ISSN 2706-6819 Vol.6, Issue 6: 25-31, DOI: 10.25236/IJFM.2024.060604

Principles of Organoid Culture

Yushu Zhang[1,a], Xiang Zheng[1,b], Yanyu Zhao[1,c], Mingsong Wu[2,3,d,*]

[1]Department of Medical Genetics, Zunyi Medical University, Zunyi, Guizhou, 563006, China
[2]Special Key Laboratory of Oral Disease Research of Higher Education Institution of Guizhou Province, Zunyi Medical University, Zunyi, Guizhou, 563006, China
[3]School of Stomatology, Zunyi Medical University, Zunyi, Guizhou, 563006, China
[a]756962366@qq.com, [b]710741195@qq.com, [c]xznuzhao@163.com, [d]mswu0909@zmu.edu.cn
[*]Corresponding author

Abstract: *Organoids are stem cell-derived in vitro culture systems that reproduce the tissue, function, and genetic characteristics of organs in vivo. Due to its similarity to the capabilities of physiological tissues, it may become an excellent model for medical research in the future. Organoid is a breakthrough in the field of medical research and has been used as an important tool for basic research and clinical application in many fields. In this review, we review the culture principles of organoids, including organoid self-assembly and culture environment, growth factors, small molecule drugs, growth matrices, and molecular markers of organoids. It then discusses current organoid applications including tumor models, gene mutations, precision medicine, and the developing organoid biobank. Finally, the perspective of the organoid field is envisaged.*

Keywords: *Organoids, self-organizing, 3D Culture, Growth Factors, Small Molecule Medicine, Extracellular Matrix*

1. Introduction

Unlike traditional two-dimensional culture, three-dimensional culture can better simulate the complex growth environment in vivo. Based on the three-dimensional culture model, a model similar to the normal organs in the human body has been constructed, namely organoids. Since organoids have (1) Humanization; (2) Stable maintenance genome; (3) Genome editing is possible; (4) Easy high-throughput screening; (5) The ability to self-organise to mimic organogenesis and have the characteristics of partial organ function. The study of organoids has gradually become one of the most influential scientific studies.

In recent years, organoid cultivation technology has made a breakthrough with the in-depth study of organoids. A large number of organoid models have been developed and used in the study of various diseases. However, organoids still have some problems that cannot be solved. These include the difficulty to fully reproduce the physiological regulatory processes of the body, the presence of potential carcinogenicity, the lack of standardized culture protocols, etc. In this review, we attempt to discuss the methods of organoid culture and their application in various fields in order to provide guidance for the creation of standardized organoid models in the future.

2. Self-assembly of Cells and the Culture Environment

Stem cells have the ability to self-renew and differentiate. Depending on the source of the cells, organoids are derived from two types of stem cells: (1) embryonic stem cells (ESCs) and their induced pluripotent stem cells (iPSCs) for induction synthesis (iPSCs, the latter two are collectively referred to as pluripotent stem cells PSCs); (2) adult stem cells (ASCs). Organoid is a model that can simulate the structure and function of natural organs driven by stem cells and constructed in the form of self-assembly. Organoids overcome spatial constraints, can better maintain cellular heterogeneity, and have the capacity for self-renewal and self-organisation [1]. It is a group of disordered cells that can spatially rearrange themselves independently, even in the same environment. Self-organisation of organoids occurs through cell sorting and spatial lineage, which depends largely on a variety of endogenous and exogenous signals. Mouse intestinal stem cells successfully form intestinal organoids with cryptovilliform structures through specific culture conditions and their embedding in a solid extracellular matrix (ECM). This also suggests that stem cells themselves have the ability to self-

ISSN 2706-6819 Vol.6, Issue 6: 25-31, DOI: 10.25236/IJFM.2024.060604

organize to form organoids. Through the study of organoids, we found three key steps required for organoid culture: (1) by inducing key signaling pathways to establish the correct regional identity of stem cells during differentiation; (2) explore and develop media that allow the desired cell types within organoids; (3) stem cells are embedded in ECM and cultured to form cell spheroids similar in structure and function to the target organ.

At present, a variety of culture environments for organoid culture have been developed. The first commonly used organoid culture method is embedding. Stem cells in vivo are cultured in a three-dimensional Matrigel's dome or flat gel. Depending on the tissue type, various growth factors or pathway inhibitors are added [2] . This allows stem cells to self-renew and differentiate. The second method is microfluidic 3D culture. Organoid spheroids can be mixed with collagen and injected into a microfluidic culture device [3]. Although there are some examples of organoids and microfluidic technology, the simulation of the fluid environment is still immature. How to use microfluidics and other technologies to control the fluid microenvironment during organoid culture is an urgent problem. A third method is cultured at the air-liquid interface. The organoids are embedded in collagen gel in an internal transporous dish. The medium in the outer dish diffuses into the inner dish through permeable turnwells. The top of the collagen layer is exposed to air through the air-liquid interface to allow cells to obtain sufficient oxygen [4]; The fourth method is to combine 3D printed culture. Biologic units (cells/proteins/DNA, etc.) and biomaterials are required by biomimetic morphology, organism function, or cell-specific microenvironment. Then, the technical means of "three-dimensional printing" are used to create personalized in vitro three-dimensional biological structure models [5].(Fig1)

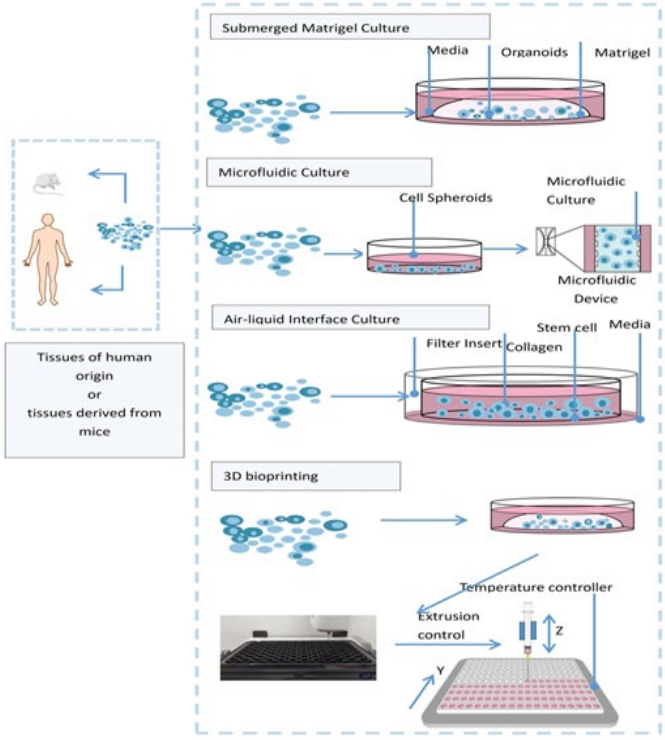

Figure 1: Organoid culture modalities

3. Growth factors

Most organoid cultures require specific exogenous signals for induction because stem cells do not have all the necessary components for self-assembly. Therefore, organoid formation requires specific growth factors to properly stimulate stem cells. Common growth factors in the process of organoid culture include Wnt agonist, bone morphogenetic protein (BMP), epidermal growth factor (EGF), fibroblast growth factor (FGF), hepatocyte growth factor (HGF), insulin-like growth factor (IGF), ROH-associated protein kinase (ROCK), transforming growth factor (TGF), vascular endothelial growth factor (VEGF), R-spondin, keratinocyte growth factor (KGF), etc.

Wnt acts as a growth factor involved in regulating cell development, multiplication, survival,

ISSN 2706-6819 Vol.6, Issue 6: 25-31, DOI: 10.25236/IJFM.2024.060604

differentiation, adhesion, polarity, cell-cell communication, and self-renewal functions. Epidermal growth factor (EGF) can bind to its receptors, inducing proliferative changes. It is commonly used in the culture of organoids such as the gastrointestinal tract, liver, thyroid, brain, and breast. R-spondin plays a role in the activation of Wnt signaling when stem cells renew themselves. It is a key factor in the maintenance and proliferation of murine and human stem cells. Fibroblast growth factor (FGF) plays an important role in mesoderm induction, limb development, neural induction and neurodevelopment in early development. Currently commonly used FGFs include organoid culture of FGF2, FGF4, FGF9, FGF10 and others. Hepatic cell growth factor (HGF) acts as a mitogen in hepatocytes. As the name suggests, it can be used as a culture for liver organoids. Bone morphogenetic protein (BMP) plays a vital role in embryogenesis, development, and maintenance of tissue homeostasis. BMP2, BMP4 are commonly used to induce iPSC and ESC production in hepatocytes, gastrointestinal tract, etc.

4. Small Molecule Medicines

Small molecules are compounds with a molecular weight of less than 1000 daltons (especially less than 400 daltons) that have biological functions. Unlike cytokines and proteins, small molecules can enter the cell directly through the cell membrane and perform appropriate functions. They are suitable for cells of different genera and have been widely used for organoid culture.

Gastrin is a very important peptide hormone in the gastrointestinal tract that can prolong the survival time of organoids and is mainly used in the culture of organoids in the intestine and liver. A83-01 is an inhibitor of the Activin/Nodal/TGF-β pathway. Generally used for cultures of liver, prostate, and mammary glands. Y27632 is a small molecule inhibitor of Rho-associated serine threonine protein kinase (ROCK) that prevents stem cell apoptosis. SB202190 is a highly potent p38 MAPK inhibitor commonly used in gastrointestinal and breast cultures. Prostaglandin E2 (PGE2) regulates the digestive system and immune system, among other things. After binding to specific receptors, it can mediate cell proliferation and differentiation for liver and prostate culture. CHIR99021 is a glycogen synthase 3β inhibitor that induces endodermal differentiation of human embryonic stem cells. N-acetylcysteine is an ROS inhibitor to be added to organoid culture.

The culture of organoids is particularly essential because it is necessary to add various appropriate and specific growth factors and small molecule drugs to the culture medium to promote the development of stem cells toward their respective destinations. They can mediate organoid formation signaling pathways and microenvironment regulation. They activate or inhibit specific signaling pathways involved in organoid formation. However, there is currently no standard specification for the concentration of these substances added to the medium. Different concentrations have an impact on organoid formation.

5. Type of Organoid Matrix

The ECM is a network of extracellular molecules that provide structural support and biochemical signals to the cell. The culture of organoids requires stem cells to be cultured in three-dimensional media, and these matrices mimic the scaffolding structure required by the ECM of tissues and organs. To grow a variety of 3D organoids, scientists provided different ECM preparations called Matrigel/Basement Membrane Extract (BME), which is composed of the basement membrane components of embryos. These include common BME, BME2 type (more tough than ordinary BME), BME3 type (more rigid, low pH and low glucose), and general-purpose UltiMatrix, which can be used for spheroids, PSC as a base layer, and organoids to cultivate. BMP type 3 can spontaneously form breast cancer cell organoids cultured with stromal cells and endothelial cells under low pH, low glucose and hypoxic conditions. In this model, tumor cells are mixed with mesenchymal stromal cells and cultured in 3D BME to spontaneously form organoids. Using BME type 2, Clevers et al. simultaneously generated normal and tumor-derived intestinal organoids from healthy and tumor tissue.

But Matrigel also has its limitations that may hinder progress in organoid development for many downstream applications and clinical translation. Moreover, there is no chemical definition of Matrigel's composition, resulting in inconsistent compositional variations, especially in high-throughput screening and large-scale organoid production. To overcome this limitation, organoids take a single ECM component, such as; Collagen I generate endometrial and gastrointestinal organoids. Type III collagen has been used as a coating on tissue culture surfaces, where it can be used as a matrix

ISSN 2706-6819 Vol.6, Issue 6: 25-31, DOI: 10.25236/IJFM.2024.060604

fold to promote cell attachment, growth, and adhesion.

Since cell adhesion ligands, matrix geometry, and mechanical properties are the main parameters affecting cell proliferation, differentiation, and maturation. The natural ECM is viscoelastic, the microtissues in the body are also of varying hardness, such as lung, brain and bone tissue. The researchers also found that 3D stem cells are sensitive to stress relaxation rates. Mesenchymal stem cells on three-dimensional hydrogels proliferate and differentiate faster under rapid relaxation. Therefore, some cell types may be more sensitive to stress relaxation under specific culture conditions. Gjorevski et al. demonstrated a modular hydrogel design that can support different stages of intestinal organoid development using a polyethylene glycol (PEG) hydrogel matrix. PEG hydrogels are rich in key ECM biopolymers such as fibronectin, laminin, collagen, etc. However, spiral-shaped oligo (ethyl)glycol polyisocyanate hydrogels exhibit specific stiffness and binding characteristics. Mesenchymal stem cells cultured in hydrogels with local hardness showed elastic materials and osteogenesis that promote the fate of fat cells. This suggests the effect of stress sclerosis on mesenchymal stem cells. They found that gelatin-Ph, which is highly hard, promotes the maturation of colorectal tumor organoids as well as survival and metabolic capacity. Mechanized gelatin-phenol hydrogels can provide a synthetic matrix that supports organoid sensitivity in vivo and in vitro.

6. Culture Condition for Various Organoids

In recent years, as organoid research has gradually become a hot spot, different studies have emerged on organoid culture conditions. Organoids of the central nervous system and various endoderm organoids induced by PSCs have been developed. Neural progenitor cells (NPCs) have the ability to aggregate and form neural tubes, and in suspension culture to form neurospheres, which then differentiate into neurons and astrocytes. Neurospheroids can also be produced from PSC-derived embryonic bodies. In 2012, Eiraku et al. cultured brain structures, retina, and pituitary by in vitro neural differentiation of ESCs. Bershteyn et al. used a patient's iPSC-derived brain organoid model to mimic the cellular features of anencephaly gyrus and reduce mitosis and neuronal migration of neuroepithelial stem cells. Trevino et al. constructed human forebrain organoids for the first time on the sixth day of suspension, and switching the medium to neural culture medium greatly extended the lifespan of human forebrain organoids for up to 300 days.

Differentiation into intestinal tissue by in vitro PSCs. This requires activin-induced deterministic endoderm-shaped organs. The main difference from adult-derived intestinal organoids is the presence of surrounding mesenchymal cells in the culture. This allows the formation of epithelium and mesenchyma supported by the mouse vascular system upon implantation in vivo. However, when intestinal organoids were optimally cultured. Mouse intestinal organoid medium and initial/passage medium were prepared based on known gastric organoid medium. The initial/passage medium is used for organoid culture and passage, and organoid medium is used for maintenance culture. Mahe et al. established a method for gastrointestinal organoids and the generation of Lgr5 single-cell-derived epithelial organoids. The stomach develops from the foregut. McCracken et al. generated the first fully derived human gastric tissue in vitro by differentiation of human pluripotent stem cells induced by Bmp, FGF, Wnt, retinoic acid, and EGF. Finally, after optimizing the culture of gastric organoids, gastric organoids were cultured, maintained, and passaged by preparing gastric organoid culture medium and gastric organoid initial/passage medium.

The early stage of liver development is from the foregut endoderm epithelium. By fibroblast growth factor (Fgf) and bone morphogenetic proteins 2 and 4 (BMP). Further liver bud structures are produced. This is followed by the production of hepatocytes, blood vessels, and bile epithelium. Recently, Huch et al. performed an optimized culture of liver organoids. They prepared three different media (i.e., initial medium, expansion medium, and differentiation medium). They cultured liver organoids with initial medium for three days, which promoted cell growth. Then, aspirate the medium and add fresh expansion medium every other day. Aspirate the expansion medium after 7-10 days of amplification and add the same volume of differentiation medium for differentiation. Pancreatic organoids can also be generated by inoculating mouse embryonic pancreatic progenitor cells in matrigel. Human pancreatic organoids are then also established that can further differentiate into ductal and endocrine lines. Georgakopoulos et al. found that human pancreatic organoids are long-term expansion from fresh and cryopreserved human pancreatic tissue in serum-free medium.

The kidneys form ureteral buds and renal stroma from the posterior end of the middle mesoderm of the embryo via Wnt and FGF signaling. In 2013, ureteral bud organoids were established by culturing

ISSN 2706-6819 Vol.6, Issue 6: 25-31, DOI: 10.25236/IJFM.2024.060604

human PSCs in Bmp4 and FGF2 and then exposed to retinoic acid, activin A, and Bmp2 to produce ureteral bud-bound renal progenitor cells. In 2015, they used a simplified and improved protocol by adding growth factors such as retinoic acid, fibroblast growth factors 2 and 9 (FGF2 and 9), CHIR99021, and small molecule drugs. Direct differentiation into complex renal organoids by human induced pluripotent stem cells was established.

Recently, a method of culturing mammary organoids using a suspension culture method using minimal matrigel has been developed. Start with a single culture with EGF or FGF. Cultures of breast organoids were maintained after 7 days using mixed cultures with the addition of EGF, FGF2, FGF10, and basal Epicult-B medium containing heparin, hydrocortisone, and insulin.

However, there are still many problems in organoid research at this stage, and many researchers are working on creating newer organoids. But they had trouble identifying an organoid that fit the bill. This needs to satisfy those individual's such as size, shape, gene expression amount and the like. These all limit the efficient research of organoids and the translation of clinical research. Organoids are models that incorporate various organ-specific cell types, tissue morphology, and function. But organoid volume is a limitation that needs to be addressed. Tissue necrosis due to hypoxia and lack of soluble factors needs to be addressed as organoid volume increases. Currently, human induced pluripotent stem cell-derived liver organoids successfully address this issue by activating angiogenic pathways, thereby vascularizing the organoids.

7. Applications

Organoids are gradually becoming one of the most important cell culture tools in biological and medical fields. Researchers have developed the most suitable culture system for their growth based on organoids from various tissue sources and cultured them in vitro for a long time. Currently, organoids are mainly used in disease research, drug screening, high-throughput screening, gene editing, etc. (Fig. 2).

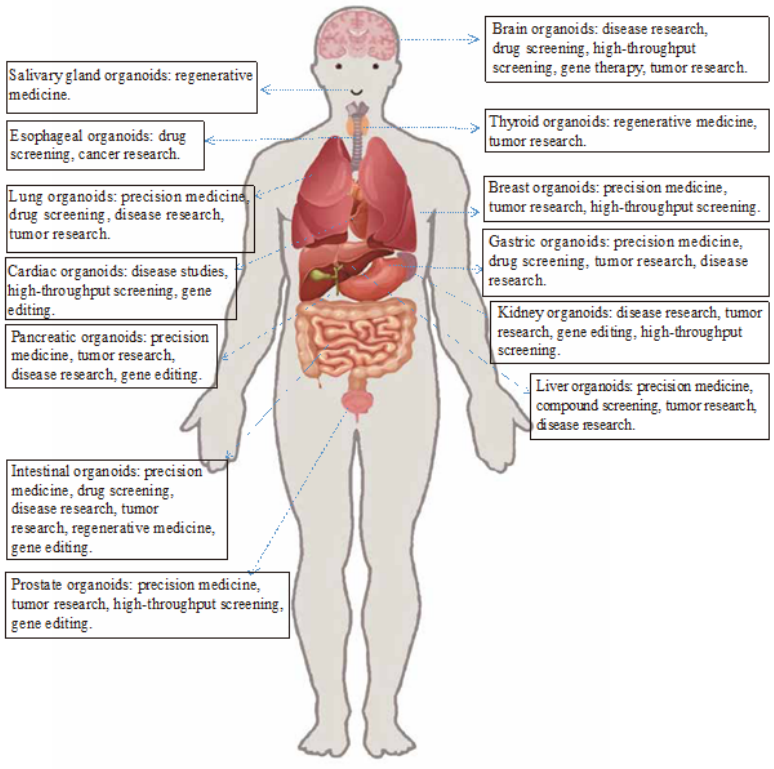

Figure 2: Application of various organoid cultures

The scholars conducted an integrated analysis of drug response data from many individual human organoids. This results in biomarker development studies for similar patients [6]. Currently, organizations including the American Type Culture Collection (ATCC) and the Dutch non-profit organoid technology organization HUB (www.hub4organoids.eu) have established organoid biobanks

ISSN 2706-6819 Vol.6, Issue 6: 25-31, DOI: 10.25236/IJFM.2024.060604

with over 800 different organoids with known genetic information and other characteristics, which are available to research institutions and companies. The Human Cancer Models Initiative (HCMI) is also building and developing tumor organoid culture models into a shared resource.

The advantage of organoids is that personalized treatment is achieved through precision medicine. Recent studies have found that using the dual targeting ability of bispecific antibodies can block the proliferation of cancer stem cells in colorectal cancer. This provides the basis for personalized treatment of cancer. Tumor organoids can use high throughput for drug screening, which can analyze drug sensitivity and improve precision medicine. Design targeted drugs with higher selectivity for patients. Instead of the traditional "one-size-fits-all" treatment option. Organoids make these possible.

8. Problems and Prospects

The establishment of organoid models is a breakthrough in the field of stem cells. But organoid techniques are still in the exploratory stage compared to traditional models. And there are a lot of problems. First, the effects that should exist between different organs are uncertain. Second, there are no standards for organoid establishment and quality control programs. And whether the organoid model needs to sertoli cell is uncertain; third, the source of the cells is difficult. The purity of the cells in the tissue cannot be determined. Fourth, for culturing the organoid, different batches of matrix gum may all affect the stability of the organoid. Moreover, the concentration of growth factors varies and affects the culture of organoids. Researchers have also proposed improvements to these problems, including animal chimera technology[7] and organ-on-a-chip technology [8].

Although current organoids cannot completely replicate the structure and function of human organs. However, because organoids are of human origin and like physiological functions, it can help solve some difficulties of the current traditional model, make up for the shortcomings of conventional cell culture and animal model research, and provide new technical support for tumor research, drug screening, new drug development, and precision treatment. Organoid technology is one of the most promising organs and disease models that meet the current ethics. It bridges the long-standing gap in developmental biology and precision medicine. The 3D structural and heterogeneous properties of organoids allow us to study cell lineage specifications using spatial and temporal information. As organoid technology continues to be optimized, organoid models will gradually be perfected, and organoids are expected to become an important tool for future life science research, continuously promoting scientific research and clinical medicine development.

Acknowledgement

We thank the reviewers who review this article and help us to edit this article. This study was supported by the Construction Projects of Medical Biomaterial Research & Development Talent Base in Guizhou Province and Zunyi City (No. [2018]3, and No. [2019]69), Science and technology project of Guizhou Province (No. 2023]260 and 5772-006).

References

[1] M.A. Lancaster, J.A. Knoblich, Organogenesis in a dish: modeling development and disease using organoid technologies, Science (New York, N.Y.), 345 (2014) 1247125.
[2] T. Seino, S. Kawasaki, M. Shimokawa, H. Tamagawa, K. Toshimitsu, M. Fujii, Y. Ohta, M. Matano, K. Nanki, K. Kawasaki, S. Takahashi, S. Sugimoto, E. Iwasaki, J. Takagi, T. Itoi, M. Kitago, Y. Kitagawa, T. Kanai, T. Sato, Human Pancreatic Tumor Organoids Reveal Loss of Stem Cell Niche Factor Dependence during Disease Progression, Cell Stem Cell, 22 (2018).
[3] A. Sontheimer-Phelps, B.A. Hassell, D.E. Ingber, Modelling cancer in microfluidic human organs-on-chips, Nat Rev Cancer, 19 (2019) 65-81.
[4] D.A. Turner, P. Baillie-Johnson, A. Martinez Arias, Organoids and the genetically encoded self-assembly of embryonic stem cells, Bioessays, 38 (2016) 181-191.
[5] E. Kim, S. Choi, B. Kang, J. Kong, Y. Kim, W.H. Yoon, H.-R. Lee, S. Kim, H.-M. Kim, H. Lee, C. Yang, Y.J. Lee, M. Kang, T.-Y. Roh, S. Jung, S. Kim, J.H. Ku, K. Shin, Creation of bladder assembloids mimicking tissue regeneration and cancer, Nature, 588 (2020) 664-669.
[6] H. Tiriac, P. Belleau, D.D. Engle, D. Plenker, A. Deschênes, T.D.D. Somerville, F.E.M. Froeling, R.A. Burkhart, R.E. Denroche, G.-H. Jang, K. Miyabayashi, C.M. Young, H. Patel, M. Ma, J.F.

ISSN 2706-6819 Vol.6, Issue 6: 25-31, DOI: 10.25236/IJFM.2024.060604

LaComb, R.L.D. Palmaira, A.A. Javed, J.C. Huynh, M. Johnson, K. Arora, N. Robine, M. Shah, R. Sanghvi, A.B. Goetz, C.Y. Lowder, L. Martello, E. Driehuis, N. LeComte, G. Askan, C.A. Iacobuzio-Donahue, H. Clevers, L.D. Wood, R.H. Hruban, E. Thompson, A.J. Aguirre, B.M. Wolpin, A. Sasson, J. Kim, M. Wu, J.C. Bucobo, P. Allen, D.V. Sejpal, W. Nealon, J.D. Sullivan, J.M. Winter, P.A. Gimotty, J.L. Grem, D.J. DiMaio, J.M. Buscaglia, P.M. Grandgenett, J.R. Brody, M.A. Hollingsworth, G.M. O'Kane, F. Notta, E. Kim, J.M. Crawford, C. Devoe, A. Ocean, C.L. Wolfgang, K.H. Yu, E. Li, C.R. Vakoc, B. Hubert, S.E. Fischer, J.M. Wilson, R. Moffitt, J. Knox, A. Krasnitz, S. Gallinger, D.A. Tuveson, Organoid Profiling Identifies Common Responders to Chemotherapy in Pancreatic Cancer, Cancer Discov, 8 (2018) 1112-1129.

[7] R.G. Rowe, G.Q. Daley, Induced pluripotent stem cells in disease modelling and drug discovery, Nat Rev Genet, 20 (2019) 377-388.

[8] S.E. Park, A. Georgescu, D. Huh, Organoids-on-a-chip, Science (New York, N.Y.), 364 (2019) 960-965.