



REVIEW

Advancement of Organoid Technology in Regenerative Medicine

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Abstract

Purpose Organoids are three-dimensional cultures of stem cells in an environment similar to the body's extracellular matrix. This is also a novel development in the realm of regenerative medicine. Stem cells can begin to develop into 3D structures by modifying signaling pathways. To form organoids, stem cells are transplanted into the extracellular matrix. Organoids have provided the required technologies to reproduce human tissues. As a result, it might be used in place of animal models in scientific study. The key goals of these investigations are research into viral and genetic illnesses, malignancies, and extracellular vesicles, pharmaceutical discovery, and organ transplantation. Organoids can help pave the road for precision medicine through genetic editing, pharmaceutical development, and cell therapy.

Methods PubMed, Google Scholar, and Scopus were used to search for all relevant papers written in English (1907–2021). The study abstracts were scrutinized. Studies on the use of stem-cell-derived organoids in regenerative medicine, organoids as 3D culture models for EVs analysis, and organoids for precision medicine were included. Articles with other irrelevant aims, meetings, letters, commentaries, congress and conference abstracts, and articles with no available full texts were excluded.

Results According to the included studies, organoids have various origins, types, and applications in regenerative and precision medicine, as well as an important role in studying extracellular vesicles.

Conclusion Organoids are considered a bridge that connects preclinical studies to clinical ones. However, the lack of a standardized protocol and other barriers addressed in this review, hinder the vast use of this technology.

Lay Summary Organoids are 3D stem cell propagations in biological or synthetic scaffolds that mimic ECM to allow inter-cellular or matrix-cellular crosstalk. Because these structures are similar to organs in the body, they can be used as research models. Organoids are medicine's future hope for organ transplantation, tumor biobank formation, and the development of precision medicine. Organoid models can be used to study cell-to-cell interactions as well as effective factors like inflammation and aging. Bioengineering technologies are also used to define the size, shape, and composition of organoids before transforming them into precise structures. Finally, the importance of organoid applications in regenerative medicine has opened a new window for a better understanding of biological research, as discussed in this study.

Keywords Organoid · Regenerative medicine · Stem cells · Precision medicine

Introduction

A three-dimensional micro-physiological platform consisting of clusters of cells called organoids was obtained with the efforts of scientists in the fields of biotechnology and regenerative medicine [1]. Organoids could imitate a series

of pathophysiological and structural features of human tissues and organs [2]. They are also already in use as disease models. Their new therapeutic ways are done through the support of regenerative medicine techniques [1–5]. They are differentiated from human pluripotent stem cells (PSCs), embryonic and adult-tissue stem cells (ESCs and ASCs) [6]. In fact, stem cells in the form of organoids can produce a variety of organ models. The range of organ models is wide, from the stomach and intestine to the brain [6, 7]. Embryonic stem cell differentiated organoids could play a role in research about organ development like what is done in the uterus [6, 8]. Interestingly, these cells could be able

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to develop into the parts of the brain in the *in vitro* environment. This process occurs without going through the natural cycle of evolution [6, 9]. Adult stem cells (ASCs) are more specific in that they differentiate into precise tissue depending on their location [6, 10]. In this way, they can replace the destroyed cells [6, 11]. Organoid models of patient-derived induced pluripotent stem cells (iPSC) can be used in the field of personalized medicine [9, 12, 13]. Literally, these are stem cells that grow in a special culture protocol. Also, they could mimic the characteristics of an organ [6, 14]. The results of animal models such as monkeys and mice cannot always be generalized to humans. That is why organoid models are projected to be superior to other *in vivo* models [3, 6–11, 14, 15]. 3D cell culture is another important advantage of organoids. With the ability to conduct more in-depth research than other cell culture protocols [9, 15]. The use of scaffold as a biological or synthetic hydrogel that can mimic the properties of the extracellular matrix (ECM) has been useful in the preparation of organoid culture protocols [10, 11, 15]. Actually, the signaling pathways and connections between cells and the extracellular matrix in the 3D culture method allow cells to function when they are in the body [10, 16]. The use of organoids derived from human tissues allows us to study the mechanisms within the human body more accurately than other culture methods [8, 10, 14–17]. Despite all the advantages mentioned, organoids have disadvantages, including the absence of all the cell types found in human organs. Also, organoid models are without vascular structure [7]. Although ASCs can be useful in repairing cells and prolonging human life, they also have harmful effects because they have the potential to change into cancerous cells more than other cells [11]. The goal of this study is to discuss the history of organoids, their origin, types, and applications in regenerative and precision medicine. The relationship between stem cells and organoid production was explained. The future of organoids in organ transplantation was considered a novel science. Also, organoid roles in studying extracellular vesicles (Evs) were discussed here. Ultimately, the various uses of current organoid models and the related challenges are discussed in detail.

Organoids: a Novel Insight to Regenerative Medicine

The knowledge of using different cell lines as animal models to achieve goals such as the discovery of new drugs for incurable diseases belongs to the twentieth century [18, 19]. Primarily, genetic studies helped the development of biological processes in order to find the mechanisms of disorders [18–20]. This trend first began in more rudimentary models such as the invertebrate model and progressed to mammalian models and finally human model systems [1]. All of this

progress has greatly contributed to the drug design process [1, 16]. At the moment, human models with three-dimensional culture systems that use different stem cells from various organs have overcome the limitations [1, 17]. Also, organoids with their new model have achieved a revolution in biomedical science [1, 17]. Also, the revolution that happened with the advent of organoids had a great impact on the future of cell therapy [21, 22]. Organoids can play a role in therapeutic techniques for diseases with unknown etiology, such as type 1 diabetes [21, 22]. Because of their complex architecture [21, 22], organoid products are significantly different from all other medical field products. Organoids can be effective in translational research as they have a role in studies about human pathophysiology and organ development [23]. But nowadays, it should be noted that the findings of organoid studies are not generalizable to be used in the field of cell therapy for the reasons stated above [21, 22]. For example, the methods could not match with standards in pharmaceuticals correctly [21, 22]. As we know, one way to treat cancer is radiation therapy, which affects the cellular response [24]. Organoids can be useful in investigating this cellular process in radiotherapy [24]. In fact, scientists need to understand all the molecular and biological events in cells and organs during radiation therapy so they can determine the maximum dose of radiation [24]. The study of the radiobiological process in the form of organoid models allows us to consider cell-to-cell interactions in more detail [24]. As a result, the results will be closer to clinical observations [24]. Another point is the ability to produce enzymes in the organoid culture protocol, which is used for defective phenotypes [24, 25]. For example, in the article by Anusha S. Shankar et al., the renin was produced by the use of kidney organoids that were differentiated from induced pluripotent stem cells (iPSCs) [24, 25]. Furthermore, organoid technology may be used in the field of organ transplantation by producing the desired organ [11, 16, 26]. Also, organoids derived from patient-derived iPSCs or other cells have been used to test gene therapy [11, 16, 26]. Identifying the wide utilization of organoids from drug mechanisms and enzyme deficiencies to gene therapy and organ transplantation, we find that their emergence is a new innovation in biotechnology with a significant history.

Historical Background of Organoids

The history of organ regeneration in an *in vitro* environment date back to 1907, when Henry Van Peters Wilson was able to explain how to produce an organ using separate sponge cells [27]. An important point in organoid models has been shown in this experimental research, where adult cells of an organism could produce multicellular complexes without external commands [27, 28]. This theory of cell isolation

and organ regeneration has long been used by laboratories in the 1950s [27, 28]. Also, the history of the construction of the first mammalian organoids goes back more than 60 years ago, and they helped biology in order to achieve great advancement [28, 29]. Scientists are investigating the possibility of transforming the body cells of more advanced animals, including vertebrates, into organs after separation [28, 29]. In subsequent experiments on chick embryos, researchers tried to produce organs from different cell types from the kidney, skin, and liver [28, 30]. In fact, this type of research was the first step in testing the cell isolation and regeneration of organs in more advanced animals [28, 30]. The emergence of PSCs has greatly influenced the progress of research in the field of organoids [31]. These cells were first extracted from mouse embryos [31]. Later on, embryonic stem cells were isolated from human blastocyst cells, and this knowledge was further developed when iPSCs were obtained [17, 32]. Biological researchers attempted to control these stem cells' behavior, including spontaneous regeneration [32]. Regenerative medicine researchers attempted to repair damaged organs by using special stem cells that could differentiate into the target cell [32]. All of these advances helped scientists think about producing organs in a laboratory environment in the form of organoids [2, 32]. Finally, the history of organoids goes back more than 40 years ago, when Howard Green tried to spread human epidermal keratinocytes by co-culture with special mouse embryonic fibroblasts [2, 32]. Mouse embryonic fibroblasts can mimic the human epidermis in the laboratory [2, 17, 32]. Considering the history of organoids, we find that they attracted attention in the twenty-first century, when scientists tried to supersede the use of animal models with new models [28]. Also, during this period, the use of stem cell derivatives greatly improved [28]. We can also mention the history of their application in various fields such as toxicology, organ transplantation, cancer, and the knowledge of embryonic development [28]. Research on the growth of epithelial breast cells on the Engelbreth-Holm-Swarm (EHS) ECM platform was then performed, resulting in three-dimensional duct production with the ability of protein synthesis in order to milk secretion. These important findings had not previously been obtained in two-dimensional culture medium [17, 33]. Furthermore, organoids aided in the development of models for studying breast cancer and the formation of new drugs in target therapy [17, 33, 34]. Interestingly, type II alveolar cells, which lose their different properties in a 2D culture in a plastic dish, are able to maintain them in an EHS matrix environment [35]. This phenomenon shows that intercellular interactions in three-dimensional culture medium with ECM respond better than in two-dimensional culture medium [35]. Intestinal organoids derived from PSCs contain mesenchymal tissue in addition to epithelial tissue with the ability to mimic intestinal function [36, 37].

These investigations progressed to make CNS models for studying the processes in the brains of human bodies [38]. Also, CNS models can be used to study neurobiological phenomena in the course of neurological diseases [38].

Current Types of Organoids and Their Application Scope

Conventionally, 2D tissue cultures and animal models have long been used to study human diseases. However, as organs are three-dimensional, 2D cell cultures lack the exact tissue architecture of the body, and although animal models have been widely used to study diseases, the gap between species hinders the full discovery of diseases [39]. For example, the use of pigs to study gastrointestinal and infectious diseases is widespread and considerable. Nevertheless, due to differences in the gastrointestinal tract of pigs and human beings, pigs serve as asymptomatic carriers of *Yersinia* infection while humans manifest severe diarrhea [39]. As another example, 2D cell culture systems can be used to study *Mycobacterium tuberculosis* infection due to the absence of confounding factors and easy infection. However, because they lack real tissue structure, they are unable to accurately predict clinical outcomes [40]. All these concerns led to the invention of 3D organoids using embryonic stem cells (ESCs) and ASCs [1, 17, 41, 42]. Organoids provide micro-environments to study infectious diseases. These 3D tissue cultures allow us to recapitulate interactions between the host and pathogens like viruses, bacteria, and parasites, thus helping the development of drugs for current diseases. [17, 41] The proof of the presented explanation is the provision of lung organoids to investigate SARS-CoV-2 infection. They are used to study the infectivity and effect of viruses on cell functions and, consequently, are used to test possible treatments like Imatinib and mycophenolic acid [43]. Organoids are used to model genetic diseases and investigate the role of genes in their pathogenesis. In order to achieve this goal, organoids are prepared by culturing cells expressing mutations obtained from affected patients or by inducing mutations in wild-type organoids [17, 41]. In the same way as infectious diseases, organoids are used to create treatments for genetic diseases [17, 41]. As an example, cystic fibrosis is a genetic disease caused by a mutation in the cystic fibrosis transmembrane conductance regulator (CFTR). Organoids can be used to screen for drugs that can compensate for and regulate these mutant channels [44]. Organoids used for cancer, which are also called tumoroids, are established by using cancer cells obtained via biopsies or independently via induction of

mutations. These organoids serve as the technology of bio banking, which is the collection of different subtypes of cancer in an *in vitro* environment with the aim of studying mutations and genotype–phenotype correlations as well as the molecular basis and pathways involved in tumorigenesis. Besides, biobanks can serve as platforms on which the responses of cancer cells to possible treatments are investigated [17, 41, 45, 46]. Tumoroids are preclinical models to elaborate on personalized drugs and screen them before applying them to patients [17, 41, 45]. In this regard, a study performed by Calandrini et al. can be mentioned in which tumoroids and matching normal kidneys of 50 children with different kidney cancers, including Wilms tumors, renal cell carcinomas, malignant rhabdoid tumors, and congenital mesoblastic nephromas, were established [47]. For this purpose, normal and cancerous tissues were obtained from kidneys that underwent nephrectomy or biopsy. Stem cells were then isolated by enzymatic and mechanical methods and used to construct organoids. Later, the constructed organoids underwent histological examinations to study phenotypic characteristics of cancerous tissues like cell marker presentations [47]. By using methods like whole-genome sequencing, genetic and epigenetic features such as mutation signature and DNA methylation profile were surveyed. Later, the current tumoroids were used as platforms to screen the efficacy of chemotherapeutic agents like actinomycin D and vincristine [47]. Moreover, organoids are used to study genome editing, the signaling pathways and mechanisms of cell and organ development [41]. Organoids can be isolated from patients with genetic disease. Then the mutation responsible for the disease can be corrected using CRISPR/Cas9 technology and, later, these edited organoids serve as sources for autologous cell therapies and can be transplanted to the appropriate site [41]. An example of this approach is the use of RPGR gene edited organoids for the treatment of retinitis pigmentosa, which leads to the gain of defects caused by the disease like photoreceptor reduction [41]. Another advantage of organoids is the use of these cell cultures as micro labs in which different signaling pathways can be manipulated to discover their roles in the maintenance or differentiation of cells. For instance, the same method was applied to study the function of Notch, Wnt, or EGFR signaling pathways in the development of enteroendocrine cells [41]. Another application of organoids is that in these microenvironments, toxic and effective doses of drugs, combinant therapies like immunotherapy, and clinical outcomes of the treatments can be predicted. Organoids cultured by isolating iPSCs from a particular patient are safe and reproducible models in which different drugs can be examined and the

most effective one is identified. They are also considered a hope for cell therapy and organ transplantation [9, 41, 45, 48]. Some of the most common organoids and their clinical applications are described in Table 1.

Organoids and Regenerative Medicine

Regenerative medicine is the science of constructing biological tissues in an *in vitro* environment using stem cells, biocompatible materials, and biochemical factors, and these tissues are similar to those in the body [72]. These manufactured tissues are used to study the mechanisms and diagnostic processes as well as probable treatments of the diseases [72–76]. Moreover, they are utilized to develop extracorporeal life support systems or to be implanted into the body to replace a damaged organ or repair injuries [72]. One of the recent accomplishments of regenerative medicine is the construction of organoids. Organoids are the 3D assemblage of organ-specific cells derived from stem cells [23]. The cells are cultured in a biologic (decellularized) or synthetic scaffold which mimics the ECM and provides proteins and growth factors necessary for the growth and differentiation of cells [17, 77–80]. Compared to previous efforts in regenerative medicine (cell transplantation, 2D cell cultures, etc.), organoids are structures that sustain their functions and features for a long time. They have a high degree of genetic stability, expansion and differentiation capacity, and self-organization. By providing an optimal chemico-physical and mechanical environment, the level of functionality and self-organization reaches an appropriate state [17, 78, 81, 82]. Moreover, they contain several cell types and thus can replicate cell–cell interactions [83]. In addition to other applications of organoids explained in the previous section, one of the anticipated uses of regenerative medicine is the usage of organoids for transplantation and substitution of damaged organs. For multiple diseases, organ transplantation is the best and most certain treatment. However, allogenic transplantation is restricted due to the limited number of matched donors and long-term immunosuppression [17, 84–86]. As the alternatives like cell transplantation and artificial organs are temporary answers, the emergence of organoids as a source of organ transplantation is considered a hopeful remedy [83]. Since organoids are able to combine with other novel technologies like gene editing and nanotechnology, they are substitutable treatments for certain diseases like chronic kidney disease (CKD), biliary atresia, inflammatory bowel disease (IBD) and metabolic hepatic disease [17, 23, 77, 78, 84, 87–90]. As proof of the current statement, a study by Yui et al. can be used. In this study, *Lgr5* + stem cells were taken from the colon epithelium and used to grow colon epithelium

Table 1 Some of the most common organoids and their clinical applications

Organoid type	Preclinical application	Clinical application	Ref
Kidney	Glomerular related disease- chronic kidney disease- polycystic kidney disease- acute kidney injury	Biobanks for Wilms tumors- malignant rhabdoid tumors- renal cell carcinomas- congenital mesoblastic nephromas	[47, 49, 50]
Intestine	Necrotizing enterocolitis- Hirschsprung's disease- diverticular disease- cystic fibrosis- Short bowel syndrome—Celiac disease-	Cystic fibrosis—celiac disease	[51–56]
Retina	Usher syndrome – retinitis pigmentosa—microphthalmia		[57–60]
Prostate	Prostate cancer	Biobanks for prostate cancer	[61–63]
Brain	Microcephaly- zika virus infection- autism. Schizophrenia—neurodegenerative diseases - Miller–Dieker syndrome - Timothy syndrome -	Biobanks for glioblastoma	[64–67]
Liver	Hepatocellular carcinoma and non-alcoholic fatty liver disease – biliary atresia- inflammatory bowel disease- cystic fibrosis-alpha-1-anti-trypsin (A1AT) deficiency-Wilson's disease-Alagille syndrome-viral hepatitis- malaria infection	Cholangiopathies	[16, 68–70]
Breast	Breast cancer		[71]
Organoid type	Preclinical application	Clinical application	Ref
Kidney	Glomerular related disease- chronic kidney disease- polycystic kidney disease- acute kidney injury	Biobanks for Wilms tumors- malignant rhabdoid tumors- renal cell carcinomas- congenital mesoblastic nephromas	[47, 49, 50, 71]
Intestine	Necrotizing enterocolitis- Hirschsprung's disease- diverticular disease- cystic fibrosis- Short bowel syndrome—Celiac disease-	Cystic fibrosis—celiac disease	[51–56]
Retina	Usher syndrome – retinitis pigmentosa—microphthalmia		[57–60]
Prostate	Prostate cancer	Biobanks for prostate cancer	[61–63]
Brain	Microcephaly- zika virus infection- autism. Schizophrenia—neurodegenerative diseases - Miller–Dieker syndrome - Timothy syndrome -	Biobanks for glioblastoma	[64–67]
Liver	Hepatocellular carcinoma and non-alcoholic fatty liver disease – biliary atresia- inflammatory bowel disease- cystic fibrosis-alpha-1-anti-trypsin (A1AT) deficiency-Wilson's disease-Alagille syndrome-viral hepatitis- malaria infection	Cholangiopathies	[16, 68–70]
Breast	Breast cancer		[71]

organoids that were identical to the real tissue. In order to investigate the functionality of the organoids, the group induced acute colitis in recipient mice and later transplanted the organoids to the injured area using enema. Shortly afterwards, the transplanted cells covered the submucosa of the injured tissue, and the mice started to gain weight. Subsequently, the graft epithelium recovered the injured tissue and manifested colon crypts which were morphologically indistinguishable from the original epithelium around [77, 91]. However, to pass from preclinical studies to clinical application, many concerns regarding safety, ethical, and legal matters still remain [17]. Lack of an optimized differentiation protocol, incomplete maturation, contamination with unwanted cells and pathogens are some of the

challenges that should be addressed in this method [23, 84, 88, 89].

A New Model for Improving Regenerative Medicine

In spite of being a hopeful treatment for many diseases, regenerative medicine should undergo different preclinical studies to be qualified for clinical administration. Before a treatment is used on people, these preclinical studies try to figure out all of its risks, safety, and effectiveness [73, 92]. Animals play an important role as models in preclinical studies. Due to physiological and anatomical

similarities between animals and humans, they are frequently used to study the pathophysiology of diseases [39]. Animals, as multi-organ creatures, recapitulate the complexity and inter-organ signaling pathways [70]. They are useful for discovering human-pathogen interactions and can be genetically modified [40]. A variety of animal models are used in regenerative medicine [1]. Danio rerio, zebrafish, for example, is an appropriate candidate for genome screening and manipulation. As zebrafish are complex enough, they can recapitulate human physiology [1]. Moreover, high reproducibility at a manageable price and a transparent embryo for developmental studies are the benefits of this model. However, it manifests huge genome complexity and limited biological similarity with the human system [1]. *Caenorhabditis elegans* is an easily established and maintained system, which is cost-effective and enables retail developmental biology. It is widely used to analyse cell lineage and cell death pathways. Yet, its metabolism, lifespan, and immune system are different from those of human beings [1]. Genetically engineered mouse models are the most preferred models, but they are expensive and compared to the egg-laying animals, the early-stage development of the mouse is inaccessible [1]. However, although general biologic processes were initially studied in animals, some human-specific mechanisms still exist that cannot be modelled by animals [1]. Using animal models is expensive and needs skilled personnel [40]. Ethical issues, anatomical differences, and difficulty in housing for large animals are some of the other obstacles. In addition, due to anatomical and physiological differences between humans and animals, the results obtained from animal studies cannot fully predict the clinical outcomes [40, 70]. Thus, some successful therapies in animal models cannot effectively be translated into humans [9]. Given the limitations listed above, the development of 2D cell architectures represented a promising avenue for disease modeling at the time. While replacing the need for animal models, this architecture has led to many successful understandings of cellular signaling pathways and drug discovery. And it has provided deeper insight into molecular mechanisms [1, 39]. As a result, the first human-specific laboratory model has emerged [1]. 2D cell cultures are cost-effective and time-saving. They are easy to maintain and can be widely manipulated in genetic studies [1]. They can be used for genome editing and biobanking. Studies based on 2D cultures are not hampered by confounding factors and impediments like the effect of the immune system, compared to animal models [40, 93]. Nevertheless, 2D cell cultures have notable limitations since they lack the spatial 3D organization of the body organs which is necessary for their function [42, 70]. They are thought to be non-physiological because of the absence of tissue complexity

and because they are immortal [17]. Furthermore, they cannot stimulate the effect of stromal factors, microenvironment, and some of the organ-specific functions. [45] As a result, 2D cell cultures failed to accurately predict clinical outcomes [40]. The emergence of 3D cell cultures, or organoids, is a recent revolution in disease modeling. Organoids are a promising laboratory tool for genome editing, drug discovery, and transplantation. They circumvent limitations on the way toward personalized medicine [70]. Organoids manifest organ-specific functions and enable the understanding of cell movement, differentiation, and tissue development. They pave the way for understanding disease pathophysiologic mechanisms [42]. These 3D cultures are able to mimic cell–cell interactions and the microenvironment present in the body [83]. One of the key factors causing all these benefits gained from organoids is the 3D structure of the ECM around cells, which leads to a gradient of nutrients and signaling pathways. Thus, cells are exposed to different levels of nutrients and growth factors [42, 83, 94]. Another key is the possibility of administration of different exogenous growth factors which guide cells to differentiate into certain cell lines [42, 83, 94]. Moreover, organoids contain different types of cells which are capable of self-organization and allow the system to mimic cell–cell and cell-ECM interactions [42, 83, 94]. Organoids are relatively cost-effective and capable of self-renewal and self-organization [1, 40]. On this basis, it is concluded that organoids maintain the benefits of the previous models, as well as bypass the majority of their limitations, opening up a new possibility in advancing regenerative medicine.

Stem Cell-Derived Organoids

As mentioned previously, organoid technology benefits from the unique properties of stem cells, which can include multi-capabilities and the power of self-renewability [95]. When we consider this ability of stem cells, we find that by providing a suitable culture method, each type of stem cell can differentiate and produce organ structures [95]. In this section we discuss different types of stem cells, including ASCs and PSCs, which consist of both ESCs and iPSCs (induced pluripotent stem cells), that can produce organoids during programmed processes [1, 95, 96]. Adult cell-derived stem cells can differentiate into specific cells of the same tissue or organ in a suitable environment with the same conditions as in the body [95, 97]. One of the advantages of these organoids is their genetic stability over a long time and the preservation of the unique characteristics of each organ [95, 97]. At the same time, ASC-derived organoids can be used to study human biology and genetics with gene modification technology [98]. Using this genetic manipulation,

the cell-to-cell connections and cell differentiation can be modified [98]. Gene modification and transgenic organization concepts can also be applied to other stem cell-derived organoids, such as iPSCs [98, 99]. These conditions can be met by observing the appropriate transgenic environment and regular and advanced protocols [98–100]. In fact, the first organoids derived from ASCs could differentiate into the small intestine of mice [2]. Thus, intestinal organoids have the ability to form from intestinal ASCs [2]. Also, two types of tissue of ectodermal origin are obtained from ASCs, which include mammary glands and salivary glands [95]. In this way, the Wnt signaling pathway plays an important role in the propagation of organoids for the long-term [95]. In general, the Wnt signaling pathway can be considered a crucial way to increase the lifespan of many organoid environments that are derived from ASCs, iPSCs, and ESCs [95]. iPSCs-derived organoids can also play a role in the production of endoderm-differentiated organs such as the small intestine, stomach, and pancreas [101]. It should be noted that the organoid environment raised from iPSCs can reconstruct the characteristics of the human gastric corpus [95, 101]. The researchers first found that they needed a retinoic acid signaling pathway to produce gastric organoid. They were able to obtain gastric organoids from the intestinal one with retinoic acid admission [95, 102]. However, in this pathway to make the gastric organoid the desired one, epidermal growth factor (EGF) and Matrigel were also needed [102, 103]. With all these measures, the end of the stomach, which is the antrum and is most similar to the intestinal tissue, was obtained [95, 103]. The WNT signaling pathway is needed to form the proximal part of the stomach, which includes the corpus with parietal cells for acid production [95, 102, 103]. Also, the human intestinal organoids (HIO) made from iPSCs can do things like contract the intestines like the nervous system of the intestines [104, 105]. This procedure necessitates the involvement of iPSC-derived neural crest [105], so the intestinal nervous system model can be simulated by merging neural crests derived from iPSCs and HIOs [105]. This results in a model that can study molecular and cellular processes in diseases such as Hirschsprung and the effects of new drugs on intestinal diseases [95, 104, 105]. iPSCs are also able to produce tissues of mesodermal origin, such as the kidneys [106]. Some factors are needed to construct the intermediate mesoderm of kidney tissue in order to produce ureteric bud and metanephric mesenchyme at the same time [106]. These factors include Activin A, Bone morphogenetic protein 4 (BMP4), and fibroblast growth factor 9 (FGF9) [95, 106, 107]. One subclass of PSC, including ESCs, can produce cerebellum, optic cup, and hippocampal-like organoids in different environmental conditions, and all of these organoids originate in ectodermal tissue [103]. Also, ESC differentiation can yield organoids that consist of neuroepithelial

tissue that mimic cortical features [103]. In fact, by inhibiting TGF and BMP signaling pathways, neuroectodermal structures can be generated spontaneously from ESCs [95, 102, 108]. Hepatic organoids were also obtained with a new method from ESCs that can be converted to both hepatocytes and cholangiocytes [109]. These hepatic organoids can proliferate and replace damaged cells with mature hepatocytes after grafting in FRG mice with liver dysfunction [95, 110–112]. The creative point is that it is possible to study drug toxicity on the liver using ESC-derived organoids and apply it to screening models [109, 113]. Thus, in a study by Shinozawa et al. in 2021, the toxicity of 238 drugs was tested on an organoid model of the liver [113]. It should be noted that the widespread use of stem cells and the communication of cells through free RNAs has greatly helped to increase the clinical application of organoids [114, 115]. This issue can be used in organ transplantation [115]. However, these connections in the intercellular environment can be formed through EVs, including exosomes, which could be produced by stem cells. [114, 115]. Figure 1 summarizes how differentiated types of organoids differ from stem cells.

Organoids as 3D Culture Models for EVs Analysis

As mentioned previously, one of the most desirable applications of organoids is their use in cancer studies. In this regard, EVs are developing tools which can increase the importance of organoids in oncologic studies [116, 117]. EVs are stable lipid bi-layered structures that contain nucleic acids, lipids, proteins, and metabolites. These vesicles are secreted by different cells under physiologic or pathologic conditions and can be considered biomarkers of diseases. Also, EVs are mediators of cell–cell crosstalk which impact the target cells through autocrine or paracrine mechanisms [116, 117]. It has been shown that tumoral cells release EVs in order to establish intercellular and matrix-cellular connections, which can later cause autocrine or paracrine oncogenesis and metastasis [118, 119]. EVs also contribute to angiogenesis and immune system modulation and regulate cancer progression. Meanwhile, as the concentration of EVs is reported to be increased in many malignancies, they play the role of a biomarker for these diseases, carrying important information about these malignancies [118, 119]. The discovery of tumoroids allowed us to study the production of EVs as well as other characteristics of cancer disease in a 3D model [120, 121]. Organoids derived from tumors (tumoids) are helpful platforms on which EVs are produced and studied [120]. One example in this regard is a study in cervical cancer tumors, which revealed that tumoroid-derived EV small RNA profiles display a high similarity to circulating EVs in the bodies of patients with cervical cancer. Also, it

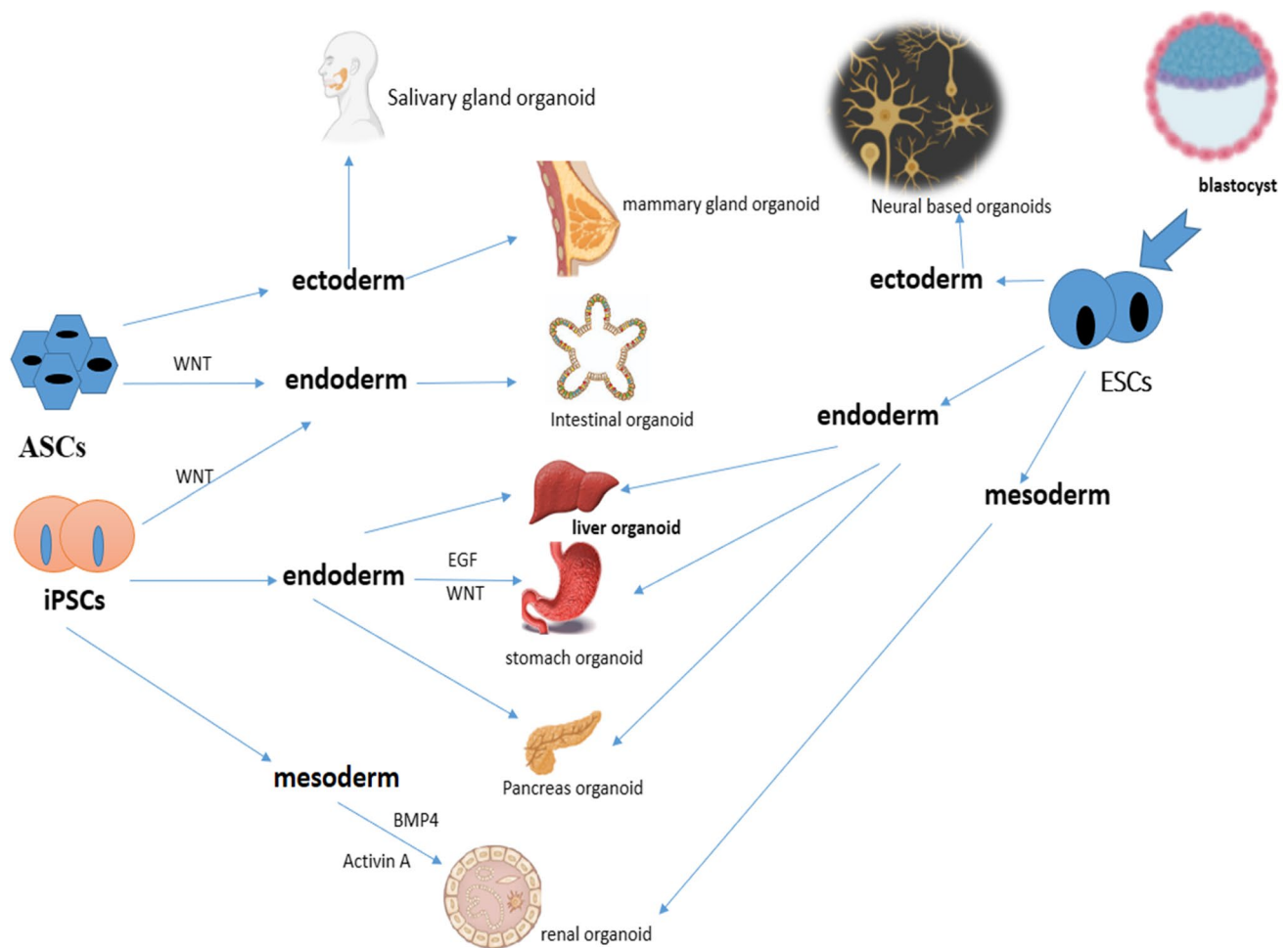


Fig. 1 Stem cells and differentiated organoids. Stem cells have the ability to transform into any types of tissues and different types of them can produce endoderm, ectoderm, and mesoderm in different conditions [104]

suggests that following EV molecular changes in interstitial fluids can be a noninvasive method to investigate tissue behaviors on a molecular scale [121]. As another example, in a study by Szvicsek et al. on colorectal cancer (CRC) organoids, it has been shown that EVs play as transmission vehicles for proteins and miRNAs. They also found that the composition of ECM is a critical factor that influences the production of EVs since the accumulation of collagen type 1 is seen to be correlated with an increased level of EVs in CRC organoids [122]. The same interaction between collagen type 1 and EVs is reported by Franchi et al. in their study on breast cancer organoids and Zeöld et al. in a study of ductal pancreatic adenocarcinoma organoids [120, 123]. Meanwhile, Szvicsek et al. reported that fibroblast-derived EVs have a promotive role in the tumorigenesis of CRCs. Finally, the effect of genetics in the production of EVs is suggested by studying the role of Apc mutation in activation of the Wnt pathway and EV production as the downstream result [122]. Similarly, in a study by Sándor et al. on lung

adenocarcinoma organoids, the role of the Wnt pathway on cell proliferation and EV secretion has been indicated [124]. In a recent study by Taha et al., it has been indicated that matrix metalloproteinase 3 (MMP3) has a crucial role in maintaining the physical integrities of EVs and any reduction in MMP3 results in disorganization of EV structures, inhibition of their protumorigenic properties, and finally a reduction in tumor proliferation and progression [116]. One more study by Namba et al. on colon cancer organoids demonstrated that the ATP-binding cassette transporter G1 (ABCG1) pump (a cholesterol lipid efflux pump) has a crucial role in tumor progression via altering EV secretion. These tumoroids suggest that depletion of ABCG1 causes intracellular accumulation of EVs and, subsequently, reduces tumor progression and metastasis. Thus, this study offers ABCG1 as a potential therapeutic target for colon cancer [125]. Ke et al. exposed gastric organoids to esophageal adenocarcinoma (EAC)-derived EVs and reported that these EVs induce neoplastic characteristics in gastric organoids,

probably due to their role in transferring miR-25 and miR-210. They also successfully lowered the cell proliferation and neoplastic phenotypes in gastroids by inhibition of miR-25 and miR-210 [117]. Besides, EVs can help in providing therapy for cancers in other ways too. For instance, Murgoci et al. used microglia-derived EVs to inhibit tumor invasion in a spheroid model of glioma, suggesting these EVs as nanotherapeutic agents for glioma [126]. In another method, EVs are loaded with certain proteins, lipids, or nucleic acids that are supposed to be carried to target cells and have an inhibitory effect on cancers. An example in this regard is the investigation by Jeong et al. into non-small cell lung cancer. They used EVs as carriers of miRNA-497 in order to suppress tumor growth and genes associated with this kind of cancer [127]. Also, miRNA-497 has an inhibitory role in angiogenesis by decreasing VEGF-A-mediated angiogenic sprouting. All together, they proposed this combination of EV-miRNA as a possible therapy for non-small cell lung cancer [127]. Despite all these benefits, the use of spheroids for EV studies has some challenges. For instance, spheroids used to model cancers have a necrotic core which secretes apoptotic bodies, interfering with EV studies. Thus, to handle this problem, spheroids should be dissociated to determine the viability of cells, the obstacle that prevents

further use of the organoid. This problem, along with the other challenges, remains to be addressed in order to gain complete knowledge about EVs [128].

Figure 2 shows an overview of EVs and their role in cell-to-cell communication in the organoid model.

Human Organoids for Precision Medicine

In 1999, Francis Collins took the first step toward precision medicine by publishing the document “Medical and Societal Consequences of the Human Genome Project,” in which he mentions the role of the human genome in prediction, treatment, and prevention of diseases [132]. The recently established concept of “precision medicine,” which is also called personalized medicine, takes advantage of new technologies such as DNA sequencing to refer to inter-individual differences as dominant factors in the evaluation and treatment of diseases [132–134]. Originally, precision medicine used to focus on DNA sequencing and genomic material as the main causes of heterogeneity in disease manifestations and management. However, nowadays, precision medicine implies the incorporation of a wide range of human features like lifestyle, socioeconomic circumstances, and family

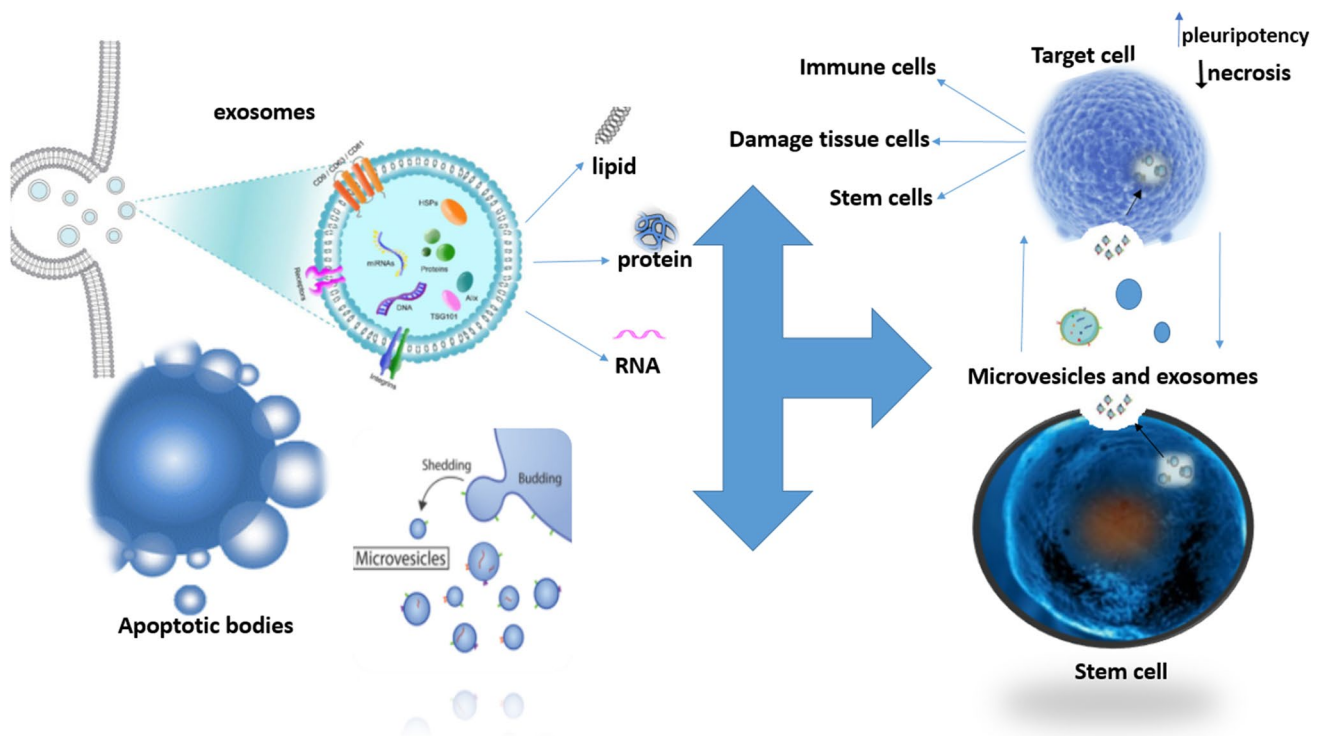


Fig. 2 Stem cell effects via EVs in an organoid model. As shown in the picture, the three major subgroups of EVs include microvesicles, exosomes, and apoptotic bodies. Since the stem cells are very dynamic, they can obtain EVs such as exosomes [129]. According to

the mentioned applications of EVs, they can be used as nanoparticles in organoid models to make them more efficient [130, 131]. EVs: *extracellular vesicles*

history as well as genetic factors in diseases [132–134]. In conventional approaches, patients with a certain type of disease were treated with a similar therapy [135]. Nonetheless, recently, it has been shown that while a particular therapy fits some patients, it does not have the expected result in others. This phenomenon is due to inter-personal variation and declares the need for personalized medicine as a “one patient, one treatment” method [135]. Despite this, therapeutic methods offered by precision medicine are applied to preclinical models in the first step to predict the treatment in humans. In this regard, organoids are suitable candidates as they can recapitulate the *in vivo* environment [136]. One of the wide applications of organoids in precision medicine is in the field of precision oncology. Precision oncology is an attempt to find the most effective therapeutic strategy for every single patient that has the maximum benefit and minimum risk for the patient. This attempt is rooted in the fact that cancer cells are genetically unstable and undergo several mutations while proliferating [137, 138]. Thus, the created cell lines manifest higher genome abnormalities than the primary cells, which leads to intra-tumor heterogeneity. It has been shown that intra-tumor heterogeneity is the main cause of cancer progression and drug resistance [137, 138]. In order to overcome this barrier, several investigations used DNA sequencing of the primary and metastatic tumor cells to identify mutations responsible for drug sensitivity and to discover the optimum treatment for patients [137, 138]. However, even when genetic alterations were figured out, a significant number of the patients failed to respond to the drugs as expected. To address this challenge, organoids derived from patients’ tumor samples were established and called “patient-derived organoids” (PDOs) [137, 138]. The PDOs are the avatars of the primary tumors but in an *ex vivo* condition as they mimic the phenotypic and genotypic features of the original tumors [17, 139]. PDO obtained from a single patient can serve as a cancer biobank and fulfill different aims of precision medicine. In this biobank, genetic material is studied, genome and drug sensitivity are integrated, and multiple tests of drug screening are performed [137–140]. Moreover, cancer responses to various therapeutic strategies like chemotherapy, radiotherapy, and combination therapy are examined. As a result, the most effective treatment for the patient is selected, and as shown by clinical trials, once this selected therapy is applied to the patient, the results match those from PDOs [17]. Also, a database is created which matches drug sensitivity and tumor genetics. On the other hand, this biobank can offer biomarkers predicting the effectiveness of drugs. These biomarkers can be molecules within tumors that are used as targets for interventions or can be environmental factors that stimulate tumor progression or augment treatment response [17, 137–142]. Another benefit of PDOs is the discovery of carcinogenic mutations and molecular subtypes of tumors

in order to categorize them [17, 143]. In addition to precision oncology, personalized medicine has taken initial steps toward other territories of medicine in recent years [1, 17]. As an example, cystic fibrosis is a genetic disease mainly caused by the loss of function of the CFTR gene. Human organoids developed to mimic this disease can be used to screen the efficacy of the current drugs for patients or to produce and test new drugs [1, 17]. Another example is the use of organoids to study inflammatory diseases. Inflammatory bowel disease is an autoimmune disorder that has impaired epithelium-microbe interaction as a main feature [144]. To confront this malfunction, intestinal organoids with the ability to mimic this disease have been developed to investigate epithelium-microbe crosstalk like ROS production and inflammatory cytokine release [144]. However, despite the current achievements, precision medicine has a long way to go to take down all the challenges and turn them into an applicable therapeutic approach [144].

Conclusion and Future Perspectives

Organoids has the ability to fulfill many of the research objectives and can replace the previous models like 2D cultures and animal models, filling the gap between preclinical studies, and clinical application of their results. Thus, in this paper, we aim to study the advancement of this technology and its application in regenerative medicine. Organoids are used to study a wide range of diseases including infectious diseases, genetic diseases, and cancers. They can be used to study the role of EVs as important aspect of cancers. Moreover, organoids are considered platforms to practice genome editing and drug screening and to investigate cell development pathways [44, 145]. However, to achieve the goals aimed for organoid technology, it has to overcome some limitations. Once an organoid is transplanted to human body, host immune system activates and rejects the transplanted tissue [87]. In order to address this challenge, an integration of bioengineering and nanotechnology has developed organoids encapsulated with natural ECM. This structure has a high immune escape ability and can prevent the rejection [87]. Another challenge is the incomplete maturation of the organoids after transplantation; as the results, organoids would not be able to perform all the expected functions [42, 84]. While transplanting an organoid, it is important to determine its capacity of maldifferentiation. According to the researches, organoids contaminated with undifferentiated pluripotent stem cells or other unrelated cells can later differentiate into unwanted and even hazardous tissues with genetic abnormalities. This matter rises concerns about safety and tumor formation of organoids [78, 84, 87]. To address this challenge, cells used to provide organoids should be purified from their surrounding stroma

by microdissection methods. Identification of biomarkers to purify subpopulation of stem cells is another solution. Adjusting the composition of the microenvironment around especially by manipulating growth factors can direct organoids to the appropriate differentiation. Genetic stability of the organoid cells should be checked regularly as well. Yet, the need for an effective differentiation protocol remains of concern [44, 78, 84, 146]. A further limitation is that organoids fail to grow a proper vascularization after engraftment. At the present time, the most encouraging solution is to depend on transplanted tissues to stimulate the growth of host vasculature. However, combining the transplanted organoids with blood vessel organoids is considered a future solution to support long-term survival of the culture [42, 145]. As mentioned above, despite all the discoveries made in the field of organoid technology, many challenges like definition of growth factors and metabolites needed for their long-term self-renewal, detection of the inter-cellular cross-talk and many other limitations remain to be answered and that is when organoid can appear as the effective treatment for the diseases and perform expected functions in human body [44].

Abbreviations ASCs: Adult stem cells; BMP: Bone morphogenetic protein; CF: Cystic fibrosis; CFTR: Cystic fibrosis transmembrane conductance regulator; CKD: Chronic kidney disease; CRC: Colorectal cancer; ECM: Extracellular matrix; EHS: Engelbreth-Holm-Swarm; ESCs: Embryonic stem cells; EGF: Epidermal growth factor; EVs: Extracellular vesicles; FGF: Fibroblast growth factors; iPSCs: Induced pluripotent stem cells; IBD: Inflammatory bowel disease; PDOs: Patient-derived organoids; PSCs: Pluripotent stem cells; TGF: Transforming growth factor; CRC: Colorectal cancer; MMP3: Matrix metalloproteinase 3; EAC: Eophageal adenocarcinoma; HIO: Human intestinal organoid

Declarations

Competing Interests The authors declare no competing interests.

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