and CHIR99021, valproic acid and IWP-2, or DAPT and IWP-2. These molecules coordinate the action of each other and induce the direct differentiation of ISCs into Paneth cells, enterocytes and secretory cell lines called goblet cells and enteroendocrine cells, respectively. It was demonstrated that the addition of DAPT or BMP molecules to the culture medium is sufficient to stimulate the differentiation of ISCs and the generation of multicellular intestinal organoids. Moreover, some studies have been carried out to search for methods to reduce the cost of culture media, which reported that the Noggin protein can be replaced by LDN-193189, and R-spondin 1 protein can be substituted by RS-246204 (Liu et al. 2018; Nam et al. 2018; Rahmani et al. 2019).

Induced human intestinal organoids (iHIOs) derived from pluripotent cells represent a new experimental model for studying intestinal pathogens (Karve et al. 2017). Karve et al. described the *in vitro* generation of iHIOs from pluripotent embryonic stem cells by triggering a process that replicated normal differentiation and made it possible to get organoids that reproduce tissue of the distal human small intestine to which *E. coli* preferentially attaches. Organoids have a luminal cavity, a microscopic brush border, villi, and crypts. The organoid epithelium contains enterocytes and major secretory lines (Paneth cells, endocrine cells, and goblet cells), and retains such functions as peptide transport and mucus secretion by goblet cells. The epithelium is surrounded by a layer of mesenchyme, which contains smooth muscle cells and subepithelial fibroblasts. iHIOs have been successfully used to study embryonic development, inflammatory bowel disease and infections (Karve et al. 2017).

Organoids obtained through cultivation of adult stem cells are formed by harvesting stem cells contained in crypts, or by isolating single Lgr5-expressing ISCs of the small intestine or human colon tissue, depending on which they are called enteroids or colonoids, respectively (Stelzner et al. 2012). Organoids derived from adult stem cells have limitations, such as the absence of mesenchymal stem cells, including myofibroblasts, endothelial cells, and smooth muscle cells, which could secrete growth factors into the medium and thus influence signaling pathway activity and cell proliferation. In this connection, it is imperative to use a cocktail of growth factors called WENR for the cultivation of enteroid organoids, enteroids (Rahmani et al. 2019).

It is known that the technology of culturing intestinal organoid (mini-gut) is actively used, including modeling diseases and therapeutic effects, studying interaction of the host and microorganism, delivery of biomolecules, biology and intestinal development (Rahmani et al. 2019).

Osteochondral organoids

Osteochondral organoids were manufactured from mouse induced pluripotent stem cells to study the interactions between bone and cartilage tissues, understanding the interaction of which is of particular importance for solving the problem of osteoarthritis. Organoids were grown by time-dependent sequential exposure to growth factors, transforming growth factor β-3 (TGF-β3) and bone morphogenetic protein 2 (BMP2) to reproduce bone development by endochondral ossification. As a result, cartilaginous regions and calcified bone regions within the organoid were obtained, with the potential for screening drugs intended for the treatment of joint diseases and studying the genetic risk in patients or the specifics of a disease development (O’Connor et al. 2020).

Use of organoids as test systems for drugs screening

One of the key reasons to establish and use 3D cellular constructs is their potential impact on the new drugs development. When used in organoid research, candidate compounds are more efficiently screened prior to *in vivo* testing, which increases the chances of success and reduces drug development costs.

Organoid models not only make it possible to perform effective testing on target tissues the drug is aimed at, but also to reveal the possible toxic effect of the substance on vital organs: heart, liver, lungs, which allows preventing unexpected complications that can lead to serious side effects (Rajkumar et al. 2013; Devarasetty et al. 2018; Miranda et al. 2018).

Most studies performed using microphysiological systems are aimed to study the toxicity of drugs, and only in some cases to identify their effectiveness (Denisuk et al. 2015; Truskey 2018; Korokin et al. 2019).

Cardiac models for toxicity research should mimic the electrical activity of cardiomyocytes and be sensitive to cytotoxic effects that damage heart muscle cells. Almost all models of cardiac organoids use cardiomyocytes as the main components of 3D structures, but they use them in different ways (Devarasetty et al. 2018; Korokin et al. 2020). For example, by using organoids it was shown that adrenaline increased the heart rate, but the developing effect can be blocked by the addition of propranolol. It was revealed that digoxin and isoproterenol have a modulating effect on the strength and timing of contractions. Numerous proarrhythmic compounds have been tested, and concentration-dependent and reversible contraction changes were demonstrated (Devarasetty et al. 2018).

Various drugs that affect the strength and duration of cardiac muscle contractility have a similar effect on the microphysiological systems consisting of cardiomyocytes derived from iPSCs (beat frequency or contractile stress) (Mathur et al. 2015; Huebsch et al. 2016; Lind et al. 2017; Truskey 2018).

A significant number of drugs with known hepatic toxicity have been tested on liver organoids. Currently, the gold standard is the inclusion of primary human hepatocytes into 3D constructs that will be used for drugs screening (Messner et al. 2013).

Vernetti et al. characterized the functional association of microphysiological systems, the joint work of the stomach and liver, which metabolized toxic terfenadine to its non-toxic and vasoactive form fexofenadine, which could not penetrate the blood-brain barrier (Vernetti et al. 2017).

An ideal lung model for drug screening and toxicity testing should provide information on the characteristics of gas exchange, respiration rate and/or cell viability. Skardal et al. formed lung organoids by layering lung epithelial cells on fibroblasts and endothelial cells. The TEER sensor was integrated into the system to demonstrate that the organoid response was similar to that of the organ *in vivo*. The study showed that when exposed to bleomycin, a chemotherapeutic agent used to treat lymphoma, pulmonary organoids secreted interleukin 8, a lung-specific marker of inflammation (Skardal et al. 2017).

Brain organoids were cultured in the lumen of microfluidic channels to provide perfusion, and then the response to nicotine was tested. Inhibition of the differentiation and organization of organoids was revealed upon exposure to nicotine concentrations recorded in smoking abusers, which, according to the authors, may account for impaired fetal neurogenesis in smoking mothers (Truskey 2018; Wang et al. 2018).

Most drugs are designed to treat specific symptoms and disease etiology, so testing is performed on 3D models of the disease of interest, sometimes referred to as “disease-in-a-dish” or “disease-on-a-chip” models (Devarasetty et al. 2018). For example, organoids were created that simulated liver fibrosis that can be used to screen drugs for the treatment of liver fibrosis (Devarasetty et al. 2018).

Leite et al. cultivated hepatocyte-like cells (HepaRG) and primary hepatic stellate cells (HSCs) in the spheroid format, after which the spheroids were treated with profibrotic compounds – allyl alcohol and methotrexate, which led to the activation of HSCs and the generation of fibrosis (Leite et al. 2016). Prestigiacomo et al. characterized a similar spheroid-based system, in which, in addition to HSCs and HepaRGs, Kupffer cells were included. Spheroids were treated with transforming growth factor-b1, methotrexate and thioacetamide to induce the activation of both HSCs and Kupffer cells for the development of fibrosis (Prestigiacomo et al. 2017).

The use of human intestinal organoids grown from crypt fragments was characterized for the study of cystic fibrosis (CF), caused by mutations in the cystic fibrosis transmembrane conductance regulator gene (CFTR) that encodes epithelial anion channels (Dekkers et al. 2016). Organoids have been used in preclinical studies to identify and develop CFTR modulating drugs and to study the mechanisms associated with differences in CFTR functioning (Van Mourik et al. 2019). In a study on organoids by Dekkers et al., CFTR mutations and additional patient specific genetic traits were shown to modify the response to CFTR modulators (Dekkers et al. 2016). Vallier et al. also showed that iPSCs obtained from CF patients differentiated into hepatic cholangiocytes and could serve as an *in vitro* model of CF (Sampaziotis et al. 2015; Lancaster and Huch 2019).

Benam et al. created a lung organoid and integrated it into a microfluidic chip containing epithelial and endothelial cells. The 3D construct was used to stimulate asthma and chronic obstructive pulmonary disease. Interleukin 13 (IL-13) treatment led to an increased number of goblet cells and increased secretion of granulocyte-colony stimulating factor (G-CSF) and granulocyte-macrophage colony-stimulating factor (GM-CSF), two inflammatory cytokines, roughly corresponding to the asthmatic response (Benam et al. 2016; Devarasetty et al. 2018). Such systems are based on cell-cell and cell-matrix interactions and are ideal for use in 3D organoids, since the disease state is directly related to changes in tissue microenvironment and cellular phenotype, which are best reflected in 3D.

Currently, a number of different tumor organoids and “tumor-on-a-chip” systems have been developed. Organoids have been successfully grown from primary tumors of the colon, prostate, breast, and pancreas. These “tumoroids” have evolved into preclinical models that can predict a patient’s individual response to treatment (Clevers 2016; Li and Izpisua Belmonte 2019). Patient-derived tumor organoids are better at replicating native tumors and potentially better models for identifying and testing new anticancer drugs (Drost and Clevers 2018; Vlachogiannis et al. 2018). For example, when drug screening using organoids of primary human liver cancer, ERK inhibition has been identified as a potential therapeutic approach for treating primary liver cancer (Broutier et al. 2017; Drost and Clevers 2018).

Boretto et al. manufactured and cultivated for a long time organoids that simulate a wide range of endometrial pathologies: from endometriosis and hyperplasia to low and high grade cancers. Organoids that model endometriosis have been shown to exhibit disease-specific features and cancer-related mutations. Organoids established from tumor-affected endometrium accurately characterize cancer subtypes, replicate altered tumor surfaces, and demonstrate patient-specific drug responses (Boretto et al. 2019).

Driehuis et al. described the creation of 30 patient-derived organoid lines (PDOs) from tumors arising in the pancreas and distal bile duct. PDOs mimic tumor histology and contain genetic changes typical of pancreatic cancer. The testing of 76 therapeutic agents, including chemotherapy drugs currently used for the treatment of pancreatic ductal adenocarcinoma (PDAC), has been characterized, which has revealed sensitivity to drugs not currently used in the clinic, which emphasizes the importance of an individualized approach to effective cancer treatment. The PRMT5 inhibitor EZP015556 has been shown to have an effect on both MTAP (a gene commonly lost in pancreatic cancer) -negative and MTAP-positive tumors (Driehuis et al. 2019).

An important advantage of using organoid technology for drug development is that both healthy and tumor tissues can be used to establish organoids, allowing for screening drugs that specifically target tumor cells while leaving healthy cells intact. This approach can lead to a decrease in the damaging effects of drugs on the organism (Drost and Clevers 2018).

The ability to create organoids from individual tumors will allow introducing a huge clinical variety of tumor tissues into the laboratory. In this connection, considerable efforts are being made to make organoids available to the scientific community, including by creating “living biobanks” (for example, the Hubrecht Organoid Technology (HUB) “living” biobank). Thus, the HUB contains about 1,000 tumor cell models, including tumors of the breast, lungs, pancreas, and prostate cancer (Weeber et al. 2017; Tuveson and Clevers 2019).

In general, it is believed that the reliability of the data obtained through organoid-based drug screening will increase as screening and data analysis methods become more standardized (Driehuis et al. 2020).

Using organoids to model infectious diseases and study adaptive responses of microorganisms when interacting with the host

Infectious diseases, predominantly diarrhea, AIDS, tuberculosis, and malaria, kill about 20 million people every year. The so-called “successful pathogens” overcome the barriers of innate and acquired immunity due to the presence of evolutionarily formed mechanisms, including bacteria evading recognition; creation of obstacles to phagocytosis and intracellular killing; the use of secretory systems in the form of a “syringe” for the introduction of dysregulating substances into the host cells; suppression or stimulation of the inflammatory response; activation of inhibitory receptors to suppress the respiratory burst in the phagosome; the effect on inflammasomes and the subsequent decrease in the synthesis of pro-inflammatory cytokines; the ability to stimulate the synthesis and secretion of cytokines that suppress the innate immune response; disruption of the functioning of key molecules of intracellular signaling pathways; impact on the processes of apoptosis and autophagy, accompanied by survival and replication within host cells (Garib and Rizopulu 2012).

In addition, pathogens (infectious diseases causative agents) have adapted to recognize the specific structures of the host organism, polarity, and changes in the stimuli of the local environment (pH, temperature, oxygen content, nutrients, hormones, physiological forces) in order to timely activate specific damaging programs during the stages of infection (Nickerson et al. 2004; Alsharif et al. 2015; Fang et al. 2016; Persat 2017).

The study of the peculiarities of the interaction of host organisms and the pathogen will reveal the evasion mechanisms of pathogenic microorganisms and use them in order to create original vaccines and fundamentally new drugs for the correction of impaired functions of the immune system in numerous diseases, such as malignant neoplasms, autoimmune and allergic diseases, as well as infectious diseases (Garib and Rizopulu 2012).

Organoids are now widely used to study the interaction of host cells with microorganisms, including viruses, bacteria, and parasites (Fatehullah et al. 2016; Barrila et al. 2018; Duque-Correa et al. 2020). The use of organoids allows the reconstruction of a three-dimensional micro-environment characteristic of the host organism and regulating infection, which includes multicellular complexes, commensal microbiota, gas exchange, and nutrient gradient, as well as physiologically relevant biomechanical forces (for example, shear stress, tension, compression), the reconstruction of which is one of the key tasks in 3D microenvironment modeling (Barrila et al. 2018).

It has been revealed that enteric microbes effect the pathogenesis of a wide range of intestinal immune-mediated diseases and systemic disorders (Hsiao et al. 2013). Various pathogens have been studied using 3D enteroid/colonoid/organoid models, including *Salmonella*, *C. difficile*, EHEC, EPEC, enterotoxic *E. coli* (ETEC), noroviruses, rotaviruses, enteroviruses, *Toxoplasma gondii*, and coronaviruses (Zhang et al. 2014; Engevik et al. 2015; Forbester et al. 2015; Bartfeld 2016; In et al. 2016a; Hill and Spence 2017; Karve et al. 2017; Barrila et al. 2018).

Induced human intestinal organoids (iHIOs) are a suitable model for studying the pathophysiology of human viral gastroenteritis caused by human rotavirus (HRV) or norovirus (HuNoV) (Yin et al. 2015; Leslie and Young 2016; Zou et al. 2017; Blutt et al. 2018). The use of iHIOs obtained from the cultivation of human stem cells was described for modeling the infectious process caused by rotaviruses (Finkbeiner et al. 2012) during co-cultivation of HIOs with rotavirus, the virus was shown to affect human IECs, mainly enterocytes and enteroendocrine cells, as well as mesenchymal cell lines (Rahmani et al. 2019).

Organoid models are used to study bacterial pathogenesis, including *Vibrio cholerae*, *Clostridium difficile*, *Shigella*, which infect intestinal organoids of murine, bovine, porcine and human origin (In et al. 2016b; Dutta and Clevers 2017; Dutta et al. 2017; Barrila et al. 2018; Derricott et al. 2019; Duque-Correa et al. 2020), as well as *Helicobacter pylori*, which colonizes stomach organoids (Duque-Correa et al. 2020). In addition, the participation of bacteria in the formation of adenocarcinoma in the gallbladder was investigated using gallbladder organoids infected with *Salmonella* (Dutta and Clevers 2017; Dutta et al. 2017). Obligate anaerobic bacteria, such as *Clostridium difficile* (*C. difficile*) survive when cultured in the cavity of HIOs. Such studies shed light on the potential of intestinal organoids for studying anaerobic bacteria, which are abundant in the gut microbiome (Rahmani et al. 2019).

Intestinal enteroids are also successfully cultured with such bacteria as *Salmonella enterica serovar Typhimurium*, which leads to the development of gastroenteritis in humans, *Escherichia coli* (*E. coli*), *Lactobacillus reuteri D8*, the presence of the latter two not only enhances the growth of enteroids, but also reduces damage caused by TNFα, which leads to ICS regeneration (Hou et al. 2018). It has also been demonstrated that lactobacilli are capable of exerting a modulating effect on the host's immune system. However, epithelial-commensal bacterial interactions with the host organism have been hardly studied due to limited access to the tissues of the human small intestine (Son et al. 2020). Some lactobacilli species have developed the ability to stimulate the generation of reactive oxygen species (ROS) in epithelial cells, which leads to the proliferation of intestinal epithelial cells through processes requiring the catalytic action of Nox1. Further studies showed that feeding the same species of lactobacilli improves wound healing and promotes the restoration of the intestinal epithelium after mechanical damage by means of the mechanisms that were dependent on the formyl peptide receptor, ROS and Nox1. However, little is known about the cellular signaling pathways that are activated in response to lactobacillus-induced ROSs in the intestinal epithelium and mediate the transmission of bacterial-initiated signals to subepithelial compartments (Darby et al. 2020). Studies have shown that lactobacilli (Lactobacillus) can effectively prevent the invasion of pathogens and protect the integrity of the intestinal mucosal barrier. However, the probiotic role of Lactobacillus mainly means the induction of low pH, secretion of antimicrobial peptides, and maintaining tight junctions (Lu et al. 2020).

An infection caused by an uropathogen, *Enterococcus faecalis*, has been studied using urothelial organoids (Horsley et al. 2018). More recently, fallopian tube organoids have served as a model to investigate long-term effects of the infection caused by *Chlamydia trachomatis* (*Ctr*) on human epithelium, which can lead to ovarian cancer (Kessler et al. 2019). It was revealed that the *Ctr*-induced infection activated the LIF signaling pathway, which is important for regulating the ability of organoid component cells to maintain an undifferentiated stemness. Infected organoids showed a less differentiated phenotype, which was confirmed by the increased efficiency of organoid formation. Moreover, *Ctr* increases DNA hypermethylation, which is an indicator of accelerated molecular aging (Kessler et al. 2019).

The complete life cycle of *Cryptosporidium parvum* can be modeled in murine and human small intestine (Heo et al. 2018; Wilke et al. 2019) and lung organoids (Heo et al. 2018). Lung organoids successfully replicate *C. parvum*-induced infections of the respiratory tract that develop in immune competent and non-immune organisms (Heo et al. 2018).

Conclusion

Thus, the creation of three-dimensional cell structures that reproduce the structural and functional characteristics of tissue *in vivo* makes it possible to study biology of the body’s development, the features of intercellular interaction under normal nicotine physiological conditions and pathology, screening drugs and co-cultivating with viruses, bacteria and parasites.

Table 1 shows the features of using various 3D cell structures in pharmacological research.

Despite the fact that the issues of vascularization of organoid systems, modeling intercellular communication with populations of stromal cells, and standardization of the procedure for creating 3D tissue-engineered constructs remain unresolved, organoid systems have great potential and provide unprecedented opportunities for improving human health.

Conflict of interests

The authors report no conflicts of interest.

Table 1. Using Different Organoid Systems in Drug Screening Research.

3D Model	Modifications	Using for drug screening, including therapies tested	Refs
Brain organoids	Organ-on-a-chip	Study of the effect of nicotine on the processes of neurogenesis	Truskey 2018; Wang et al. 2018.
	Organoids derived from primary tumors	Anti-cancer drug screening	Truskey 2018; Wang et al. 2018
Lung organoids	Organoids derived from primary lung tumor	Anti-cancer drug screening	Sachs et al. 2019
	Layering of lung epithelial cells on fibroblasts and endothelial cells, adding a sensor to the TEER system	Study of the effect of bleomycin on the secretion of inflammatory markers	Skardal et al. 2017
	Organ-on-a-chip, models of asthma and chronic obstructive pulmonary disease	Interleukin 13 (IL-13) exposure, study of asthmatic response	Benam et al. 2016; Devarasetty et al. 2018
Heart organoids	Cardiac organoid models with cardiomyocytes as major components	Study of the effect of adrenaline, digoxin, isoproterenol and a variety of proarrhythmic compounds on the strength and timing of contractions	Devarasetty et al. 2018
	Microphysiological systems consisting of cardiomyocytes derived from iPSCs	Study of the effect of drugs on beat frequency or contractile stress	Mathur et al. 2015; Huebsch et al. 2016; Lind et al. 2017; Truskey 2018
Mammary gland (breast) organoids	Organoids from primary breast tumor – “tumoroids”	Anti-cancer drug screening	Sachs et al. 2018; levers 2016; Li and Izpisua Belmonte 2019
Liver 3D constructs	Liver organoids, including primary human hepatocytes	Screening liver-toxic drugs	Messner et al. 2013
	Organoids grown from a liver tumor	Anti-cancer drug screening, search for therapeutic approaches	Broutier et al. 2017; Drost and Clevers 2018
	Functional microphysiological systems that combine stomach and liver organoids	Study of the metabolism of toxic terfenadine to its non-toxic and vasoactive form fexofenadine	Vernetti et al. 2017
	Formation of spheroids from hepatocyte-like cells (HepaRG) and primary hepatic stellate cells (HSCs) (and possibly Kupffer cells), followed by the generation of a fibrotic state	Effect of transforming growth factor-b1, methotrexate and thioacetamide to induce simultaneous activation of HSCs and Kupffer cells for the development of fibrosis	Leite et al. 2016; Prestigiacomo et al. 2017
Pancreas organoids	30 patient-derived organoid lines (PDOs) from tumors originating in the pancreas and distal bile duct, “tumoroids”	Testing of 76 therapeutic agents was characterized, including chemotherapy drugs currently used to treat pancreatic ductal adenocarcinoma (PDAC)	Driehuis et al. 2019; Clevers 2016; Li and Izpisua Belmonte 2019
Stomach organoids	Organoids derived from primary stomach tumors	Anti-cancer drug screening	Yan et al. 2018; Driehuis et al. 2019
Intestinal organoids	Intestinal tumor-derived organoids	Anti-cancer drug screening	Yan et al. 2018
	Intestinal organoids grown from crypt fragments as a model for the study of cystic fibrosis	Organoids were used in preclinical studies to identify and develop CFTR modulating drugs and to study the mechanisms associated with differences in CFTR functioning	Dekkers et al. 2016; Van Mourik et al. 2019
	iPSCs obtained from CF patients differentiated into hepatic cholangiocytes, CF *in vitro* model	*In vitro* CF study	Sampaziotis et al. 2015
Fallopian tube, endometrium organoids	Organoids grown from primary tumors	Anti-cancer drug screening	Tamura et al. 2018
	Organoids that model a wide range of endometrial pathologies: from endometriosis and hyperplasia to low and high grade cancers	Organoids that model endometriosis were shown to exhibit disease-specific features and cancer-related mutations. Organoids formed from tumor-affected endometrium accurately characterize cancer subtypes, replicate altered tumor surfaces, and demonstrate patient-specific drug responses	Boretto et al. 2019
Bladder, prostate organoids	Organoids grown from primary tumors	Anti-cancer drug screening	Gao et al. 2014; Lee et al. 2018; Driehuis et al. 2020; Kim et al. 2020
Colorectal organoids	Organoids grown from a primary tumor of the colon	Anti-cancer drug screening	Van de Wetering 2015; Clevers 2016; Li and Izpisua Belmonte 2019

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