Kidney Diseases

Review Article

Kidney Dis 2025;11:1–10
DOI: 10.1159/000541919

Received: May 9, 2024
Accepted: October 3, 2024
Published online: November 11, 2024

Progress of Induced Pluripotent Stem Cell-Derived Renal Organoids in Clinical Application

Na Ning[a, b, c] Zhiting Liu[a, c] Xinyu Li[a] Yi Liu[b] Wei Song[a]

[a]Department of Minimally Invasive Comprehensive Treatment of Cancer, Shandong Provincial Hospital Affiliated to Shandong First Medical University, Jinan, China; [b]Pediatric Research Institute, Children's Hospital Affiliated to Shandong University, Jinan, China; [c]Fuwei Biotechnology (Shandong) Co., LTD, Jinan, China

Keywords
Human-induced pluripotent stem cells · Kidney organoids · Three-dimensional model · Drug screen

Abstract
Background: Kidney disease has become a growing public health problem worldwide, and there is an urgent need to develop reliable models for investigating novel and effective treatment strategies. In recent years, kidney organoids, as novel models different from traditional two-dimensional cells and model animals, have attracted more and more attention. Current advances have allowed the generation of kidney organoids from the directed differentiation of induced pluripotent stem cells (iPSCs), which possess similar characteristics to embryonic stem cells, but bypass ethical constraints and have a wide range of sources. ***Summary:*** Herein, the methods of generating renal organoids from iPSCs, the applications of iPSC-derived renal organoids in disease modeling, drug effectiveness detection, and regenerative medicine as well as the challenges were reviewed. ***Key Messages:*** iPSC-derived renal organoids can be used to model kidney diseases and are great models for studying kidney injury and toxicity. Many efforts are needed to finally apply organoids into clinical application.

Introduction

Organoids are in vitro three-dimensional (3D) structures generated from pluripotent stem cells or adult stem cells, which closely mimic the structures and function of native organs [1, 2]. They can be maintained in a long term in vitro while keeping the stability of physiological and genetic information [3]. Induced pluripotent stem cells (iPSCs) refer to those that reprogram terminally differentiated somatic cells into pluripotent stem cells by introducing specific transcription factors. iPSCs possess similar characteristics to embryonic stem cells, but bypass ethical constraints and have a wide range of sources [4]. In recent years, the application of iPSCs has shifted from the cellular level to organoid-based investigations since iPSC-derived organoids possess multicellular and multifunctional features as well as organ-like nature [4].

The kidney, an important organ that functions in the production and discharge of urine, maintenance of electrolyte acid-base balance, regulation of synthesis, and secretion of a variety of hormones, plays a vital role in maintaining the stability of internal environment [5]. In recent years, kidney disease has become a growing public health problem that influences more than 850 million people worldwide [6, 7]. It is particularly important to

karger@karger.com
www.karger.com/kdd

Correspondence to:
Xinyu Li, lixinyu@sdfmu.edu.cn
Yi Liu, y_liu99@sina.com
Wei Song, songwei@sdfmu.edu.cn

create highly biomimetic models that can simulate the normal physiological function of kidney and the occurrence and progression of kidney disease in vitro for drug development or curative effect prediction. At present, iPSC-derived renal organoids have been reported, providing strong support for disease modeling, drug screening, and organ regeneration. Herein, we reviewed the advances in the generation of iPSC-derived renal organoids, the efforts that have been made in modeling genetic kidney diseases and other kidney diseases, and the potential applications of iPSC-derived renal organoids in disease therapy.

Generation of iPSC-Derived Renal Organoids

Several initial core protocols have been developed to generate 3D renal organoids from human pluripotent stem cells (hPSCs) in 2014–2015, with variations in culture medium, combination and concentration of growth factors used for directed differentiation, etc. (Fig. 1; Table 1) [8–13]. In 2014, Taguchi et al. [8] successfully induced metanephric nephron progenitors from human iPSCs in vitro. iPSCs were treated with bone morphogenetic protein 4 (Bmp4) for 24 h and then treated with activin A and fibroblast growth factor 2 (Fgf2) for another 48 h. The cells were induced into mesodermal lineage cells. Then, the cells were treated with Bmp4 and a high concentration of Wnt agonist (CHIR99201 [CHIR], 10 mM) for 6 days to further posteriorize and maintain the immature mesoderm state. By further induction with activin A, Bmp4, CHIR (3 mM), and retinoic acid for 2 days, the posterior intermediate mesoderm was formed. After that, CHIR (1 mM) and fibroblast growth factor 9 (Fgf9) were added. Metanephric mesenchyme (MM) was obtained 3 days later (Fig. 1a). The metanephric nephron progenitor markers (Wt1, Pax2, Sall1, and Six2) were found to be positive in 20%–70% of the MM stage cells. MM needs to be further cocultured with mouse spinal cord to obtain organoids with proximal and distal tubules and podocyte structures [8].

Similar to Taguchi's method, Morizane et al. also differentiated hPSCs into nephron progenitor cells (NPCs) and then constructed kidney organoids from NPCs. But Morizane's protocol did not need to coculture NPCs with mouse spinal cord for the generation of kidney organoids and could produce NPCs and organoids using chemically defined components without supplementation of non-purified factors. In brief, hPSCs were induced to form late primitive streak by regulating Wnt signaling and then exposed to activin A to form posterior intermediate mesoderm, followed by being treated with Fgf9 to generate NPCs. Using this protocol, the researchers generated NPCs that co-expressed the critical markers (Six2, Sall1, Wt1, and Pax2) from hESCs and hiPSCs through 8–9 days of differentiation with 80%–90% efficiency. Kidney organoids were then induced from NPCs on 96-well plates that were suitable for chemical screening (Fig. 1b). The kidney organoids possess nephron-like structures, expressing markers of podocytes, proximal tubules, loops of Henle, and distal tubules in a contiguous, ordered arrangement similar to that of nephrons [9, 10].

Unlike the above-reviewed methods for kidney differentiation directly from hPSCs, Freedman et al. [11] reported a two-step protocol that formed spheroid first and then inhibited glycogen synthase kinase-3β (GSK3β). Exogenous addition of Fgf2, activin A, or Bmp was not required in this protocol (Fig. 1c). Segmented, nephron-like kidney organoids that were composed of cell populations with characteristics of proximal tubules, podocytes, and endothelial cells were differentiated from spheroids via suppression of GSK3β. However, the formation of vascularized glomerulus from hPSC podocyte-like cells and neighboring endothelial cells was not observed in organoids generated by this method.

Little's team reported the generation of kidney micro-organoids from hPSCs using a suspension culture method, which was a modified protocol of their previous investigation [12, 13]. The modified differentiation procedure is shown in Figure 1d [13]. The transcriptional equivalence of all anticipated renal cell types in the micro-organoids was consistent with the organoids produced by a previous method, as detected by single-cell transcription profiling. Using this method, the final cell yield increased by 3–4 folds compared to the static culture and thereby reducing costs [13].

Kidney organoids generated in static culture are usually variable, vascularless, immature, and low throughput. A comparison of Morizane's and Takasato's protocols using single-cell transcriptomics revealed that both protocols generated multiple kidney cells with different proportions, but the organoid-derived cell types were immature and 10%–20% of cells were nonrenal [14]. Differences in the proportion of cell types in organoids are possibly due to differences in iPSCs and induced batches [14, 15]. Homan et al. [16] developed a millifluidic culture system that cultivated kidney organoids underflow on 3D-printed millifluidic chips, enlarging their endogenous pool of endothelial progenitor cells and producing vascular networks with perfusable lumens

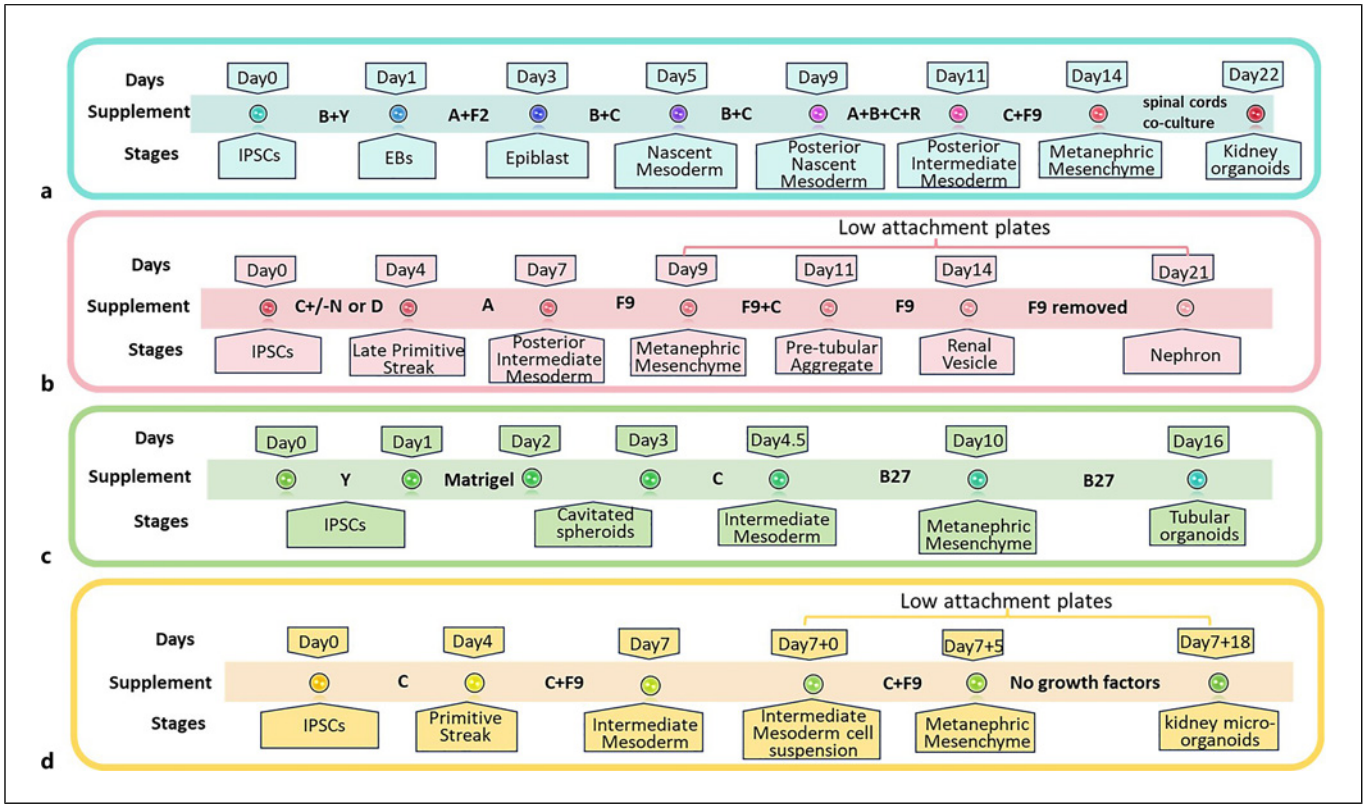

Fig. 1. Different differentiation protocols into kidney organoids from hiPSCs. **a** Taguchi et al. protocol of metanephric nephron progenitor induction from hiPSCs. **b** Protocols for generation of kidney organoids from hPSCs reported by Morizane's team. **c** Schedule of spheroid-to-organoid culture protocol published by Freedman and colleagues. **d** Outline of the kidney micro-organoid differentiation protocol from iPSCs (CRL1502.C32 cells) reported by Little's team. A, activin A; B, Bmp4; C, CHIR99201; D, dorsomorphin; F2, Fgf2; F9, Fgf9; N, noggin; R, retinoic acid; Y, Y27632.

surrounded by mural cells. Kidney organoids generated under flow presented more mature podocyte and tubular compartments. Zeng and colleagues generated an expandable, 3D branching ureteric bud (UB) organoid culture model which could be derived from both mouse and human fetal kidneys' primary UB progenitors, or generated de novo from hPSCs or hiPSCs. UB organoids generated collecting duct organoids, with differentiated principal and intercalated cells adopting spatial assemblies reflective of the adult kidney's collecting system [17]. Little's team found that prolonged maintenance and delayed epithelialization of the nephron progenitor population improved the maturation and functionality of the proximal tubules [18, 19]. In 2024, researchers found that rapamycin treatment and indirect coculture of differentiating iPSCs with iPSC macrophages and peripheral blood mononuclear cells activated autophagy and prevented apoptosis induced by CHIR. Moreover, monocytes promote the development of organoids via the release of extracellular vesicles [20]. ETS translocation variant 2 (ETV2) has been reported to play a central role in directing endothelial cell differentiation. Engineered ETV2-expressing cells contributed to the formation of complex vascular-like network in human cortical organoids [21]. Through mixing iETV2-hiPSCs with nontransgenic hiPSCs, vascularized kidney organoids were obtained. The kidney organoids exhibited extensive endothelialization and increased maturation of nephron structures [22]. Using different bio-ink materials, a variety of printing processes, and flexible 3D space design, 3D bioprinting technology can manufacture fine 3D structures composed of a variety of materials and realize the integrated printing of bionic structural units and biological functional units [23]. Lawlor et al. [24] generated nephron organoids from iPSC-derived nephron progenitors employing extrusion-based bioprinting. Using this technology, the size, cell number, and conformation of the nephron organoids can be

Table 1. Protocols of generation of hPSC-derived renal organoids

Protocols	Cell source	Organoid morphology	The efficiency of generating NPCs	Components in organoids	Culture dimension	Advantages	Limitations
Taguchi et al. [8] protocol	Mouse ESC/ hiPSCs	Spherical organoids	Induced SIX2 + NPCs with ~62% efficiency	Wt1/ nephrin^{+}glomeruli; cadherin6^{+} proximal tubules; E-cadherin^{+} distal tubules	3D	A big step toward kidney reconstruction in vitro	Needs to coculture with mouse embryonic spinal cords; lower efficiency of generating NPCs; immaturity
Morizane et al. [9, 10] protocol	hESCs/ hiPSCs	Spherical organoids	Induced SIX2 + NPCs with 80–90% efficiency	Multi-segmented nephron structures with characteristics of podocytes, proximal tubules, loops of Henle, and distal tubules	2D and 3D	Using monolayer culture for NPC generation; using fully defined medium; higher efficiency of generating NPCs	The differentiation efficiency is affected by the variability intrinsic to hPSC lines; no endothelia-like cells and CD-like cells; immaturity
Freedman et al. [11] protocol	hESCs/ hiPSCs	Tubular organoids	/	Segmented nephron structures containing cell populations with characteristics of proximal tubules, podocytes, and endothelium	2.5D, Matrigel sandwich spheroids	No exogenous addition of Fgf2, activin, or Bmp; low cost; high throughput; sample steps	Organoids are random in size, not uniform; no CD structure; more off-target cells; immaturity
Little's team's protocol [12, 13]	iPSC/ hESC	Spherical organoids	/	Six to ten nephrons surrounded by endothelial and stromal populations	3D	Specifying to IM before aggregate formation; higher cell yield; low cost	Immaturity

CD, collecting duct; ESC, embryonic stem cell.

precisely manipulated, and the nephron yield per starting cell number was substantially improved. Although modifications of the protocols for the differentiation of kidney organoids from hiPSCs have been made since the publication in 2014, there is still a significant gap in obtaining vascularized, mature kidney organoids.

Application of iPSC-Derived Renal Organoids

Modeling Kidney Diseases

One of the attractive applications for establishing patient iPSC-derived kidney organoids is disease modeling. As the most common inherited kidney disease, autosomal dominant polycystic kidney disease (ADPKD) accounts for 7–10% of all patients receiving renal replacement therapy all over the world [25]. PKD1 and PKD2, which encode ciliary proteins, are the most common pathogenic genes of ADPKD, of which PKD1 accounts for about 85% [26, 27]. Using CRISPR/Cas9 gene editing technology, PKD1 biallelic mutation was introduced into hPSCs. The kidney organoids derived from these PKD1 gene-edited hPSCs formed large as well as translucent cyst-like structures in the proximal tubule of organoids [11, 28].

Recently, Shimizu and colleagues generated new kidney organoids utilizing hiPSCs derived from an ADPKD patient. This novel ADPKD kidney organoid model could reproduce renal cysts and possesses the potential to serve as a drug screening platform [29]. Compared with the mouse models of polycystic kidney disease, the ADPKD organoid models established from hPSCs or hiPSCs eliminate the differences between

species and are more conducive to the in-depth study of the pathogenesis and drug screening.

Autosomal recessive polycystic kidney disease, correlated with enlarged kidney and biliary dysgenesis, is the neonatal form of PKD with PKHD1 as the pathogenesis [30]. Low et al. [31] successfully generated kidney organoids from autosomal recessive polycystic kidney disease iPSCs, which recapitulated renal cyst formation in the proximal tubules following cAMP stimulation. The cystic phenotype of the kidney organoids can be ameliorated by gene correction using CRISPR-Cas9 or drug treatment. This kidney organoid model provides new avenues for studying human kidney development, modeling disease pathogenesis, and patient-specific drug screening.

Mutations in ADAMTS9 are correlated with the development of nephronophthisis-related ciliopathies (NPHP-RC). Glomeruli of the NPHP-RC patients are mostly normal, and proteinuria is negligible. Recently, Yu et al. [32] discovered new compound-heterozygous ADAMTS9 variants in two NPHP-RC siblings with glomerular manifestations and proteinuria. They generated kidney organoids from ADAMTS9 knockout hiPSCs and found that knockout of ADAMTS9 exhibited no influence on the differentiation of nephrons but decreased the number of primary cilia. Arai et al. [33] established kidney organoids from NPHP1-overexpressing hiPSCs and NPHP1-deficient hiPSCs and found that cyst formation in patient-derived kidney organoids was rescued by NPHP1 overexpression. In kidney organoids, NPHP1-deficient hiPSCs resulted in the downregulation of cilia-related genes in epithelial cells and the upregulation of cell cycle-related genes.

Using karyomegalic interstitial nephritis patient-derived hiPSCs (FAN1-mutant) as well as FAN1-edited hiPSCs (WTC-11 FAN1+/−) that edited by CRISPR/Cas9 system, researchers generated kidney organoids modeling FAN1-deficient kidney disease. When treated with mitomycin C, the level of healthy viable cells was reduced while the levels of DNA damage markers were enhanced in the FAN1-mutant and FAN1-edited organoids compared to wild type (WT), suggesting the effective recapitulation of the phenotype of FAN1-deficient kidney disease [34].

Cui et al. [35] revealed that kidney organoids generated from WT-hiPSC and Fabry disease (FD) patients-derived hiPSCs both exhibited typical nephron marker expression without structural deformity. In kidney organoids derived from FD patients, alpha-galactosidase A activity was declined and globotriaosylceramide deposition was enhanced relative to that in WT. Notably, globotriaosylceramide deposition was more significant in CMC-Fb-001 organoids (from a patient with a classic type of FD) than in CMC-Fb-003 organoids (non-classic FD), suggesting that these kidney organoids not only recapitulate the phenotype but also represent the severity of FD in line with the GLA mutation type. In the same year, this group reported that CRISPR/Cas9-mediated repression of A4GALT expression could rescue the phenotype of FD nephropathy in the kidney organoid model [36]. Similarly, Lim et al. [37] established kidney organoids from Gitelman's disease (GIT) patient-derived hiPSCs and proved that this organoid model recapitulates the GIT phenotype in terms of NCCT expression decrease. Correction of SLC12A3 gene mutation mediated by CRISPR/Cas9 system rescued the disease phenotype of GIT kidney organoids. Taken together, these investigations suggest that hiPSC-based kidney disease modeling can contribute to elucidating molecular pathogenesis.

Organoid Models for Researching Kidney Injury and Toxicity

Potential drugs might exhibit unexplained toxicity during drug development, thus resulting in pharmaceutical companies' significant financial loss. Renal organoids, which largely mimic the structure and function of natural organs, have the potential to be a more accurate model system for overcoming the gaps between preclinical drug efficacy studies in cell cultures and animal models and clinical trials [18, 38]. Morizane et al. [9] investigated the potential of hESC-derived kidney organoids for researching kidney injury and toxicity in vitro. The expression of kidney injury molecule-1 (KIM-1) (a biomarker of acute kidney injury [39]) was upregulated in gentamicin (proximal tubular toxicity)-treated organoids at the luminal surface of LTL+ tubules in a dose-dependent manner; meanwhile, cisplatin (proximal tubular toxicity) treatment remarkably enhanced KIM-1 in LTL+ tubules and repressed E-cadherin expression, suggesting kidney organoids as patient-specific models of toxic kidney injury for testing the nephrotoxicity of drugs and other chemicals [9]. Dilz and colleagues performed a quantitative and optical assessment of chemotherapeutic agent doxorubicin-induced toxicity in hiPSC-derived renal organoids. After being treated by doxorubicin (0.08–5 μg/mL) for 48 h, cell viability of the organoids was significantly and dose-dependently reduced. At high drug concentrations (1.25 and 5 μg/mL), tubular disintegration and loss of cellular boundaries were observed using confocal microscopy, which were further confirmed by a dose-dependent reduction of the nuclear area [38]. Notably, in this system, each component of organoids is

equally exposed to the drug and all cell types rather than only podocytes would be influenced, which may be different from the physiological effect that glomerulus and proximal tubules were typically exposed to the highest drug concentration since kidney's filtering function [38, 40]. hiPSC-derived HMOX1-reporter renal organoids could forecast toxicity to kidneys, while they are variable in their individual responses to drug treatment and certain organoid formats (flat or 3D) might be better suitable to distinct drugs [41]. Gu et al. [42] explored nephrotoxicity induced by esculentoside A utilizing iPSC-derived kidney organoids. After being treated by esculentoside A, podocytes and proximal tubular endothelial cells in organoids were injured; meanwhile, KIM-1, β2-microglobulin, and cystatin C were induced. These investigations support that iPSC-derived kidney organoids are good models for researching the nephrotoxicity of drugs.

Drug Screening

Drug development has always been a process of "high investment, high risk, and low return." The lack of suitable animal models, high cost, ethical issues, and differences between species are important factors hindering preclinical trials [43]. Organoids can well simulate the function of human organs and better reflect the human body's treatment, therefore are considered as a promising model for better drug screening.

Freedman and colleagues developed a high-throughput automation platform and examined the influences of eight compounds on hPSC-derived PKD organoids. Blebbistatin, an inhibitor of nonmuscle myosin II ATPase activity, was identified as a specific activator of PKD cystogenesis in organoids [44]. Hollywood et al. [45] developed iPSCs and kidney organoid models of cystinosis. Inhibition of mTOR using everolimus could recover basal autophagy to a healthy control level. Combined treatment with everolimus and cysteamine rescued all of the observed cystinotic phenotypes in the models, implying that combined treatment may improve curative effects in cystinosis patients. Recently, Oishi and colleagues exposed hiPSC-derived kidney organoids to cisplatin and mimic severe acute kidney injury. And then, Food and Drug Administration (FDA)-approved drugs were tested for therapeutic and nephrotoxicity screening. Using fully automated 3D imaging, imatinib (a tyrosine kinase inhibitor used in hematological malignancies) was recognized as a cisplatin-induced kidney injury's possible preventive therapy [46]. In 2022, the US FDA for the first time, based on preclinical efficacy data obtained from human organoid-on-a-chip studies, combined with existing safety data, approved a sutimlimab antibody targeting the complement C1s protein for the treatment of demyelinating diseases into clinical trials, reflecting the drug developer and the US FDA's confidence and endorsement of organoid research [47]. In China, the State Medical Products Administration has repeatedly included new in vitro evaluation techniques such as organoids and microfluidic into the preclinical evaluation guidelines for drugs. Organoids and microfluidic models can be used to provide useful complementary information for efficacy and safety evaluation when relevant animal models are not available. The governments' positive attitude toward the application of organoid technology will promote the application of organoids in the research and development of new drugs. Moreover, as organoids can reflect the genetic mutations and physiological characteristics of patients, the use of patient iPSC-derived organoids for drug screening can help achieve personalized precision therapy.

Cell Therapy and Regenerative Medicine

It has been reported that transplantation of OSR1 + SIX2+ NPCs derived from hiPSCs in the renal capsule has therapeutic effectiveness for acute kidney injury induced by ischemia/reperfusion in mice [48]. Cell therapy using NPCs may contribute to renoprotective factor generation, yet much further research is needed to develop cellular therapies to effectively generate new nephrons or expand renal tubules in vivo [49].

Regenerative medicine refers to reconstructing diseased or damaged tissues through innovative medical treatments, or supporting the regeneration of diseased and damaged tissues. Organoids are considered a potential source of transplantable tissue and functional cell types, and attempts to apply organoids to the field of organ transplantation have never been interrupted. Several studies have revealed that impaired organ function in animals could be repaired by transplantation of organoids [50]. For example, by replacing the native colonic epithelium with ileum-derived organoids in mice, a highly vascularized and functional small intestinalized colon was generated. In short-bowel syndrome rats, small intestinalized colon can restore its absorption function and significantly improve intestinal failure [51]. In 2021, it was first reported in science that transplanting cholangiocyte organoids into human livers undergoing ex vivo normothermic perfusion could repair the damaged biliary tract [52]. Recently, Wu et al. [53] reported the first-in-human tissue replacement therapy using islet tissue (E-islets) generated in vitro from autologous endoderm stem cells for islet function-impaired type 2

diabetes. The first 27-month data showed significant improvements in glycemic control with no tumor formation or severe graft-related adverse events. These reports indicate the prospect and unique advantage of organoids in regenerative medicine.

Patients with end-stage renal disease require dialysis or kidney transplantation for sustained life. However, the limited supply of healthy kidney sources as well as the inherent complication of tissue rejection emphasizes the requirement for additional kidney sources. If immune-compatible grafts could be differentiated from the individual-derived iPSCs, more patients would benefit. PSC-derived kidney organoids in vitro possess nephron structures, while they are often disorganized and immature [54]. The vascularization of organoids is a critical step in transplantation, as they need to form a connection to the host circulatory system. It has been reported that transplantation of kidney organoids under immunodeficient mice's kidney capsule allows mouse endothelial cells to infiltrate the organoids and facilitate vascularization [31, 54–57]. Functional vascularization is needed for ongoing morphogenesis and maturation of kidney organoids [54]. Kidney organoid xenografts could form vascularized, glomerulus-like structures, and exhibit filtration function to some degree, whereas the lack of appropriate collecting duct outlets and adequate stromal-like cells restricts the function and raises safety concerns [58, 59]. A combination of nephron-like as well as ureteric-like organoids together with renal stromal cells might provide help for generating more functional grafts [58]. Different from the organoids generated from primary tissues, embryonic stem cell/iPSC-derived organoids often present cell types other than the intended lineage since the factors utilized for directed differentiation are not able to completely drive all the cells into the lineage of choice [60]. scRNA-seq analysis identified off-target cells, such as paraxial mesoderm-like cells and neuronal cells in nephron organoids [14]. Single-cell census revealed diminished off-target cells after the transplantation of organoids under the mouse kidney capsule [57]. It is worth noting that transplantation of nephron organoids sometimes led to the formation of cartilage-like tissues [56] and Wilms tumor-like tissues [61], which might be attributed to the off-target cells and/or insufficient epigenetic programming of nephron progenitors that would back to the undifferentiated tumor-like state [62].

Another problem for transplantation is immune rejection. Both autologous iPSC-derived retinal pigment epithelial cells [63] and human leukocyte antigen (HLA)-matched allogeneic iPSCs-derived retinal pigment epithelium [64] have been reported to successfully transplant into age-related macular degeneration and remain intact and safe in 1 year. In 2020, Schweitzer et al. [65] reported the transplantation of autologous iPSC-derived dopamine progenitor cells in the putamen of Parkinson's disease patients. The implanted cells survived for 2 years without side effects. The symptoms of Parkinson's disease after implantation were stable or ameliorative. However, these successes were limited to the immune-privileged sites. A powerful immune response will be potentially elicited after the transplantation of tissue or cells in the kidney. To address this issue, Gaykema et al. [66] knocked out beta-2 microglobulin (B2M) in iPSCs using CRISPR-Cas9 and successfully obtained iPSCs without surface expression of HLA class I. Knockout of B2M protected the iPSC-derived kidney organoids from T-cell rejection in vitro. However, this protection became inadequate to restrain T-cell-mediated immune rejection in mice with human peripheral blood mononuclear cells, partially because the preexisting expression of HLA class II posed an additional immunological challenge to its transplantation. Shankar et al. [67] revealed that both exposure of kidney organoids to immune cells in vitro and in vivo resulted in the robust infiltration of immune cells. Infiltration of immune cells in kidney organoids formed a more realistic model for drug research and alloreactivity studies. In the future, the safety issues including the remained off-target cells with pluripotency and immune attack should be solved before considering clinical trials for the transplantation of synthetic kidneys generated from PSCs [62].

Advantages and Limitations

Using iPSC-derived kidney organoids, we can screen effective drugs and assess the potential renal toxicity of the drugs in an environment closer to the in vivo microenvironment, thus reducing the usage of animals, accelerating the development of new drugs, and saving research and development costs. Using this 3D model, we can not only bridge gaps between animal models and human tissues, but also provide more sophisticated 3D human kidney tissues than 2D cell cultures [68–70].

However, the limitations of iPSC-derived kidney organoids should not be ignored. First, the generation of kidney organoids from iPSCs required a series of growth factors and supplements to simulate the microenvironment of cell growth, which resulted in a high cultivation cost. The types and dosages of growth factors and nutrients used in iPSC-derived organoid culture medium also vary from laboratory to laboratory. Although several companies have commercialized the culture media, there

are also significant differences between their culture systems. More importantly, the addition of growth factors possibly causes gene mutation, resulting in a deviation between the results of the mechanism exploration and the drug susceptibility test, and the actual situation [50]. Matrigel is usually needed for the cultivation of iPSC-derived renal organoids. Its ingredients are complex and often present different properties between batches. Freezing and thawing, packaging, storage, and use of the operating conditions are strict and complex. The temperature-sensitive cross-linking conditions are uncertain, and the gel strength is uncontrollable. In addition, the presence of heterogeneous contaminants is another important limitation. The development of materials with specific compositions, such as hydrogels, will be a promising succedaneum. Recently, Ivan Krupa et al. [71] described the protocols for the generation of hiPSC-derived kidney organoids and their maturation within self-assembling peptide hydrogels and gelatin methacryloyl hydrogels. In the future, optimization of the culture medium, reduction of the cost, establishment of uniform standardized organoid construction processes, and quality control standards will promote the popularization of iPSC-derived kidney organoid technology and accelerate its development. Second, despite the advances in the methods for the generation of kidney organoids from iPSCs, the immature tissue development, insufficient vascularization, inability to form effective collection tubes, and the presence of several nonrenal cells (such as muscle cells and neurons) during induced differentiation are still important factors hindering the progress and application of iPSC-derived renal organoids [72, 73]. The organoids induced by iPSCs resemble fetal tissue; therefore, they are applicable for modeling diseases exhibiting abnormalities in early embryonic development. For modeling late-onset diseases, more mature organoids need to be developed. To realize kidney organoid transplantation, major efforts are required to achieve functional vascular perfusion and ureters that can connect to the host's tissue.

Conclusion

Kidney organoids induced by hiPSCs in vitro resemble human kidney and exhibit many advantages and application prospects as a novel research method different from conventional research techniques (2D cell culture and model animals). The potential applications of iPSC-derived renal organoids include disease model, kidney injury and toxicity model, drug screening, cell therapy, and regenerative medicine. However, the research and application of iPSC-derived renal organoids face many challenges since a wide gap still exists between the structure and the function of the renal organoids generated from iPSCs and the mature kidney at present. Protocol modifications toward improving the efficiency of organoid induction and obtaining more mature and functional kidney organoids still need a lot of exploration. These efforts will finally facilitate clinical transformation in the future.

Conflict of Interest Statement

The authors declare that they have no competing interests.

Funding Sources

This work was funded by National Human Genetic Resources Sharing Service Platform Special Funded Project (YCZYPT[2020] 10-1) and Key Research and Development Program of Shandong Province (2022CXGC020501).

Author Contributions

Na Ning: methodology, investigation, and writing original draft. Zhiting Liu: methodology and investigation. Xinyu Li: methodology, investigation, and writing – review and editing. Yi Liu: conceptualization, methodology, investigation, funding acquisition, project administration, supervision, and writing – review and editing. Wei Song: conceptualization, methodology, investigation, funding acquisition, project administration, and supervision.

References

1 Kretzschmar K, Clevers H. Organoids: modeling development and the stem cell niche in a dish. Dev Cell. 2016;38(6):590–600. https://doi.org/10.1016/j.devcel.2016.08.014

2 Andrews MG, Kriegstein AR. Challenges of organoid research. Annu Rev Neurosci. 2022; 45:23–39. https://doi.org/10.1146/annurev-neuro-111020-090812

3 Tuveson D, Clevers H. Cancer modeling meets human organoid technology. Science. 2019;364(6444):952–5. https://doi.org/10.1126/science.aaw6985

4 Zhang T, Qian C, Song M, Tang Y, Zhou Y, Dong G, et al. Application prospect of induced pluripotent stem cells in organoids and cell therapy. Int J Mol Sci. 2024;25(5):2680. https://doi.org/10.3390/ijms25052680

5 Takasato M, Little MH. The origin of the mammalian kidney: implications for recreating the kidney in vitro. Development. 2015; 142(11):1937–47. https://doi.org/10.1242/dev.104802

6 Foreman KJ, Marquez N, Dolgert A, Fukutaki K, Fullman N, McGaughey M, et al. Forecasting life expectancy, years of life lost, and all-cause and cause-specific mortality for 250 causes of death: reference and alternative scenarios for 2016-40 for 195 countries and territories. Lancet. 2018; 392(10159):2052–90. https://doi.org/10.1016/S0140-6736(18)31694-5

7 Liang J, Liu Y. Animal models of kidney disease: challenges and perspectives. Kidney360. 2023;4(10):1479–93. https://doi.org/10.34067/KID.0000000000000227
8 Taguchi A, Kaku Y, Ohmori T, Sharmin S, Ogawa M, Sasaki H, et al. Redefining the in vivo origin of metanephric nephron progenitors enables generation of complex kidney structures from pluripotent stem cells. Cell Stem Cell. 2014;14(1):53–67. https://doi.org/10.1016/j.stem.2013.11.010
9 Morizane R, Lam AQ, Freedman BS, Kishi S, Valerius MT, Bonventre JV. Nephron organoids derived from human pluripotent stem cells model kidney development and injury. Nat Biotechnol. 2015;33(11):1193–200. https://doi.org/10.1038/nbt.3392
10 Morizane R, Bonventre JV. Generation of nephron progenitor cells and kidney organoids from human pluripotent stem cells. Nat Protoc. 2017;12(1):195–207. https://doi.org/10.1038/nprot.2016.170
11 Freedman BS, Brooks CR, Lam AQ, Fu H, Morizane R, Agrawal V, et al. Modelling kidney disease with CRISPR-mutant kidney organoids derived from human pluripotent epiblast spheroids. Nat Commun. 2015;6:8715. https://doi.org/10.1038/ncomms9715
12 Takasato M, Er PX, Chiu HS, Maier B, Baillie GJ, Ferguson C, et al. Kidney organoids from human iPS cells contain multiple lineages and model human nephrogenesis. Nature. 2016;536(7615):238. https://doi.org/10.1038/nature17982
13 Kumar SV, Er PX, Lawlor KT, Motazedian A, Scurr M, Ghobrial I, et al. Kidney micro-organoids in suspension culture as a scalable source of human pluripotent stem cell-derived kidney cells. Development. 2019;146(5):dev172361. https://doi.org/10.1242/dev.172361
14 Wu H, Uchimura K, Donnelly EL, Kirita Y, Morris SA, Humphreys BD. Comparative analysis and refinement of human PSC-derived kidney organoid differentiation with single-cell transcriptomics. Cell Stem Cell. 2018;23(6):869–81.e8. https://doi.org/10.1016/j.stem.2018.10.010
15 Phipson B, Er PX, Combes AN, Forbes TA, Howden SE, Zappia L, et al. Evaluation of variability in human kidney organoids. Nat Methods. 2019;16(1):79–87. https://doi.org/10.1038/s41592-018-0253-2
16 Homan KA, Gupta N, Kroll KT, Kolesky DB, Skylar-Scott M, Miyoshi T, et al. Flow-enhanced vascularization and maturation of kidney organoids in vitro. Nat Methods. 2019;16(3):255–62. https://doi.org/10.1038/s41592-019-0325-y
17 Zeng Z, Huang B, Parvez RK, Li Y, Chen J, Vonk AC, et al. Generation of patterned kidney organoids that recapitulate the adult kidney collecting duct system from expandable ureteric bud progenitors. Nat Commun. 2021;12(1):3641. https://doi.org/10.1038/s41467-021-23911-5
18 Vanslambrouck JM, Wilson SB, Tan KS, Groenewegen E, Rudraraju R, Neil J, et al. Enhanced metanephric specification to functional proximal tubule enables toxicity screening and infectious disease modelling in kidney organoids. Nat Commun. 2022;13(1):5943. https://doi.org/10.1038/s41467-022-33623-z
19 Vanslambrouck JM, Tan KS, Mah S, Little MH. Generation of proximal tubule-enhanced kidney organoids from human pluripotent stem cells. Nat Protoc. 2023;18(11):3229–52. https://doi.org/10.1038/s41596-023-00880-1
20 Pecksen E, Tkachuk S, Schröder C, Vives Enrich M, Neog A, Johnson CP, et al. Monocytes prevent apoptosis of iPSCs and promote differentiation of kidney organoids. Stem Cell Res Ther. 2024;15(1):132. https://doi.org/10.1186/s13287-024-03739-8
21 Skylar-Scott MA, Huang JY, Lu A, Ng AHM, Duenki T, Liu S, et al. Orthogonally induced differentiation of stem cells for the programmatic patterning of vascularized organoids and bioprinted tissues. Nat Biomed Eng. 2022;6(4):449–62. https://doi.org/10.1038/s41551-022-00856-8
22 Maggiore JC, LeGraw R, Przepiorski A, Velazquez J, Chaney C, Vanichapol T, et al. A genetically inducible endothelial niche enables vascularization of human kidney organoids with multilineage maturation and emergence of renin expressing cells. Kidney Int. 2024:S0085-2538(24)00407-1. https://doi.org/10.1016/j.kint.2024.05.026
23 Daly AC, Prendergast ME, Hughes AJ, Burdick JA. Bioprinting for the biologist. Cell. 2021;184(1):18–32. https://doi.org/10.1016/j.cell.2020.12.002
24 Lawlor KT, Vanslambrouck JM, Higgins JW, Chambon A, Bishard K, Arndt D, et al. Cellular extrusion bioprinting improves kidney organoid reproducibility and conformation. Nat Mater. 2021;20(2):260–71. https://doi.org/10.1038/s41563-020-00853-9
25 Ong AC, Devuyst O, Knebelmann B, Walz G; ERA-EDTA Working Group for Inherited Kidney Diseases. Autosomal dominant polycystic kidney disease: the changing face of clinical management. Lancet. 2015;385(9981):1993–2002. https://doi.org/10.1016/S0140-6736(15)60907-2
26 Cornec-Le Gall E, Torres VE, Harris PC. Genetic complexity of autosomal dominant polycystic kidney and liver diseases. J Am Soc Nephrol. 2018;29(1):13–23. https://doi.org/10.1681/ASN.2017050483
27 Bergmann C, Guay-Woodford LM, Harris PC, Horie S, Peters DJM, Torres VE. Polycystic kidney disease. Nat Rev Dis Primers. 2018;4(1):50. https://doi.org/10.1038/s41572-018-0047-y
28 Cruz NM, Song X, Czerniecki SM, Gulieva RE, Churchill AJ, Kim YK, et al. Organoid cystogenesis reveals a critical role of microenvironment in human polycystic kidney disease. Nat Mater. 2017;16(11):1112–9. https://doi.org/10.1038/nmat4994
29 Shimizu T, Mae SI, Araoka T, Okita K, Hotta A, Yamagata K, et al. A novel ADPKD model using kidney organoids derived from disease-specific human iPSCs. Biochem Biophys Res Commun. 2020;529(4):1186–94. https://doi.org/10.1016/j.bbrc.2020.06.141
30 Harris PC, Torres VE. Polycystic kidney disease. Annu Rev Med. 2009;60:321–37. https://doi.org/10.1146/annurev.med.60.101707.125712
31 Low JH, Li P, Chew EGY, Zhou B, Suzuki K, Zhang T, et al. Generation of human PSC-derived kidney organoids with patterned nephron segments and a de novo vascular network. Cell Stem Cell. 2019;25(3):373–87.e9. https://doi.org/10.1016/j.stem.2019.06.009
32 Yu S, Choi YJ, Rim JH, Kim HY, Bekheirnia N, Swartz SJ, et al. Disease modeling of ADAMTS9-related nephropathy using kidney organoids reveals its roles in tubular cells and podocytes. Front Med. 2023;10:1089159. https://doi.org/10.3389/fmed.2023.1089159
33 Arai Y, Ito H, Shimizu T, Shimoda Y, Song D, Matsuo-Takasaki M, et al. Patient-derived and gene-edited pluripotent stem cells lacking NPHP1 recapitulate juvenile nephronophthisis in abnormalities of primary cilia and renal cyst formation. Front Cell Dev Biol. 2024;12:1370723. https://doi.org/10.3389/fcell.2024.1370723
34 Lim SW, Na D, Lee H, Fang X, Cui S, Shin YJ, et al. Modeling of *FAN1*-deficient kidney disease using a human induced pluripotent stem cell-derived kidney organoid system. Cells. 2023;12(18):2319. https://doi.org/10.3390/cells12182319
35 Cui S, Fang X, Lee H, Shin YJ, Koh ES, Chung S, et al. Modeling of Fabry disease nephropathy using patient derived human induced pluripotent stem cells and kidney organoid system. J Transl Med. 2023;21(1):138. https://doi.org/10.1186/s12967-023-03992-0
36 Cui S, Shin YJ, Fang X, Lee H, Eum SH, Ko EJ, et al. CRISPR/Cas9-mediated A4GALT suppression rescues Fabry disease phenotypes in a kidney organoid model. Transl Res. 2023;258:35–46. https://doi.org/10.1016/j.trsl.2023.02.005
37 Lim SW, Fang X, Cui S, Lee H, Shin YJ, Ko EJ, et al. CRISPR-Cas9-Mediated correction of SLC12A3 gene mutation rescues the Gitelman's disease phenotype in a patient-derived kidney organoid system. Int J Mol Sci. 2023;24(3):3019. https://doi.org/10.3390/ijms24033019
38 Dilz J, Auge I, Groeneveld K, Reuter S, Mrowka R. A proof-of-concept assay for quantitative and optical assessment of drug-induced toxicity in renal organoids. Sci Rep. 2023;13(1):6167. https://doi.org/10.1038/s41598-023-33110-5

39 Vaidya VS, Ozer JS, Dieterle F, Collings FB, Ramirez V, Troth S, et al. Kidney injury molecule-1 outperforms traditional biomarkers of kidney injury in preclinical biomarker qualification studies. Nat Biotechnol. 2010;28(5):478–85. https://doi.org/10.1038/nbt.1623

40 Nunez-Nescolarde AB, Nikolic-Paterson DJ, Combes AN. Human kidney organoids and tubuloids as models of complex kidney disease. Am J Pathol. 2022;192(5):738–49. https://doi.org/10.1016/j.ajpath.2022.01.009

41 Lawrence ML, Elhendawi M, Morlock M, Liu W, Liu S, Palakkan A, et al. Human iPSC-derived renal organoids engineered to report oxidative stress can predict drug-induced toxicity. iScience. 2022;25(3):103884. https://doi.org/10.1016/j.isci.2022.103884

42 Gu S, Wu G, Lu D, Meng G, Wang Y, Tang L, et al. Nephrotoxicity assessment of Esculentoside A using human-induced pluripotent stem cell-derived organoids. Phytother Res. 2024;38(10):4893–903. https://doi.org/10.1002/ptr.7721

43 Lee SJ, Lee HA. Trends in the development of human stem cell-based non-animal drug testing models. Korean J Physiol Pharmacol. 2020;24(6):441–52. https://doi.org/10.4196/kjpp.2020.24.6.441

44 Czerniecki SM, Cruz NM, Harder JL, Menon R, Annis J, Otto EA, et al. High-throughput screening enhances kidney organoid differentiation from human pluripotent stem cells and enables automated multidimensional phenotyping. Cell Stem Cell. 2018;22(6):929–40.e4. https://doi.org/10.1016/j.stem.2018.04.022

45 Hollywood JA, Przepiorski A, D'Souza RF, Sreebhavan S, Wolvetang EJ, Harrison PT, et al. Use of human induced pluripotent stem cells and kidney organoids to develop a cysteamine/mTOR inhibition combination therapy for cystinosis. J Am Soc Nephrol. 2020;31(5):962–82. https://doi.org/10.1681/ASN.2019070712

46 Oishi H, Tabibzadeh N, Morizane R. Advancing preclinical drug evaluation through automated 3D imaging for high-throughput screening with kidney organoids. Biofabrication. 2024;16(3):035003. https://doi.org/10.1088/1758-5090/ad38df

47 Rumsey JW, Lorance C, Jackson M, Sasserath T, McAleer CW, Long CJ, et al. Classical complement pathway inhibition in a "Human-On-A-Chip" model of autoimmune demyelinating neuropathies. Adv Ther. 2022;5(6):2200030. https://doi.org/10.1002/adtp.202200030

48 Toyohara T, Mae S, Sueta S, Inoue T, Yamagishi Y, Kawamoto T, et al. Cell therapy using human induced pluripotent stem cell-derived renal progenitors ameliorates acute kidney injury in mice. Stem Cells Transl Med. 2015;4(9):980–92. https://doi.org/10.5966/sctm.2014-0219

49 Morizane R, Miyoshi T, Bonventre JV. Concise review: kidney generation with human pluripotent stem cells. Stem Cell. 2017;35(11):2209–17. https://doi.org/10.1002/stem.2699

50 Yang S, Hu H, Kung H, Zou R, Dai Y, Hu Y, et al. Organoids: the current status and biomedical applications. MedComm. 2023;4(3):e274. https://doi.org/10.1002/mco2.274

51 Sugimoto S, Kobayashi E, Fujii M, Ohta Y, Arai K, Matano M, et al. An organoid-based organ-repurposing approach to treat short bowel syndrome. Nature. 2021;592(7852)99–104. https://doi.org/10.1038/s41586-021-03247-2

52 Sampaziotis F, Muraro D, Tysoe OC, Sawiak S, Beach TE, Godfrey EM, et al. Cholangiocyte organoids can repair bile ducts after transplantation in the human liver. Science. 2021;371(6531)839–46. https://doi.org/10.1126/science.aaz6964

53 Wu J, Li T, Guo M, Ji J, Meng X, Fu T, et al. Treating a type 2 diabetic patient with impaired pancreatic islet function by personalized endoderm stem cell-derived islet tissue. Cell Discov. 2024;10(1):45. https://doi.org/10.1038/s41421-024-00662-3

54 van den Berg CW, Ritsma L, Avramut MC, Wiersma LE, van den Berg BM, Leuning DG, et al. Renal subcapsular transplantation of PSC-derived kidney organoids induces neovasculogenesis and significant glomerular and tubular maturation in vivo. Stem Cell Rep. 2018;10(3):751–65. https://doi.org/10.1016/j.stemcr.2018.01.041

55 Xinaris C, Benedetti V, Rizzo P, Abbate M, Corna D, Azzollini N, et al. In vivo maturation of functional renal organoids formed from embryonic cell suspensions. J Am Soc Nephrol. 2012;23(11):1857–68. https://doi.org/10.1681/ASN.2012050505

56 Bantounas I, Ranjzad P, Tengku F, Silajdžić E, Forster D, Asselin MC, et al. Generation of functioning nephrons by implanting human pluripotent stem cell-derived kidney progenitors. Stem Cell Rep. 2018;10(3):766–79. https://doi.org/10.1016/j.stemcr.2018.01.008

57 Subramanian A, Sidhom EH, Emani M, Vernon K, Sahakian N, Zhou Y, et al. Single cell census of human kidney organoids shows reproducibility and diminished off-target cells after transplantation. Nat Commun. 2019;10(1):5462. https://doi.org/10.1038/s41467-019-13382-0

58 Freedman BS, Dekel B. Engraftment of kidney organoids in vivo. Curr Transpl Rep. 2023;10(2):29–39. https://doi.org/10.1007/s40472-023-00397-2

59 Pan B, Fan G. Stem cell-based treatment of kidney diseases. Diseases. 2020;245(10):902–10. https://doi.org/10.1177/1535370220915901

60 Fatehullah A, Tan SH, Barker N. Organoids as an in vitro model of human development and disease. Nat Cell Biol. 2016;18(3):246–54. https://doi.org/10.1038/ncb3312

61 Shankar AS, Du Z, Tejeda Mora H, Boers R, Cao W, van den Bosch TPP, et al. Kidney organoids are capable of forming tumors, but not teratomas. Stem Cell. 2022;40(6):577–91. https://doi.org/10.1093/stmcls/sxac009

62 Nishinakamura R. Advances and challenges toward developing kidney organoids for clinical applications. Cell Stem Cell. 2023;30(8):1017–27. https://doi.org/10.1016/j.stem.2023.07.011

63 Mandai M, Watanabe A, Kurimoto Y, Hirami Y, Morinaga C, Daimon T, et al. Autologous induced stem-cell-derived retinal cells for macular degeneration. N Engl J Med. 2017;376(11):1038–46. https://doi.org/10.1056/NEJMoa1608368

64 Sugita S, Mandai M, Hirami Y, Takagi S, Maeda T, Fujihara M, et al. HLA-matched allogeneic iPS cells-derived RPE transplantation for macular degeneration. J Clin Med. 2020;9(7):2217. https://doi.org/10.3390/jcm9072217

65 Schweitzer JS, Song B, Herrington TM, Park TY, Lee N, Ko S, et al. Personalized iPSC-derived dopamine progenitor cells for Parkinson's disease. N Engl J Med. 2020;382(20):1926–32. https://doi.org/10.1056/NEJMoa1915872

66 Gaykema LH, van Nieuwland RY, Lievers E, Moerkerk WBJ, de Klerk JA, Dumas SJ, et al. T-cell mediated immune rejection of beta-2-microglobulin knockout induced pluripotent stem cell-derived kidney organoids. Stem Cells Transl Med. 2024;13(1):69–82. https://doi.org/10.1093/stcltm/szad069

67 Shankar AS, Tejeda-Mora H, Du Z, Nlandu Q, Palomares-Cabeza V, van den Bosch TPP, et al. Interactions of the immune system with human kidney organoids. Transpl Int. 2024;37:12468. https://doi.org/10.3389/ti.2024.12468

68 Andersen ML, Winter LMF. Animal models in biological and biomedical research: experimental and ethical concerns. An Acad Bras Cienc. 2019;91(Suppl 1):e20170238. https://doi.org/10.1590/0001-3765201720170238

69 Liu Q, Yue L, Deng J, Tan Y, Wu C. Progress and breakthroughs in human kidney organoid research. Biochem Biophys Rep. 2024;39:101736. https://doi.org/10.1016/j.bbrep.2024.101736

70 Na DH, Cui S, Fang X, Lee H, Eum SH, Shin YJ, et al. Advancements in research on genetic kidney diseases using human-induced pluripotent stem cell-derived kidney organoids. Cells. 2024;13(14):1190. https://doi.org/10.3390/cells13141190

71 Krupa I, Treacy NJ, Clerkin S, Davis JL, Miller AF, Saiani A, et al. Protocol for the growth and maturation of hiPSC-derived kidney organoids using mechanically defined hydrogels. Curr Protoc. 2024;4(7):e1096. https://doi.org/10.1002/cpz1.1096

72 Gupta N, Dilmen E, Morizane R. 3D kidney organoids for bench-to-bedside translation. J Mol Med. 2021;99(4):477–87. https://doi.org/10.1007/s00109-020-01983-y

73 Long HY, Qian ZP, Lan Q, Xu YJ, Da JJ, Yu FX, et al. Human pluripotent stem cell-derived kidney organoids: current progress and challenges. World J Stem Cells. 2024;16(2):114–25. https://doi.org/10.4252/wjsc.v16.i2.114