



Progress, prospects, and limitations of organoid technology

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Organoids are mini-organs generated through *in vitro* 3-dimensional culture that mimic some of the structural and physiological functions of real organs. In recent research, various organoids have been derived from pluripotent stem cells or multipotent organ-specific adult stem cells *in vitro* and have been used in regenerative medicine, disease modeling, precision medicine, toxicology studies, and drug discovery. However, research on reproduction-related organoids has not been comprehensive, and some limitations need to be addressed for culturing these organoids. In this review, we discuss the historical advances, major recent developments, limitations, and potential of organoid culture, including human reproductive organoids.

Keywords: Organoids; Stem cells; Regenerative medicine; Reproduction; Microfluidics

Introduction

Embryonic stem cells were first isolated in 1981 from mouse blastocysts [1] and subsequently from human blastocysts in 1998 [2]. Stem cells have been recently used in regenerative medicine owing to their ability to differentiate into all types of cells and maintain tissue homeostasis in adults. However, most stem cells are cultured in a 2-dimensional (2D) non-physiological environment. These cultured stem cells differ greatly in their expression of genes and proteins from those cultured in a 3-dimensional (3D) environment [3]. Since various cells form a 3D network with the extracellular matrix (ECM) *in vivo* that affects their growth, proliferation, differentiation, and apoptosis [4,5], creating a 3D environment to mimic *in vivo* conditions while culturing stem cells *in vitro* is important. Many studies have successfully cultured stem cells in a 3D environment mimicking the *in vivo* ECM using various biomaterials.

With continuous efforts in stem cell research for the develop-

ment of a 3D culture system, Sato et al. [6] finally generated intestinal organoids from adult intestinal stem cells using a Matrigel-based 3D culture method. When stem cells are cultured in a 3D environment with ECM-containing Matrigel *in vitro*, they self-organize into tiny 3D organoid structures with similar features and functionality to real organs. Several organoids, such as testis [7], fallopian tube [8], endometrium [9], brain [10], gut [11], and liver [12], have been cultured either from directly sourced tissue-resident adult stem cells (ASCs) from biopsy samples or pluripotent stem cells (PSCs). These organoids have been successfully used for many clinical applications (Fig. 1). However, research on reproduction-related organoids has not been comprehensive, as certain limitations need to be addressed for culturing these organoids. Here, we highlight the progress and briefly review the applications and current limitations of various organoids, including reproduction-related organoids, in biomedical research.

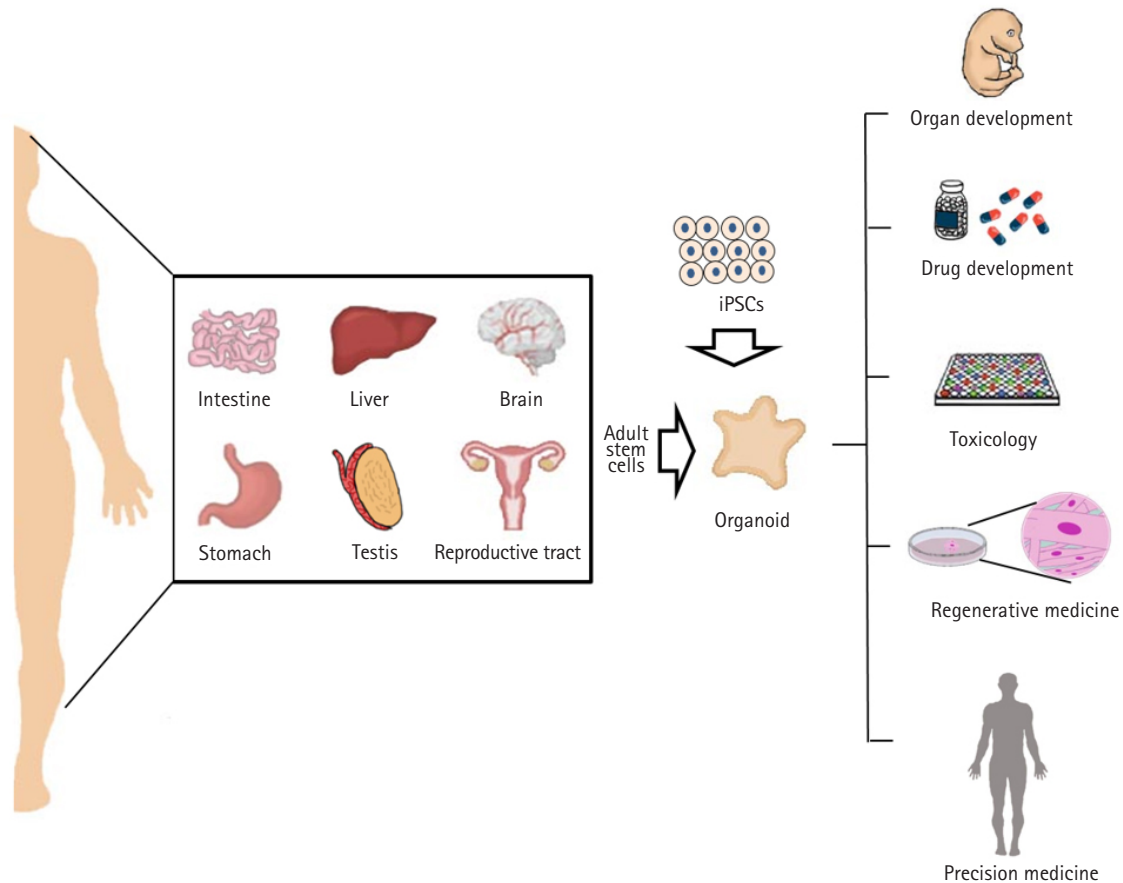


Fig. 1. Derivation and application of various organoids from induced pluripotent stem cells (iPSCs) or adult stem cells.

Ethics statement: This study was a literature review of previously published studies and was therefore exempt from institutional review board approval.

Progress in organoid research

1. Testis organoids

Approximately 7% of men are infertile [13]. The causes of male-factor infertility are diverse and complex. Chromosomal and genetic diseases (e.g., Klinefelter syndrome, Y chromosome deletions, trisomy 21), exposure to environmental chemicals, radiation, cancer, and chemotherapeutic agents can lead to infertility [14] due to their adverse effects on sperm function and quality in the testis. Despite the development of various organoids over the past decade, testicular organoids have received increasing attention. Testicular organoids are similar to the testes in structure and function and serve as highly useful model systems to study male infertility and the mechanism of germ cell niche, germ cell functions, and the interaction between germ cells for spermatogenesis [15,16]. In addition, testis organoids

can be used as high-throughput drug and toxicity screening tools that can replace animal experiments owing to ethical issues [17].

2. Fallopian organoids

The fallopian tubes play an important role in the female reproductive system as sites for gamete and embryo transport, fertilization, sperm reservoir, and embryonic development [18]. Improper functioning of fallopian tubes can cause infertility in females. Fallopian tube organoids were derived from the female reproductive system by isolating cells using enzymatic digestion and culturing the cells by embedding them in Matrigel [8]. These organoids were able to maintain the structure and functionality of fallopian tubes, as evident from their ability to respond to female hormones (estrogen and progesterone), the presence of cilia and secretions, and folding of the epithelium [19].

3. Endometrium organoids

The human endometrium is a complex multicellular and dy-

namic tissue that undergoes the menstrual cycle in response to female steroid hormones [20]. During pregnancy, the blastocyst first implants at the functional layer of the endometrium, which is the outermost layer of the uterus [21]. Thus, the endometrium is essential for female reproduction in mammals, and defects in its function or cyclic remodeling can cause implantation failure, pregnancy disorders, endometriosis, or endometrial cancers [22]. Endometriosis is a chronic inflammatory disease that causes pelvic pain and excessive bleeding due to the proliferation of endometrial tissue outside the uterus [23]. Endometrial or uterine cancer is the 4th most common gynecological malignancy in women in the United States [24]. Although many studies have investigated endometrial cancer, little is known about the cellular and molecular mechanisms behind its pathology. The main limitation of these studies was the lack of an accurate research model system, as mouse models do not physiologically resemble human endometrial development and function *in vivo* [9]. For instance, the process of endometrial decidualization varies between mice and humans [25]. Therefore, results obtained from animal models cannot be directly translated to humans. To address these obstacles, human endometrial organoids were derived from primary endometrial cells to mimic the human endometrium. Endometrial organoids have been shown to recapitulate the morphology and function of the adult human endometrium in response to estrogen and progesterone [26]. Endometrial organoids can be used not only to elucidate the mechanism of pathological diseases, such as endometriosis and endometrial cancer, but also to contribute to the development of therapeutic agents.

4. Brain organoids

The human brain is divided into three regions—the forebrain, midbrain, and hindbrain—which are primarily composed of neurons and glial cells [27]. However, the function, development, and pathology of many disorders of the human brain are not yet fully understood, and it is difficult to study their mechanisms *in vivo*, as the human brain is the central system that regulates the body and cannot be cultured outside the body. Therefore, an *in vitro* model to understand human brain development and disorders is needed. Efforts have been made toward 2D culturing of brain tissues or organs by differentiating neural stem cells and PSCs into neurons; however, the structure and function of the brain could not be recapitulated [28]. Watanabe et al. [29] developed a 3D culture method to generate different brain regions from mouse or human PSCs. Brain organoids were then generated *in vitro*, as tiny organs that could recapitulate many features of the brain. In 2013, 3D cerebral organoids were

generated *in vitro* that could grow up to a few millimeters from various brain tissues such as the retina, dorsal cortex, ventral forebrain, midbrain-hindbrain boundary, choroid plexus, and hippocampus [30]. In addition, organoids from specific brain regions, such as the midbrain and hippocampus, were established through the organoid culture system [31]. Brain organoids provide an excellent research platform for understanding the development of various disorders of the human brain, such as schizophrenia and Alzheimer disease.

5. Gut organoids

In 2009, Sato et al. [6] first established intestinal organoids derived from leucine-rich repeat-containing G protein-coupled receptor 5 (*Lgr5*)+ stem cells using a Matrigel 3D culture system. These organoids form crypt-villus structures with cellular heterogeneity to mimic the physiology and organization of the actual intestine. When transplanted into mice, intestinal organoids showed long-term survival, and their regenerative ability was also confirmed [32]. Hindgut spheroids were cultured from human PSCs in Matrigel to promote the maturation of intestinal organoids. Although intestinal organoids are derived from PSCs, gastric and lingual organoids—derived from parts of the digestive tract—are generated from adult pyloric stem cells and adult tongue epithelium, respectively [33]. Organoid technology can be used as a powerful tool to study gastrointestinal diseases, intestine–microbe interactions, and colorectal cancer.

6. Liver organoids

The liver is a critical organ that performs various functions such as detoxification, protein synthesis, and the production of bile, which is necessary for digestion [34]. To conduct studies on liver function and regeneration, liver organoids resembling the adult liver in various species were generated from induced PSCs, hepatoblasts, and adult tissue-derived cells [35,36]. Liver organoids can be used as *in vitro* culture models to replace animal models for studying drug toxicity, metabolism, personalized medicine, and regenerative medicine.

Current limitations in organoid research

Various organoids have been established from the stem or progenitor cells to recapitulate the cellular complexity of actual organs *in vitro*. Most organoids are cultured in Matrigel, which is derived from the secretion of Engelbreth-Holm-Swarm mouse sarcoma cells and is rich in ECM proteins, including laminin, collagen IV, heparan sulfate proteoglycan, nidogen/ entactin, and other undefined factors [37]. However, these factors make

it difficult to elucidate the precise structure and function of organoids cultured in Matrigel. In addition, the murine origin of Matrigel impedes organoid use for human clinical transplantation. Therefore, to overcome these limitations, organoid culture systems have been developed using a wide range of naturally derived biomaterials (collagen, alginate, fibronectin, laminin, hyaluronic acid, and alginate–chitosan mixtures) and synthetic materials (polyethylene glycol and nanocellulose) [38].

Blood vessels constitute the circulatory system of the body. Blood constantly flows through the blood vessels, ensuring cell survival by a constant exchange of nutrients, oxygen, and waste [39]. From the perspective of cell culture, the human body is dynamic. However, organoids are cultured through static methods rather than the dynamic environment of blood vessels in the body. To bypass this limitation, a microfluidic chip platform has been developed to culture organoids [40]. It can constantly carry oxygen, nutrients, and waste using a pump connected to a chip-like blood vessel with embedded organoids. Several microfluidic “organoids-on-chips” mimicking the *in vivo* physiology of specific organs have been designed, with tremendous implications in organoid research [41,42].

Organoids derived from PSCs and tissue-specific stem cells *in vitro* are a few millimeters in size, unlike *in vivo* organs; therefore, they are still insufficient for clinical organ transplantation [43]. However, convergence research in the fields of stem cell biology, developmental biology, bioengineering, biomaterials, tissue engineering, and microfluidics can address this problem in the future.

Organs in the body comprise different kinds of cells, including immune cells, vascular cells, fibroblasts, and microbes [44–46]. These cells all interact with each other to perform a variety of organ functions. However, organoids are currently derived from only one stem cell type. Therefore, to establish a physiological replica of human organs *in vitro*, a co-culture system between organoids and different cell types is required.

Conclusion

In this review, we summarized the progress, applications, and limitations of various organoids. Organoids are mini-organs cultured *in vitro* that resemble their organs of origin in terms of functions and characteristics. Various organoids aiming to replicate organs such as the brain, gut, liver, testis, uterus, fallopian tube, and endometrium have been derived from PSCs and ASCs for use in a variety of applications, including organ development models, disease modeling, drug development, regenerative medicine, precision medicine, and transplantation. Although

organoid technology has rapidly expanded in recent years, its applications remain at the proof-of-principle level. The convergence of organoid research with stem cell biology, biomaterials research, tissue engineering, and microfluidics would make it possible to overcome several limitations of current organoid technology and to advance its implementation in preclinical and clinical research.

Notes

Conflict of interest

No potential conflict of interest relevant to this article was reported.

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Author contributions

Conceptualization: JHP, DGB, JKC; Data curation: JHP, JKC; Formal analysis: JHP, JKC; Investigation: JHP, DGB, JKC; Project administration: JKC; Resources: JHP, DGB; Supervision: JKC; Writing—original draft: JHP, JKC; Writing—review & editing: JHP, JKC.

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Data availability

Please contact the corresponding author for data availability.

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