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Progress and breakthroughs in human kidney organoid research

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ABSTRACT

The three-dimensional (3D) kidney organoid is a breakthrough model for recapitulating renal morphology and function *in vitro*, which is grown from stem cells and resembles mammalian kidney organogenesis. Currently, protocols for cultivating this model from induced pluripotent stem cells (iPSCs) and patient-derived adult stem cells (ASCs) have been widely reported. In recent years, scientists have focused on combining cutting-edge bioengineering and bioinformatics technologies to improve the developmental accuracy of kidney organoids and achieve high-throughput experimentation. As a remarkable tool for mechanistic research of the renal system, kidney organoid has both potential and challenges. In this review, we have described the evolution of kidney organoid establishment methods and highlighted the latest progress leading to a more sophisticated kidney transformation research model. Finally, we have summarized the main applications of renal organoids in exploring kidney disease.

1. Introduction

Organoid refers to 3D cellular aggregate containing the target organ's fundamental structural unit and possessing basic physiologic function. This process is achieved by offering growth factors sequentially and providing an appropriate supportive microenvironment. Related signaling pathways are then activated; consequently, either human embryonic stem cells (ESCs), induced pluripotent stem cells (iPSCs), or tissue-resident adult stem cells (ASCs) can undergo self-organization and differentiate directly into various cell lineages of specific organs [1]. In 1981, scientists successfully isolated pluripotent stem cells from mouse embryos for the first time. Until 1998, human embryonic cells were isolated and cultured *in vitro*, and then iPSCs were established by reprogramming mouse and human fibroblasts, which marked the milestone progress of stem cell and organoids research [2]. Although recapitulating mammalian organ structure *in vitro* is challenging, many protocols have been reported over the past 15 years, including organoids of the intestine [3], stomach [4], retina [5], cerebra [6], liver [7,8], kidney [9], prostate [10], lung [11], and others.

It is particularly difficult to construct a highly organized organoid of the kidney *in vitro,* because the kidney is one of the most complex and vital organs in the human body, which is responsible for blood filtering and metabolic waste secretion in order to maintain the balance of fluid, electrolyte, temperature, and pH in the internal environment [12]. Fortunately, efforts have been made for years by many scientists. For example, in 2014, Taguchi et al. reported the first kidney organoid protocol, which could induce pluripotent stem cells (PSCs) into metanephric mesenchyme (MM), which generates most nephrons components, including glomeruli and renal tubules [13]. In 2015, Takasato et al. successfully generated kidney organoids containing both collecting duct and nephron components derived from two distinct kidney progenitors based on human PSCs [14]. At the same time, Morizane et al. established a robust protocol for differentiating hPSCs into multipotent nephron progenitor cells (NPCs), and then kidney organoids involving podocytes, proximal and distal tubules, and loops of Henle could be produced [15]. Afterward, many researchers made modifications and optimizations based on these core protocols in order to achieve scale-up cultivation and improve functional maturation and vascularization [16,17]. In 2019, Clevers et al. used ASCs isolated from urine or tissue biopsy to induce tubuloids; the cystic structures were obtained within one week after seeding in Matrigel. ASC-derived organoids produce a cost-effective and personalized *in vitro* model for patients [18].

Compared with traditional 2D cell lines and animal models, 3D kidney organoids provide better histological features and bridge gaps

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between interspecies [19]. However, this new model still has limitations in terms of maturation and functional recapitulation [20]. Fortunately, with the assistance of single-cell transcriptomics, researchers can comprehensively track the development of kidney cells and keep optimizing the differentiation protocols [21] Furthermore, with new technique like microfluidic bioreactor devices [22,23], kidney organoids are promising to become convenient platforms for scientific research, such as modeling renal disease, accelerating drug screening, and therapeutic applications [24,25]. The following sections of this review sequentially introduce the human embryonic kidney's development process and then summarize the evolution history of landmark protocols, including cultivation methods and achieved results. The progress and challenges of these new methods have also been discussed, providing theoretical support and direction for future research.

2. The developmental process of fetal kidneys

The development of the mammalian embryonic kidney has three steps: pronephros, mesonephros, and metanephros. The first two parts degenerate and disappear successively during human the first trimester. The metanephros, the so-called permanent kidney, starts developing in the fifth week of gestation. The glomeruli is formed in the ninth week, and until the tenth week, the fetal kidney is capable of generating urine [26].

Kidney organogenesis depends on the bidirectional inductive interaction between the ureteric bud (UB) lineage and the metanephric mesenchyme (MM), both of them derived from the intermediate mesoderm (IM). The outgrowth of UB precursors is induced by Glial cell–derived neurotrophic (GDNF) signaling from MM [27,28] GDNF activates Gfral/Ret receptor tyrosine kinase in the UB cells and promotes their consecutive rounds of branching to support the whole kidney structure, including the renal calyx, renal pelvis, ureter, and bladder [29]. This precise process lasts until 20 weeks of pregnancy. MM is initially dispersed in the posterior region of the embryo near the hindlimb buds. Under the induction by the UB-derived Wnt signal, MM starts proliferation and condenses into a comet-shaped mass surrounding the UB tips, called cap mesenchyme (CM). This condensation gradually undergoes a mesenchymal-to-epithelial transition (MET) and forms epithelial cysts with lumen-like structure referred to as the renal vesicles (RVs) [30,31]. RVs subsequently propagates into comma-shaped bodies and then extend to S-shaped bodies. This S-shaped structure has three segments, of which the part farthest from UB forms podocyte and Bowman's capsule, the middle part differentiates into proximal tubules, the segment near UB develops into descending branch, ascending branch, and distal tubules of the Henle's loop, and finally connects with the middle end of UB to form the main body of the nephron [32,33] (Fig. 1). In addition, the formation of mature nephrons is inseparable from the support of stromal cells and endothelial lineages. Groups of stromal progenitors originate from the MM, giving rise to the interstitium, mesangium, and numerous vascular support cells, such as vascular smooth muscle cells and pericytes [34,35]. Other stromal cells responsible for *in vivo* organ morphogenesis and proper cellular interactions possibly migrated from external embryonic tissues into the metanephric kidney [36]. This may explain the different features between current PSC-derived kidney organoids and human fetal kidney in terms of architecture and molecular patterning. Vascularization of the renal system is crucial for its specialized functions because the blood vascular system is responsible for oxygen and nutrient transportation, blood filtration, and pH maintenance. However, the detailed process of the generation of renal blood vessels is poorly understood. It is known that populations of endothelial progenitor cells (EPCs) promote the formation of de novo vessels [37,38]. Several attempts have been made to improve renal organoids' vascularization and the formation of glomerular capillaries, such as transplanting hPSC-derived renal cells into highly vascularized sites in animals, taking fluid shear stress into consideration combined with microfluidic devices. These topics will be introduced thoroughly in the following part.

3. Exploration and evolution of hiPSC-derived kidney organoids

The initial knowledge and lineage tracking of human kidney embryonic development originated from studies based on mice, thanks to the evolutionary conservation between species, which means that both embryonic precursor progenitor cells in mice and humans under specific developmental stages express highly similar molecular markers [39]. By utilizing lineage-tracing experiments and fluorescence-activated cell sorting (FACS) analyses, researchers have monitored the developmental trajectory of kidney *in vitro* in order to further clarify the process of human kidney development [40]. Besides, different organogenesis duration between mice and humans should be considered. Specifically, the embryonic day (E) of the mouse is no longer than E21, while human pregnancy is divided into three trimesters: the first trimester is from week 1 to the end of week 12, the second trimester is from week 13 to the end of week 26, and the third trimester is from week 27 to the end of pregnancy. Therefore, time extension adjustments should be made based on the induction protocol of mouse kidney organoids to adapt to the development process of human embryonic kidney.

Metanephros derives from the posterior region of the intermediate mesoderm (IM), which migrates from primitive streak (presomitic mesoderm, PSM) [41]. As mentioned above, IM gives rise to both key kidney progenitor populations, the UB and MM, with distinct temporospatial origins. UB derives from the anterior intermediate mesoderm (aIM), while MM arises from the posterior intermediate mesoderm (pIM) [42]. Critical growth factors are responsible for this precise regulation.

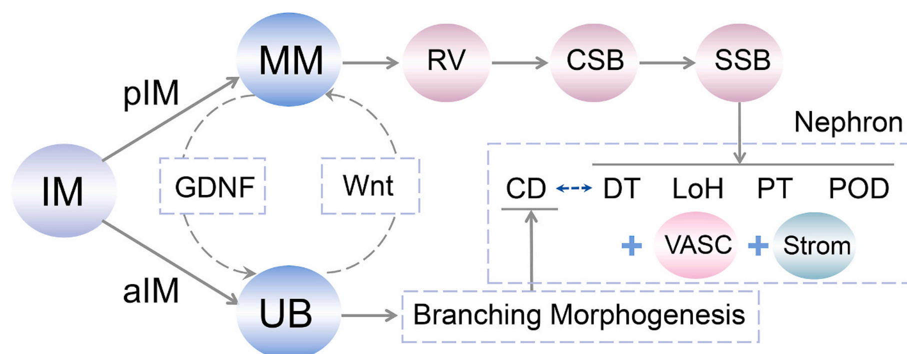

Fig. 1. Diagram of human fetal kidney development, differentiating from imtermediate mesoderm (IM) to metanephric mesenchyme (MM) and ureteric bud (UB), and finally form nephron. (pIM) posterior intermediate mesoderm; (aIM) anterior intermediate mesoderm; (GDNF) Glial cell–derived neurotrophic; (RV) renal vesicles; (CSB) comma-shaped bodies; (SSB) S-shaped bodies; (CD) Collecting duct; (DT) Distal tubule; (LoH) loop of Henle; (PT) proximal tubule; (POD) podocyte; (VASC) vasculature; (STROM) stroma.

Retinoic acid (RA) and FGF9 are required to regulate the anterior-posterior patterning, and exposure to varied duration and concentration of CHIR99021, an inhibitor of GSK-3, is utilized to control the fate selection and growth balance of UB and MM [43,44].

3.1. Initial core protocols establishing the research foundation

In 2014, Taguchi et al. separately identified the development origins of MM in mice and humans and successfully derived MM from pluripotent stem cells (PSCs), and then they used these progenitors to induce 3D kidney organoids by co-culturing with mouse embryonic spinal cords [13]. The organoids contained glomeruli with podocytes, the proximal and distal regions of renal tubules with clear lumina, which are the basic elements of the nephron. By performing microarray and quantitative RT-PCR analyses, they drew the conclusion that $Osr1^+/Wt1^+/Pax2^-/Six2^-$/posterior Hox^+ IM, located posteriorly at E9.5, was the precursor population for metanephric nephron progenitors. They proposed the combination of 10 μM CHIR99021 (Wnt agonist) and Bmp4 inducing the formation of nascent mesoderm (step 1, BC10). Next, activin and retinoic acid, combining 3 μM CHIR and Bmp4, determined the pIM (step 2, ABC3). Finally, 1 μM CHIR and FGF9 could promote pIM evolving into metanephric nephron progenitors (step 3, C1F). As a result, the MM stage of the mouse appeared at E8.5, while the human MM population was harvested at day 14 with 20–70 % cells expressing metanephric nephron progenitor markers (Wt1/Pax2/-Sall1/Six2). In terms of human iPSC-derived MM cells, when co-cultured with mouse embryonic spinal cord for 8 days, three-dimensional (3D) kidney structures were formed, consisting of Wt1/$nephrin^+$ glomeruli, cadherin6+ proximal tubules and $E\text{-}cadherin^+$ distal tubules.

In 2015, Morizane et al. defined pIM development fate and generated nephron progenitor cells (NPCs) from human pluripotent stem cells (hPSCs) with 90 % efficiency in 9 days [15]. Instead of using a mouse embryonic spinal cord, which would not be suitable for further clinical applications, their protocol applied GSK-3β inhibitor CHIR99021 and FGF9 to induce self-organizing toward renal vesicle (RV) stage, enabling the continuous development of RV to form nephron organoids consisting of podocytes, proximal tubules, loops of henle, and distal tubules. In this protocol, high dose CHIR99021 (8uM) together with Bmp4, FGF2, FGF8, FGF9, IDE-1, JAG1, Noggin, and Y-27632 was added in the beginning four days, resulting in T+/TBX6+ late primitive streak cells (step 1). Subsequently, activin and lower dose of CHIR combined with series of growth factors were used until day 7 to induce WT1+/HOXD11+ exact pIM cells, but not aIM (step 2). On day 7, pIM cells were treated with low dose of FGF9, and SIX2+/SALL1+/WT1+/PAX2+, where marked NPCs appeared within 1–2 days (step 3). From day 10 onward, the cell aggregation gradually formed renal vesicles and they could intrinsically differentiate into epithelial structures of nephron in the absence of exogenous FGF9 stimulation (step 4). Immunocytochemistry confirmed that the acquired kidney organoids at day 16 expressed tetragonolobus lectin (LTL), N-cadherin (CDH2), and Nephrin $(NPHS1)^+$Podocalyxin $(PODXL)^+$ cells, respectively, representing proximal tubules, distal tubules and podocytes.

Compared to other researchers in the same period, who assumed that simultaneous generation of ureteric epithelium (UE) and metanephric mesenchyme (MM) was paradoxical, because UE and MM have distinct temporospatial origins. In 2015, Takasato et al. provided a method to modulate the differentiation fate of both IM-derived kidney progenitor populations [14]. By using quantitative PCR and Immunofluorescence analyses, they proposed that a shorter duration of Wnt signaling (CHIR99021, an inhibitor of GSK-3) application prefer the expression of aIM markers (LHX1, GATA3), while longer period CHIR99021 induces pIM markers (HOXD11, EYA1). These observations were further confirmed in the detection of Day 18 kidney organoids. They showed ureteric epithelium characteristics ($GATA3^+/PAX2^+/ECAD^+$) under fewer days of CHIR application but derived a nephron-like structure after more days in the presence of CHIR. Then, they established an effective protocol to guide human embryonic stem (ES) cells or human induced pluripotent stem cells (iPSCs) to form kidney organoids containing both collecting duct and nephrons. Initially, four days of CHIR99021 was proved to be the best length of time to regulate the proportion of UE and MM populations (step 1). Followed by three days of FGF9, cells were changed from monolayer culture to organoid culture stage, resulting in the formation of aggregates spontaneously. Each organoid comprised renal components, including collecting duct ($GATA3^+/ECAD^+$), early distal tubule ($GATA3^-/LTL^-/ECAD^+$), early proximal tubule ($LTL^+/ECAD^-$), glomeruli ($WT1^+$) and stroma ($FOXD1^+/MEIS1^+$). Transcriptional profiling indicated that the kidney organoids at day 18 showed the highest equivalence to the first trimester embryonic kidney.

Originated from the method raised by Takasato et al. (2015), Kumar et al. proposed a modified suspension culture approach, which was more suitable for scale-up production and brought benefit to high throughput biological applications [45]. In this way, each kidney formed 6 to 10 nephrons surrounded by endothelial and stromal cells within 18 days. The combination and concentration of growth factors used in the first 7 days were almost the same as the previous method. Changes had been made since the seventh day onwards: monolayer cultures of IM cells were shifted to low adhesion 6 cm 2 cell culture dishes placed on an orbital shaker (60 rpm). Except for the basal media containing FGF9+heparin + CHIR99021, 0.1 % polyvinyl alcohol (PVA) and methyl-cellulose (MC) were added to promote cell aggregation. Przepiorski et al. came up with another adjusted suspension protocol with lower cost and higher efficiency. They used 15 % KnockOut Serum Replacement (KOSR) instead of the FGF9 factor, which is prohibitively expensive. The kidney organoids were cultured in spinner flasks bioreactors since day 8; after another 6 days, hundreds of organoids containing tubular kidney tissue were formed. At day 26, the early stages of the foot process of podocyte could be observed by electron microscopy, while apical microvilli failed to develop, indicating the relatively immature organoids [46] (Fig. 2).

3.2. Improvements and optimization in new protocols for kidney organoid

Despite the methods of generating kidney organoids from mouse embryonic stem cells (ESCs) and human induced pluripotent stem cells (hiPSCs) described above provided exciting progress in replicating renal morphogenesis *in vitro*, current protocols still fall short of what would be required for organ regeneration or accurate disease modeling. On one hand, most aIM precursors derived from UB cells could not show branching morphogenesis, which is an essential process for the organization of the kidney's elaborate geometry. For example, most kidney organoids lack higher-order structure, showing randomly arranged nephrons' contents and lacking the module-module connections. On the other hand, kidney organoids have a finite lifespan and maturity [35,47]. For instance, kidney organoids obtained by suspension culture environment would present fibrosis and in increase in off-target cells after day. The immaturity of kidney organoids can partly be attributed to the absence of functional vascular supply, because the formation of glomerular capillaries and perfused endothelial networks are challenging. Here, we introduce the latest research findings attempting to overcome these limitations (Table 1).

In 2017, Taguchi et al. defined distinct molecular cues to derive UB and MM from pluripotent stem cells of mice and humans. They manually isolated a single bud and co-cultured it with MM, including nephron progenitors (NPs) and stromal progenitors (SPs) at the same developmental stage, resulting in the recapitulation of higher-order kidney organoids [48]. The branching capacity was confirmed by time-course fluorescence images, showing that the induced UB (i-UB) underwent dichotomous branching up to 6 to 7 generations (1 generation/day). On day 7 after reconstitution, Six2+ NPs were maintained on each i-UB tip in the periphery of the organoid, mimicking the nephrogenic zone of embryonic kidneys. Meanwhile, the i-UB was proved to have nephron

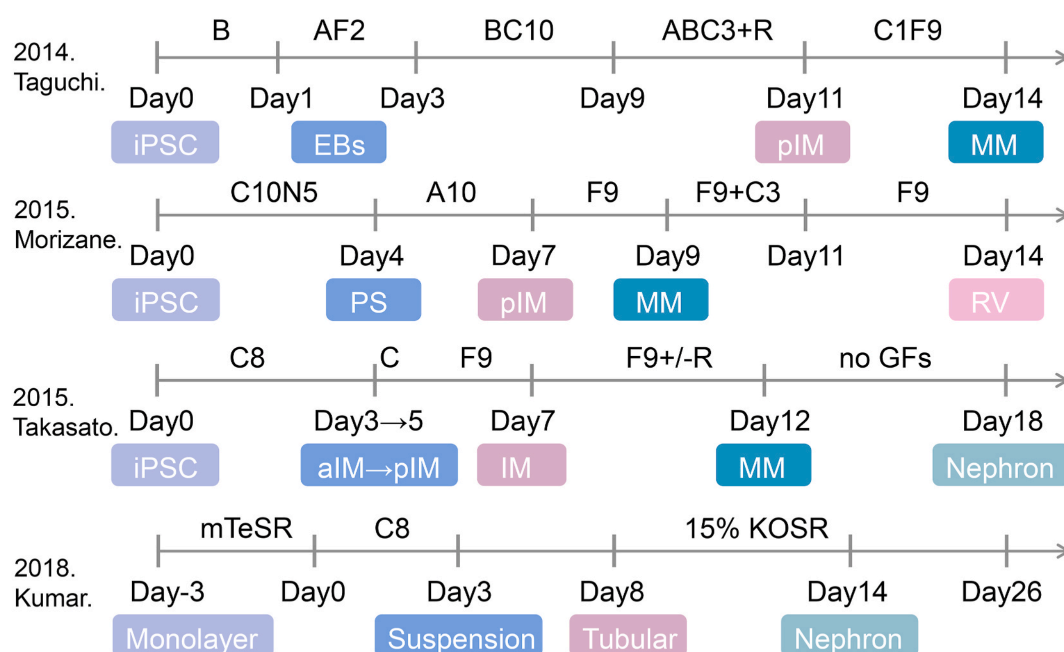

Fig. 2. Overview of several initial kidney organoid protocols, including culture timeline, growth factors used and developmental stage. (A) ActivinA (following number indicates micromolar concentration); (B) BMP4; (C) CHIR99201 (Wnt pathway agonist; following number indicates micromolar concentration); (F2) FGF2; (R) retinoic acid; (F9) FGF9; (N) Noggin; (no GFs) no growth factors added to basal medium; (mTeSR) differentiation medium (Stem Cell Technologies); (KOSR) KnockOut Serum Replacement.

Table 1
Novel improvements in human kidney organoids induction.

Protocol reference	Cell sources	Improvement	Method
Taguchi et al., 2017 [48]	Mouse and human PSCs	Higher-order kidney-like architecture; branching capacity.	Clearify molecular cues of UB and MM; Co-culture MM, NPCs and SPs.
Van den Berg et al., 2018 [50]	Human PSCs	Vascularization; plasma circulation in glomerular.	7 + 18 differentiation; Transplant under the renal capsule of mice for 28 days.
Garreta et al., 2019 [49]	Human PSCs	Large-scale cultivation; maturation (matched fetal kidneys at 22 weeks); vascularization.	Implant into CAM
Homan et al., 2019 [51]	Human PSCs	Formation of vascular networks; polarization; form brush border; upregulation of transporter.	Organoid-on-chips + fluidic shear stress
Lee et al., 2021 [52]	Human PSCs	Promotion of podocytes' maturity and angiogenesis; sensitivity to nephrotoxic drugs	Organoid-on-chips + biomechanical stimuli
Amanda et al., 2022 [53]	Human iPSCs	Formation of open lumen structure resembling vessels	co-culture with HUVECs
Lawlor et al., 2021 [54]	Human PSCs	Reproducibility, less heterogeneity, control size, number, conformation; scale-up, quality control	Automoated 3D-bioprinting

*(PSCs): pluripotent stem cells; (UB): ureteric bud; (MM): metanephric mesenchyme; (NPCs): nephron progenitor cells; (SPs): stromal progenitors; (CAM): chick chorioallantoic membrane; (ASCs): Adult stem cells; (ECM): Extracellular Matrix; (HUVECs): human umbilical vein endothelial cells.

induction capacity because distal tubule segments (E-cadherin+), proximal tubule segments (LTL+), loop of Henle (NKCC2), and glomerular (Nephrin^{+}) structures were observed on the inner side of the organoids. Vascular endothelial cells were missing, since they were not contained in the Pdgfra^{+} SPs fraction. It is noteworthy that the distal end of each nephron was connected to the ureteric tips, indicating that these organoids had higher-order kidney-like architecture.

In 2019, Garreta et al. successfully cultivated more mature kidney-like organoids than ever before, which transcriptionally resembled second-trimester human fetal kidneys (around 22 weeks). Firstly, the human iPSCs were cultured in a monolayer state, including 8 μM CHIR99021 for 3 days and FGF9+activin A for another day. The purpose was to derive iPSC toward (PAX2+) IM cells, which contain (OSR1, HOXD11) pIM and (GATA3) aIM. Then, these cells were operated to self-aggregate into three-dimensional (3D) spheroids. After 8 days, under the induction of CHIR (3 μM) for 3 days and the continuous effect of FGF, RV-stage organoids were formed, which transcriptionally matched human fetal kidneys at 16 weeks of gestation. Until day 16, relatively mature kidney organoids were obtained, with proximal tubular segment (SLC3A1) and the glomerular markers (NPHS1, PODXL, SYNPO, WT1). Additionally, they performed transmission electron microscopy (TEM) analysis and observed podocytes with deposition of a basement membrane and epithelial tubular cells with brush borders. In order to achieve vascularization, day 16 kidney organoids were implanted into the chick chorioallantoic membrane (CAM). It is a highly vascularized extracellular tissue with advantages including a naturally immunodeficient environment and minimally invasive access compared to transplantation under the kidney capsule of immunodeficient mice. Five days after implantation, multiple blood vessels from the CAM invaded kidney organoids and formed the circulation of chick blood. CAM implantation also promoted the glomeruli to form enlarged Bowman's space. Injection of cisplatin into chick vasculature, a well-known nephrotoxic agent, resulted in upregulated levels of KIM-1 (a tubule toxicity marker) and CASPASE 3 (an apoptotic marker) in proximal tubular structures of kidney organoids [49]. One year ago, Van den Berg et al. (2018) used a similar kidney organoid differentiation protocol modified from Takasato et al. (2015). After 7 + 18 days of differentiation, the organoid showed

segmenting nephron structures surrounded by the $CD31^+$ endothelial cells and $PDGFR^-\beta^+$ pericytes, which are essential for angiogenesis. Then, they transplanted the organoids under the renal capsule of recipient immunocompromised mice, and observed relatively mature podocyte and tubular epithelial structures for up to 28 days. Importantly, circulating plasma was detected in glomerular structures by FITC-labelled dextran [50].

In 2019, Homan et al. tried to promote the vascularization and maturation of renal organoids by embedding them in gelatin-fibrin ECM coated on 3D-printed millifluidic chips and providing a flow medium to form fluidic shear stress (FSS). They tested flow rates from 0.04 mL/min (low FSS, ~0.0001 dyn/cm^2) to 1–4.27 mL/min (high FSS, 0.008–0.035 dyn/cm^2) and confirmed that perfusion under higher FSS could improve the formation of $MCAM^+$/$PECAM1^+$ vascular networks in organoids [51]. It was proved that higher FSS enhanced better morphogenesis and improved the functional potential of the tubules. Specifically, the tubular structures underwent polarization and formed a brush border, indicated by apical enrichment of the brush border marker (LTL^+). The ciliary assembly was also observed. In addition, the upregulation of tubular epithelial transporters was confirmed, including solute transporters (SLC34A1, ATP1A1, SLC6A19, SLC9A3, SLC2A2) and drug transporters (ABCB1, LRP2). During the 10 days of flowing culture under high FSS, glomeruli started vascularization in a stepwise process. Beginning with a single capillary loop invading an S-shape body (SSB), then podocytes formed a primitive Bowman's capsule (capillary loop stage, CLS), and finally vascular expanded to form nascent glomerular tufts in early corpuscles. Afterward, Lee et al. provided the kidney-on-a-chip with microfluidic system, they confirmed that biomechanical stimuli can increase podocytes' maturity and promote angiogenesis. In addition, they found that perfusion-cultured kidney organoids are more sensitive for prediction of toxic responses, showing more severe injury after 24 h treatment with tacrolimus (a nephrotoxicity drug) compared with those cultured in static conditions [52]. In 2022, Amanda et al. achieved co-culture of kidney organoids and human umbilical vein endothelial cells (HUVECs) on microfluidic chip. HUVECs successfully infiltrated into kidney organoids and interconnected with endogenous endothelial, contributing to vascular development [53]. Organoid-on-a-chip facilitate endothelial populations maturation and replicate blood flow condition, providing an approach for large-scale cultivation and not restricted by animal hosts, which is more accurate for *in vitro* applications.

Currently, kidney organoids generated by existing protocols exhibit variations in cell type composition and morphological structure. Even based on the same approach, batch-to-batch variation is inevitable as the result of differences in manually operated processes, cell line source and reagents used. Automated 3D-bioprinting technology is a promising method to achieve organoid's higher reproducibility and less heterogeneity. In 2021, Lawlor et al. demonstrated that 3D bioprinting could precisely control kidney organoids' biophysical properties, including organoid size, cell number, and conformation [54]. Compared with manually generated organoids, automated extrusion-based bioprinting ones show improvement in scale-up and quality control. In specific, after monolayer culture of iPSCs to intermediate mesoderm stage for 7-day, dissociation to single cell suspension was used as the cellular bioinks, which were then loaded into a Novo Gen MMX extrusion-based 3D cellular bioprinter. This bioprinter enables manipulation of tip positioning (defined in the X, Y and Z axes), tip movement, and speed, approximately 200 organoids in just 10 min. The resulting renal organoids on day 20 comprised podocytes, proximal tubules, distal tubules, the loop of Henle's thick ascending limb, connecting segments, endothelial cells, and renal stroma, with higher maturity and consistent volume. This method combines developmental biology, biomaterials, and bioengineering, providing a method for industrialized large-scale production of renal organs, bringing hope for the future application of high-throughput drug screening [55].

4. ASC-derived renal tubular organoids

Adult stem cells (ASCs) are obtained from biological samples of renewable tissues, which can differentiate and self-organize into 3D organoids under the support of several growth factors and extracellular matrix (ECM) [56]. Unlike PSC-derived organoids, which reproduce the growth and development process, the formation of ASC-based organoids represents the self-renewal and repair of mature tissues by mimicking the niche environment *in vivo* [57]. In 2009, Sato and Clevers et al. first achieved the morphogenesis of intestine epithelial structures *in vitro*. They sorted Lgr5+ stem cells and embedded them in Matrigel (a 3D laminin and collagen-rich matrix), employing a Wnt, Notch, and EGF cocktail in order to maintain stem cell fate and promote mitogenic effects. The mini-guts showed a symmetric cyst structure, with a central lumen surrounded by highly polarized villus epithelium. They can be passaged for at least one year with stable phenotype and karyotype [3,55,58].

Inspired by the ASC-organoid protocol for the intestine, many researchers have extended the approach to establish kidney tubuloids [59]. ASCs originated from patient kidney biopsies or human urine have a multipotent differentiation capacity, which can undergo expansion and form 3D cystic structures consisting of a simple cuboidal epithelium. Especially, human urine-derived stem cells (USCs) provide a simple, safe, non-invasive, and low-cost approach to establish kidney tubule-like organoids [18]. In specific, several essential growth factors need to be added to the culture media, including R-spondin (a Wnt signaling enhancer), FGF-10 (responsible for the survival of kidney progenitor cells), A8301 (an inhibitor of TGF-β receptors, preventing growth arrest), mitogen epidermal growth factor (EGF), and Rho-kinase inhibitor Y-27632 (prevent the apoptosis of cells). Besides, it is confirmed that kidney-specific extracellular matrix (k-ECM) can enhance the multiple differentiation potential of USC, resulting in higher expression of tubule epithelial cell marker (such as AQP1) and podocyte markers (like nephrin) compared to traditional and universal ECM culture [60]. The ASC-derived kidney organoid mainly contains a tubular nephron system, such as kidney epithelial, proximal tubule, collecting duct, loop of Henle, and distal tubule. These structures form a highly level of polarized epithelia and show an apical primary cilium on tubuloid cells. Renal tubular epithelial cells are responsible for absorbing glucose, amino acids, drugs, and other substances from urine. Thus, kidney tubuloids could be used as an ideal tool for personalized nephrotoxicology testing.

5. Application of iPSC and ASC-derived kidney organoid in disease modeling

With the continuous improvement of culture techniques for kidney organoids, this novel and powerful tool has been widely applied in renal disease modeling, including drug-induced nephrotoxicity [61,62], genetic defect disease, inflammatory nephropathy, and infectious diseases [46,63].

Freedman et al. developed a 3D culture system using Matrigel in a sandwich-like structure, then mature tubular organoids with polarized epithelium and a hollow lumen were formed, containing proximal tubules, nephron progenitor, distal tubule, podocytes and endothelial cords [64]. To confirm transport and absorption functions, they performed fluorescent assays using compounds of different sizes. The researchers then co-incubated the organoids with cisplatin (chemotherapy medication for cancer treatment) and gentamicin (an antibiotic damages nephrons) in a dose-dependent pattern, and they found that the expression level of kidney injury molecule-1 (KIM-1) gradually increased with treatment duration [65]. This research confirmed that renal organs contain drug transporters and can serve as a model for drug nephrotoxicity research. Kidney organoids have proven to model genetic abnormalities such as autosomal-dominant forms of PKD or autosomal-recessive PKD. The former is caused by heterozygous mutations in PKD1 or PKD2, while the latter is the result of biallelic mutations

in PKHD1. PKD is characterized by the accumulation of fluid-filled cysts in the kidney, leading to kidney function decline and failure eventually. Freedman et al. used CRISPR/Cas9 gene editing to generate knockout PKD1/2$^-$ iPSCs derived kidney organoids, which developed fluid-filled cysts phenotype [64]. Low et al. used autosomal-recessive PKD patient-derived iPSCs to establish cystogenesis in kidney organoids and tested therapeutic compounds [66]. These studies show that kidney organoids can be used as a genetic kidney disease functional model and drug screening platform.

Furthermore, Lemos et al. induced renal organoids toward fibrosis model using the inflammatory factor IL-1β, which mediately promotes upregulation of the downstream target gene MYC through the IRAK4 receptor, ultimately leading to renal tubulointerstitial fibrosis, manifested as activation of aSMA$^+$ myofibroblasts and collagen deposition [67]. In addition, ASC-derived kidney organoids provide new avenues to study virus infection and personalized drug screening. For example, Schutgens et al. isolated ASCs from human urine, developed them into kidney tubuloids, and then treated them with the BK virus. BK virus could expand in the tubuloids successfully, and Cidofovir (an antiviral drug) was able to decrease the number of BK copies significantly [18].

iPSC-derived organoids can recapitulate kidney development, so they are more capable of serving as research tool for inborn kidney genetic lesions, in order to investigate disease etiology and pathogenesis. However, reprogramming patient specific fibroblasts into iPSC takes months, and iPSC-derived kidney organoid has finite lifespan. On the contrary, ASC derived tubuloids can be established as soon in half a month and they are able to be expanded for passages, so this method is time-saving and more convenient for individualized diseases modeling. While tubuloids have more matured epithelium, they do not contain glomeruli and renal stroma cells. Both iPSC and ASC organoids have advantages and drawbacks, they may complement each other in future research.

6. Conclusions

Scientists and clinicians are continuously deepening our understanding of the kidney's developmental mechanisms and physiological functions. Renal organoids have gradually become equal to human embryonic kidneys in terms of structural complexity, maturity, and vascularization function. By combining microfluidic equipment, 3D printing techniques, and gene editing technologies, renal organoids have shown great potential in certain disease transformation research. However, in order to serve as a stable drug screening platform that completely replaces animal models, improvements are still needed for kidney organoids, such as a unified industrialized standard culture process, further reducing costs, increasing efficiency, and reducing batch-to-batch differences. Additionally, we hope that kidney organoids can one day shine brightly in the field of regenerative medicine, solving the shortage of kidney transplant resources and the problem of immune rejection.

Data availability

Not applicable.

CRediT authorship contribution statement

Qi Liu: Writing – original draft. **Liang Yue:** Writing – review & editing. **Jiu Deng:** Writing – review & editing, Funding acquisition. **Yingxia Tan:** Writing – review & editing. **Chengjun Wu:** Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

[1] B.E. Chambers, N.E. Weaver, R.A. Wingert, The "3Ds" of growing kidney organoids: advances in nephron development, disease modeling, and drug screening, Cells (2023) 12.
[2] B. Artegiani, H. Clevers, Use and application of 3D-organoid technology, Hum. Mol. Genet. 27 (2018) R99–r107.
[3] T. Sato, R.G. Vries, H.J. Snippert, M. van de Wetering, N. Barker, D.E. Stange, J. H. van Es, A. Abo, P. Kujala, P.J. Peters, H. Clevers, Single Lgr5 stem cells build crypt-villus structures in vitro without a mesenchymal niche, Nature 459 (2009) 262–265.
[4] K.W. McCracken, E.M. Catá, C.M. Crawford, K.L. Sinagoga, M. Schumacher, B. E. Rockich, Y.H. Tsai, C.N. Mayhew, J.R. Spence, Y. Zavros, J.M. Wells, Modelling human development and disease in pluripotent stem-cell-derived gastric organoids, Nature 516 (2014) 400–404.
[5] M. Eiraku, N. Takata, H. Ishibashi, M. Kawada, E. Sakakura, S. Okuda, K. Sekiguchi, T. Adachi, Y. Sasai, Self-organizing optic-cup morphogenesis in three-dimensional culture, Nature 472 (2011) 51–56.
[6] K. Muguruma, A. Nishiyama, H. Kawakami, K. Hashimoto, Y. Sasai, Self-organization of polarized cerebellar tissue in 3D culture of human pluripotent stem cells, Cell Rep. 10 (2015) 537–550.
[7] M. Huch, H. Gehart, R. van Boxtel, K. Hamer, F. Blokzijl, M.M. Verstegen, E. Ellis, M. van Wenum, S.A. Fuchs, J. de Ligt, M. van de Wetering, N. Sasaki, S.J. Boers, H. Kemperman, J. de Jonge, J.N. Ijzermans, E.E. Nieuwenhuis, R. Hoekstra, S. Strom, R.R. Vries, L.J. van der Laan, E. Cuppen, H. Clevers, Long-term culture of genome-stable bipotent stem cells from adult human liver, Cell 160 (2015) 299–312.
[8] S. Nantasanti, B. Spee, H.S. Kruitwagen, C. Chen, N. Geijsen, L.A. Oosterhoff, M. E. van Wolferen, N. Pelaez, H. Fieten, R.W. Wubbolts, G.C. Grinwis, J. Chan, M. Huch, R.R.G. Vries, H. Clevers, A. de Bruin, J. Rothuizen, L.C. Penning, B. A. Schotanus, Disease modeling and gene therapy of copper storage disease in canine hepatic organoids, Stem Cell Rep. 5 (2015) 895–907.
[9] B. Phipson, P.X. Er, A.N. Combes, T.A. Forbes, S.E. Howden, L. Zappia, H.J. Yen, K. T. Lawlor, L.J. Hale, J. Sun, E. Wolvetang, M. Takasato, A. Oshlack, M.H. Little, Evaluation of variability in human kidney organoids, Nat. Methods 16 (2019) 79–87.
[10] W.R. Karthaus, P.J. Iaquinta, J. Drost, A. Gracanin, R. van Boxtel, J. Wongvipat, C. M. Dowling, D. Gao, H. Begthel, N. Sachs, R.G.J. Vries, E. Cuppen, Y. Chen, C. L. Sawyers, H.C. Clevers, Identification of multipotent luminal progenitor cells in human prostate organoid cultures, Cell 159 (2014) 163–175.
[11] A.P. Wong, C.E. Bear, S. Chin, P. Pasceri, T.O. Thompson, L.J. Huan, F. Ratjen, J. Ellis, J. Rossant, Directed differentiation of human pluripotent stem cells into mature airway epithelia expressing functional CFTR protein, Nat. Biotechnol. 30 (2012) 876–882.
[12] M. Tekguc, R.C.V. Gaal, S.G.M. Uzel, N. Gupta, L.V. Riella, J.A. Lewis, R. Morizane, Kidney organoids: a pioneering model for kidney diseases, Translational research, J. Lab. Clin. Med. 250 (2022) 1–17.
[13] A. Taguchi, Y. Kaku, T. Ohmori, S. Sharmin, M. Ogawa, H. Sasaki, R. Nishinakamura, Redefining the in vivo origin of metanephric nephron progenitors enables generation of complex kidney structures from pluripotent stem cells, Cell Stem Cell 14 (2014) 53–67.
[14] M. Takasato, P.X. Er, H.S. Chiu, B. Maier, G.J. Baillie, C. Ferguson, R.G. Parton, E. J. Wolvetang, M.S. Roost, S.M. Chuva de Sousa Lopes, M.H. Little, Kidney organoids from human iPS cells contain multiple lineages and model human nephrogenesis, Nature 526 (2015) 564–568.
[15] R. Morizane, A.Q. Lam, B.S. Freedman, S. Kishi, M.T. Valerius, J.V. Bonventre, Nephron organoids derived from human pluripotent stem cells model kidney development and injury, Nat. Biotechnol. 33 (2015) 1193–1200.
[16] M.H. Little, A.N. Combes, Kidney organoids: accurate models or fortunate accidents, Genes Dev. 33 (2019) 1319–1345.
[17] N. Khoshdel-Rad, A. Ahmadi, R. Moghadasali, Kidney organoids: current knowledge and future directions, Cell Tissue Res. 387 (2022) 207–224.
[18] F. Schutgens, M.B. Rookmaaker, T. Margaritis, A. Rios, C. Ammerlaan, J. Jansen, L. Gijzen, M. Vormann, A. Vonk, M. Viveen, F.Y. Yengej, S. Derakhshan, K.M. de Winter-de Groot, B. Artegiani, R. van Boxtel, E. Cuppen, A.P.A. Hendrickx, M. M. van den Heuvel-Eibrink, E. Heitzer, H. Lanz, J. Beekman, J.L. Murk, R. Masereeuw, F. Holstege, J. Drost, M.C. Verhaar, H. Clevers, Tubuloids derived from human adult kidney and urine for personalized disease modeling, Nat. Biotechnol. 37 (2019) 303–313.
[19] M.L. Andersen, L.M.F. Winter, Animal models in biological and biomedical research - experimental and ethical concerns, An. Acad. Bras. Cienc. 91 (2019) e20170238.
[20] N. Gupta, E. Dilmen, R. Morizane, 3D kidney organoids for bench-to-bedside translation, J. Mol. Med. 99 (2021) 477–487.

[21] A.N. Combes, L. Zappia, P.X. Er, A. Oshlack, M.H. Little, Single-cell analysis reveals congruence between kidney organoids and human fetal kidney, Genome Med. 11 (2019) 3.

[22] S. Musah, A. Mammoto, T.C. Ferrante, S.S.F. Jeanty, M. Hirano-Kobayashi, T. Mammoto, K. Roberts, S. Chung, R. Novak, M. Ingram, T. Fatanat-Didar, S. Koshy, J.C. Weaver, G.M. Church, D.E. Ingber, Mature induced-pluripotent-stem-cell-derived human podocytes reconstitute kidney glomerular-capillary-wall function on a chip, Nat. Biomed. Eng. 1 (2017).

[23] P. Romagnani, L. Lasagni, Modeling the glomerular filtration barrier: are you kidney-ing me? Cell Stem Cell 21 (2017) 7–9.

[24] T. Miyoshi, K. Hiratsuka, E.G. Saiz, R. Morizane, Kidney organoids in translational medicine: disease modeling and regenerative medicine, Dev. Dynam. : an official publication of the American Association of Anatomists 249 (2020) 34–45.

[25] S.M. Czerniecki, N.M. Cruz, J.L. Harder, R. Menon, J. Annis, E.A. Otto, R. E. Gulieva, L.V. Islas, Y.K. Kim, L.M. Tran, T.J. Martins, J.W. Pippin, H. Fu, M. Kretzler, S.J. Shankland, J. Himmelfarb, R.T. Moon, N. Paragas, B.S. Freedman, High-throughput screening enhances kidney organoid differentiation from human pluripotent stem cells and enables automated multidimensional phenotyping, Cell Stem Cell 22 (2018) 929–940.e924.

[26] H.G. Preuss, Basics of renal anatomy and physiology, Clin. Lab. Med. 13 (1993) 1–11.

[27] F. Costantini, R. Kopan, Patterning a complex organ: branching morphogenesis and nephron segmentation in kidney development, Dev. Cell 18 (2010) 698–712.

[28] G.R. Dressler, Advances in early kidney specification, development and patterning, Development (Cambridge, England) 136 (2009) 3863–3874.

[29] F. Costantini, GDNF/Ret signaling and renal branching morphogenesis: from mesenchymal signals to epithelial cell behaviors, Organogenesis 6 (2010) 252–262.

[30] J. Schnell, M. Achieng, N.O. Lindström, Principles of human and mouse nephron development, Nat. Rev. Nephrol. 18 (2022) 628–642.

[31] K. Georgas, B. Rumballe, M.T. Valerius, H.S. Chiu, R.D. Thiagarajan, E. Lesieur, B. J. Aronow, E.W. Brunskill, A.N. Combes, D. Tang, D. Taylor, S.M. Grimmond, S. S. Potter, A.P. McMahon, M.H. Little, Analysis of early nephron patterning reveals a role for distal RV proliferation in fusion to the ureteric tip via a cap mesenchyme-derived connecting segment, Dev. Biol. 332 (2009) 273–286.

[32] L.L. O'Brien, A.P. McMahon, Induction and patterning of the metanephric nephron, Semin. Cell Dev. Biol. 36 (2014) 31–38.

[33] M. Little, K. Georgas, D. Pennisi, L. Wilkinson, Kidney development: two tales of tubulogenesis, Curr. Top. Dev. Biol. 90 (2010) 193–229.

[34] A. Das, S. Tanigawa, C.M. Karner, M. Xin, L. Lum, C. Chen, E.N. Olson, A. O. Perantoni, T.J. Carroll, Stromal-epithelial crosstalk regulates kidney progenitor cell differentiation, Nat. Cell Biol. 15 (2013) 1035–1044.

[35] S. Tanigawa, E. Tanaka, K. Miike, T. Ohmori, D. Inoue, C.L. Cai, A. Taguchi, A. Kobayashi, R. Nishinakamura, Generation of the organotypic kidney structure by integrating pluripotent stem cell-derived renal stroma, Nat. Commun. 13 (2022) 611.

[36] T. Bohnenpoll, E. Bettenhausen, A.C. Weiss, A.B. Foik, M.O. Trowe, P. Blank, R. Airik, A. Kispert, Tbx18 expression demarcates multipotent precursor populations in the developing urogenital system but is exclusively required within the ureteric mesenchymal lineage to suppress a renal stromal fate, Dev. Biol. 380 (2013) 25–36.

[37] J.W. Mugford, P. Sipilä, J.A. McMahon, A.P. McMahon, Osr1 expression demarcates a multi-potent population of intermediate mesoderm that undergoes progressive restriction to an Osr1-dependent nephron progenitor compartment within the mammalian kidney, Dev. Biol. 324 (2008) 88–98.

[38] N. Khoshdel Rad, N. Aghdami, R. Moghadasali, Cellular and molecular mechanisms of kidney development: from the embryo to the kidney organoid, Front. Cell Dev. Biol. 8 (2020) 183.

[39] M. Takasato, M.H. Little, The origin of the mammalian kidney: implications for recreating the kidney in vitro, Development (Cambridge, England) 142 (2015) 1937–1947.

[40] M.H. Little, A.P. McMahon, Mammalian kidney development: principles, progress, and projections, Cold Spring Harbor Perspect. Biol. 4 (2012).

[41] H. Tsujimoto, T. Kasahara, S.I. Sueta, T. Araoka, S. Sakamoto, C. Okada, S.I. Mae, T. Nakajima, N. Okamoto, D. Taura, M. Nasu, T. Shimizu, M. Ryosaka, Z. Li, M. Sone, M. Ikeya, A. Watanabe, K. Osafune, A modular differentiation system maps multiple human kidney lineages from pluripotent stem cells, Cell Rep. 31 (2020) 107476.

[42] N. Gupta, R. Morizane, Kidney development to kidney organoids and back again, Semin. Cell Dev. Biol. 127 (2022) 68–76.

[43] T.J. Carroll, A. Das, Defining the signals that constitute the nephron progenitor niche, J. Am. Soc. Nephrol. : JASN (J. Am. Soc. Nephrol.) 24 (2013) 873–876.

[44] C.M. Karner, A. Das, Z. Ma, M. Self, C. Chen, L. Lum, G. Oliver, T.J. Carroll, Canonical Wnt9b signaling balances progenitor cell expansion and differentiation during kidney development, Development (Cambridge, England) 138 (2011) 1247–1257.

[45] S.V. Kumar, P.X. Er, K.T. Lawlor, A. Motazedian, M. Scurr, I. Ghobrial, A. N. Combes, L. Zappia, A. Oshlack, E.G. Stanley, M.H. Little, Kidney micro-organoids in suspension culture as a scalable source of human pluripotent stem cell-derived kidney cells, Development (Cambridge, England) (2019) 146.

[46] A. Przepiorski, A.E. Crunk, E.B. Espiritu, N.A. Hukriede, A.J. Davidson, The utility of human kidney organoids in modeling kidney disease, Semin. Nephrol. 40 (2020) 188–198.

[47] H. Wu, K. Uchimura, E.L. Donnelly, Y. Kirita, S.A. Morris, B.D. Humphreys, Comparative analysis and refinement of human PSC-derived kidney organoid differentiation with single-cell transcriptomics, Cell Stem Cell 23 (2018) 869–881. e868.

[48] A. Taguchi, R. Nishinakamura, Higher-order kidney organogenesis from pluripotent stem cells, Cell Stem Cell 21 (2017) 730–746.e736.

[49] E. Garreta, P. Prado, C. Tarantino, R. Oria, L. Fanlo, E. Martí, D. Zalvidea, X. Trepat, P. Roca-Cusachs, A. Gavaldà-Navarro, L. Cozzuto, J.M. Campistol, J. C. Izpisúa Belmonte, C. Hurtado Del Pozo, N. Montserrat, Fine tuning the extracellular environment accelerates the derivation of kidney organoids from human pluripotent stem cells, Nat. Mater. 18 (2019) 397–405.

[50] C.W. van den Berg, L. Ritsma, M.C. Avramut, L.E. Wiersma, B.M. van den Berg, D. G. Leuning, E. Lievers, M. Koning, J.M. Vanslambrouck, A.J. Koster, S.E. Howden, M. Takasato, M.H. Little, T.J. Rabelink, Renal subcapsular transplantation of PSC-derived kidney organoids induces neo-vasculogenesis and significant glomerular and tubular maturation in vivo, Stem Cell Rep. 10 (2018) 751–765.

[51] K.A. Homan, N. Gupta, K.T. Kroll, D.B. Kolesky, M. Skylar-Scott, T. Miyoshi, D. Mau, M.T. Valerius, T. Ferrante, J.V. Bonventre, J.A. Lewis, R. Morizane, Flow-enhanced vascularization and maturation of kidney organoids in vitro, Nat. Methods 16 (2019) 255–262.

[52] H.N. Lee, Y.Y. Choi, J.W. Kim, Y.S. Lee, J.W. Choi, T. Kang, Y.K. Kim, B.G. Chung, Effect of biochemical and biomechanical factors on vascularization of kidney organoid-on-a-chip, Nano convergence 8 (2021) 35.

[53] A. Bas-Cristóbal Menéndez, Z. Du, T.P.P. van den Bosch, A. Othman, N. Gaio, C. Silvestri, W. Quirós, H. Lin, S. Korevaar, A. Merino, J. Mulder, M.J. Hoogduijn, Creating a kidney organoid-vasculature interaction model using a novel organ-on-chip system, Sci. Rep. 12 (2022) 20699.

[54] K.T. Lawlor, J.M. Vanslambrouck, J.W. Higgins, A. Chambon, K. Bishard, D. Arndt, P.X. Er, S.B. Wilson, S.E. Howden, K.S. Tan, F. Li, L.J. Hale, B. Shepherd, S. Pentoney, S.C. Presnell, A.E. Chen, M.H. Little, Cellular extrusion bioprinting improves kidney organoid reproducibility and conformation, Nat. Mater. 20 (2021) 260–271.

[55] H. Clevers, Modeling development and disease with organoids, Cell 165 (2016) 1586–1597.

[56] C. Corrò, L. Novellasdemunt, V.S.W. Li, A brief history of organoids, Am. J. Physiol. Cell Physiol. 319 (2020) C151–c165.

[57] F.A. Yousef Yengej, J. Jansen, M.B. Rookmaaker, M.C. Verhaar, H. Clevers, Kidney organoids and tubuloids, Cells (2020) 9.

[58] T. Sato, H. Clevers, Growing self-organizing mini-guts from a single intestinal stem cell: mechanism and applications, Science (New York, N.Y.) 340 (2013) 1190–1194.

[59] L. Gijzen, F.A. Yousef Yengej, F. Schutgens, M.K. Vormann, C.M.E. Ammerlaan, A. Nicolas, D. Kurek, P. Vulto, M.B. Rookmaaker, H.L. Lanz, M.C. Verhaar, H. Clevers, Culture and analysis of kidney tubuloids and perfused tubuloid cells-on-a-chip, Nat. Protoc. 16 (2021) 2023–2050.

[60] H. Guo, N. Deng, L. Dou, H. Ding, T. Criswell, A. Atala, C.M. Furdui, Y. Zhang, 3-D human renal tubular organoids generated from urine-derived stem cells for nephrotoxicity screening, ACS Biomater. Sci. Eng. 6 (2020) 6701–6709.

[61] J.L.M. Digby, T. Vanichapol, A. Przepiorski, A.J. Davidson, V. Sander, Evaluation of cisplatin-induced injury in human kidney organoids, Am. J. Physiol. Ren. Physiol. 318 (2020) F971–f978.

[62] M.L. Lawrence, M. Elhendawi, M. Morlock, W. Liu, S. Liu, A. Palakkan, L.F. Seidl, P. Hohenstein, A.K. Sjögren, J.A. Davies, Human iPSC-derived renal organoids engineered to report oxidative stress can predict drug-induced toxicity, iScience 25 (2022) 103884.

[63] S. Rauth, S. Karmakar, S.K. Batra, M.P. Ponnusamy, Recent advances in organoid development and applications in disease modeling, Biochimica et biophysica acta, Reviews on cancer 1875 (2021) 188527.

[64] B.S. Freedman, C.R. Brooks, A.Q. Lam, H. Fu, R. Morizane, V. Agrawal, A.F. Saad, M.K. Li, M.R. Hughes, R.V. Werff, D.T. Peters, J. Lu, A. Baccei, A.M. Siedlecki, M. T. Valerius, K. Musunuru, K.M. McNagny, T.I. Steinman, J. Zhou, P.H. Lerou, J. V. Bonventre, Modelling kidney disease with CRISPR-mutant kidney organoids derived from human pluripotent epiblast spheroids, Nat. Commun. 6 (2015) 8715.

[65] B. Ding, G. Sun, S. Liu, E. Peng, M. Wan, L. Chen, J. Jackson, A. Atala, Three-dimensional renal organoids from whole kidney cells: generation, optimization, and potential application in nephrotoxicology in vitro, Cell Transplant. 29 (2020) 963689719897066.

[66] J.H. Low, P. Li, E.G.Y. Chew, B. Zhou, K. Suzuki, T. Zhang, M.M. Lian, M. Liu, E. Aizawa, C. Rodriguez Esteban, K.S.M. Yong, Q. Chen, J.M. Campistol, M. Fang, C.C. Khor, J.N. Foo, J.C. Izpisua Belmonte, Y. Xia, Generation of human PSC-derived kidney organoids with patterned nephron segments and a de novo vascular network, Cell Stem Cell 25 (2019) 373–387.e379.

[67] D.R. Lemos, M. McMurdo, G. Karaca, J. Wilflingseder, I.A. Leaf, N. Gupta, T. Miyoshi, K. Susa, B.G. Johnson, K. Soliman, G. Wang, R. Morizane, J. V. Bonventre, J.S. Duffield, Interleukin-1β activates a MYC-dependent metabolic switch in kidney stromal cells necessary for progressive tubulointerstitial fibrosis, J. Am. Soc. Nephrol. : JASN (J. Am. Soc. Nephrol.) 29 (2018) 1690–1705.