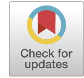
INVITED REVIEW ARTICLE

Kidney organoids: a pioneering model for kidney diseases



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The kidney is a vital organ that regulates the bodily fluid and electrolyte homeostasis via tailored urinary excretion. Kidney injuries that cause severe or progressive chronic kidney disease have driven the growing population of patients with end-stage kidney disease, leading to substantial patient morbidity and mortality. This irreversible kidney damage has also created a huge socioeconomical burden on the healthcare system, highlighting the need for novel translational research models for progressive kidney diseases. Conventional research methods such as *in vitro* 2D cell culture or animal models do not fully recapitulate complex human kidney diseases. By contrast, directed differentiation of human induced pluripotent stem cells enables *in vitro* generation of patient-specific 3D kidney organoids, which can be used to model acute or chronic forms of hereditary, developmental, and metabolic kidney diseases. Furthermore, when combined with biofabrication techniques, organoids can be used as building blocks to construct vascularized kidney tissues mimicking their *in vivo* counterpart. By applying gene editing technology, organoid building blocks may be modified to minimize the process of immune rejection in kidney transplant recipients. In the foreseeable future, the universal kidney organoids derived from HLA-edited/deleted induced pluripotent stem cell (iPSC) lines may enable the supply of bioengineered organotypic kidney structures that are immune-compatible for the majority of the world population. Here, we summarize recent advances in kidney organoid research coupled with novel technologies such as organoids-on-chip and biofabrication of 3D kidney tissues providing convenient platforms for high-throughput drug screening, disease modelling, and therapeutic applications. (Translational Research 2022; 250:1–17)

Abbreviations: iPSC = induced pluripotent stem cell; CKD = chronic kidney disease; ESKD = end-stage kidney disease; IM = intermediate mesoderm; MM = metanephric mesenchyme; UB = ureteric bud; NPCs = nephron progenitor cells; MET = mesenchymal-to-epithelial transition; SPCs = stromal progenitor cells; EPCs = endothelial progenitor cells; HDR = homology-directed repair; APCs = antigen-presenting cells; PKD = polycystic kidney disease; GBM = glomerular basement

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membrane; CNS = congenital nephrotic syndrome; PEC = parietal epithelial cell; NPs = nephron progenitors; TMA = thrombotic microangiopathy; HLA = human leukocyte antigen;

INTRODUCTION

The kidney is responsible for the regulation of fluid, electrolyte, and pH balance in the human body via plasma filtration, reabsorption of essential nutrients, and secretion of the metabolic waste.[94] The kidney is composed of more than 20 distinct cell types, that are necessary for its function to maintain homeostasis. While tubular cells have the capacity to self-repair after injury, repeated and/or severe renal injury cause irreversible damage to the kidney, leading to its dysfunction.[21,94] Chronic kidney disease (CKD) constitutes a major global healthcare problem causing a heavy financial burden with its high prevalence in 13.4% of the global population and 35% of the people over 70 years.[44,53] In addition, CKD is an independent risk factor for cardiovascular diseases.[53] CKD is directly or indirectly caused by many types of disorders including hereditary diseases, metabolic syndrome and chronic autoimmune and/or inflammatory diseases or as a cumulative result of drug-induced nephrotoxicity.[46] Loss of functional units of the kidney causes progressive loss of renal function over time due to its limited regeneration capacity, leading to end-stage kidney disease (ESKD), which requires patients to initiate renal replacement therapy.

There have been many efforts to create novel sources for kidney regeneration during the past decade. One approach is to derive kidney cells and tissues from human pluripotent stem cells (hPSCs). Detailed analyses of the embryonic origins of kidney lineages have enabled researchers to induce its differentiation process using pluripotent stem cells *in vitro*.[75,92] By mimicking signal cascades and growth factor stimulation of embryonic kidney development, directed differentiation protocols have been developed to generate kidney organoids that consist of segmented-nephrons and stromal cells.[45,68] These kidney organoids can now serve as a new tool for patient-specific drug screening and disease modeling. Coupled with CRISPR/Cas9 genome editing, genetic kidney disease models are established with their isogenic control organoids, complementing animal experimentation.[32] Towards the ultimate goals of kidney regeneration, significant advances have been made in biomanufacturing organ-specific tissues at scale to overcome the challenge of large tissue fabrication *in vitro*.[89] Moreover, the generation of hypoimmunogenic hPSCs by genome editing may mitigate the immune rejection against the bioengineered organs. In this review, we summarize the recent advances in kidney organoids, disease models, and efforts that are underway to build transplantable kidneys that might revolutionize the care of patients with ESKD.

Kidney structure and development. Nephrons are the functional unit of the kidney, responsible for the production and excretion of urine via blood filtration and active transport. Each nephron consists of glomerular and tubular segments. Glomerular podocytes form filtration units with capillary networks integrated within the Bowman's capsule, filtering blood to produce ultrafiltrate as primitive urine. Glomeruli are structurally continuous with the proximal tubules, loops of Henle, distal tubules, and connecting tubules and/or collecting ducts[45] (Fig 1). After filtration of the renal blood from the glomerular capillaries through their highly fenestrated endothelium,[60] this glomerular ultrafiltrate flows from Bowman's space to the tubular lumens. Tubular cells are surrounded by peritubular capillaries, and 99% of the glomerular ultrafiltrate is reabsorbed from the tubular lumens to peritubular capillaries. The remaining portion is further subject to tubular secretion of ions and organic solutes, determining the final composition of urine.[43,84] Resorptive and secretory processes are facilitated by active transport in the tubular epithelial cells together with the peritubular capillary endothelia. Finally, urine produced by nephrons is collected to the bladder through the collecting duct and ureter.

Kidneys developmentally originate from the intermediate mesoderm (IM). During the formation of the mammalian kidney, three distinct renal rudiments namely pronephros, mesonephros, and metanephros arise from anterior to posterior areas in the IM.[45] Pronephros, mesonephros, and the ureteric bud (UB) are derived from the anterior IM (aIM) whereas metanephric mesenchyme (MM) is derived from the posterior IM (pIM) (Fig 2).[29,43] While the pronephros and mesonephros spontaneously degenerate as non-functional structures, the Wolffian duct persists as the collecting duct of the metanephric kidneys.[34,86] Although the previous literature pointed out the $Osr1^+$ metanephric progenitors in the IM as the common ancestry of the MM and UB,[76] the subsequent lineage-tracing mouse studies demonstrated the distinct origins of UB and MM lineages. The UB, the caudal outpouching of the Wolffian duct, arises from $Pax2^+Osr1^+$ progenitors in the anterior IM while the MM is derived from $Pax2^-Osr1^+$ progenitors in the posterior IM, that latter further differentiating into $Osr1^+Six2^+Sall1^+WT1^+$ nephron progenitor cells (NPCs).[92] NPCs give rise to segmented nephron structures through reciprocal induction with the UB.[45,76] When invaded by the UB, the MM

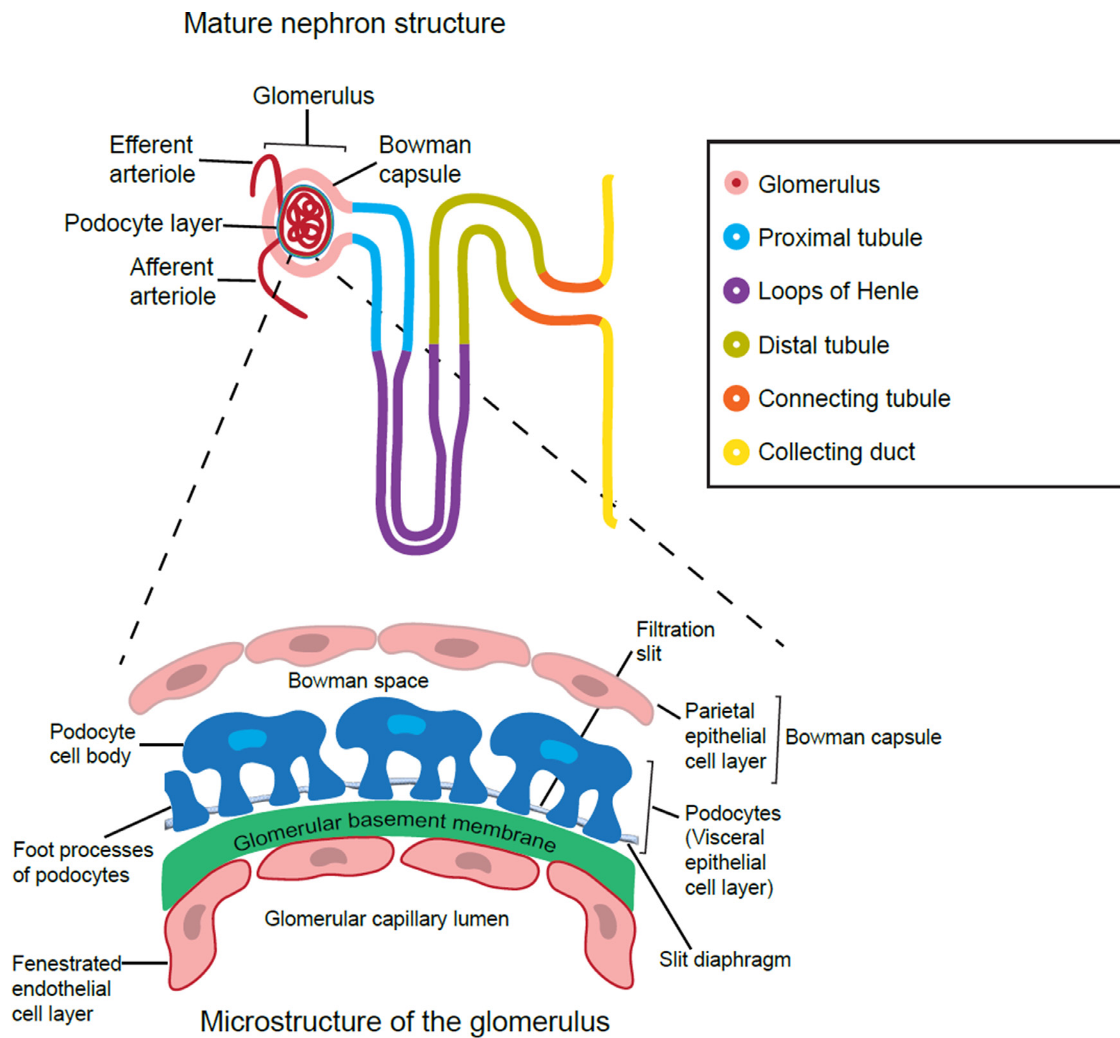

Fig 1. The structure of a mature nephron unit. The upper schema designates each unit of a nephron with a different function. The glomerulus, composed of Bowman's capsule and the glomerular capillary network, is responsible for the filtration of glomerular blood flow. The glomerular capillary network, formed by the afferent arteriole, is assembled into the efferent arteriole. The lower illustration shows the magnified microstructure of the glomerular filtration barrier, which is composed of glomerular fenestrated endothelial cells, glomerular basement membrane, and podocytes (visceral epithelial cells). The secondary foot processes of podocytes are attached to each other by intervening filtration slits forming the slit diaphragm. On the other hand, Bowman space is lined by the parietal epithelial cell layer. The ultrafiltrate, drained from the Bowman space into the tubules (proximal tubule, loops of Henle, and distal tubule), is further processed into the urinary output by absorption and secretion. The urinary drainage is finally transmitted into the collecting duct via the connecting tubule.

supports the extensive branching of UB tips through the secretion of glial cell-derived neurotrophic factor (GDNF), while the cap mesenchyme surrounding the UB tips undergoes a mesenchymal-to-epithelial transition (MET) via UB-derived Wnt signal activation.[24,29,111] The pIM-derived MM is composed of the heterogeneous cell populations of NPCs, stromal progenitor cells (SPCs), and endothelial progenitor cells (EPCs), which give rise to the nephron structures and their surrounding interstitial tissue (Fig 2). aIM-derived UB differentiates into the collecting duct network which is responsible for draining the urine to the bladder via ureters.[45]

Differentiation of kidney organoid from hPSCs. Based on the knowledge of mammalian kidney development, protocols have been established to generate nephron kidney organoids from hPSCs.[43,46] These protocols are based on the approach of "directed differentiation" whereby sequential treatment of growth factors is developed to follow the developmental stages of the

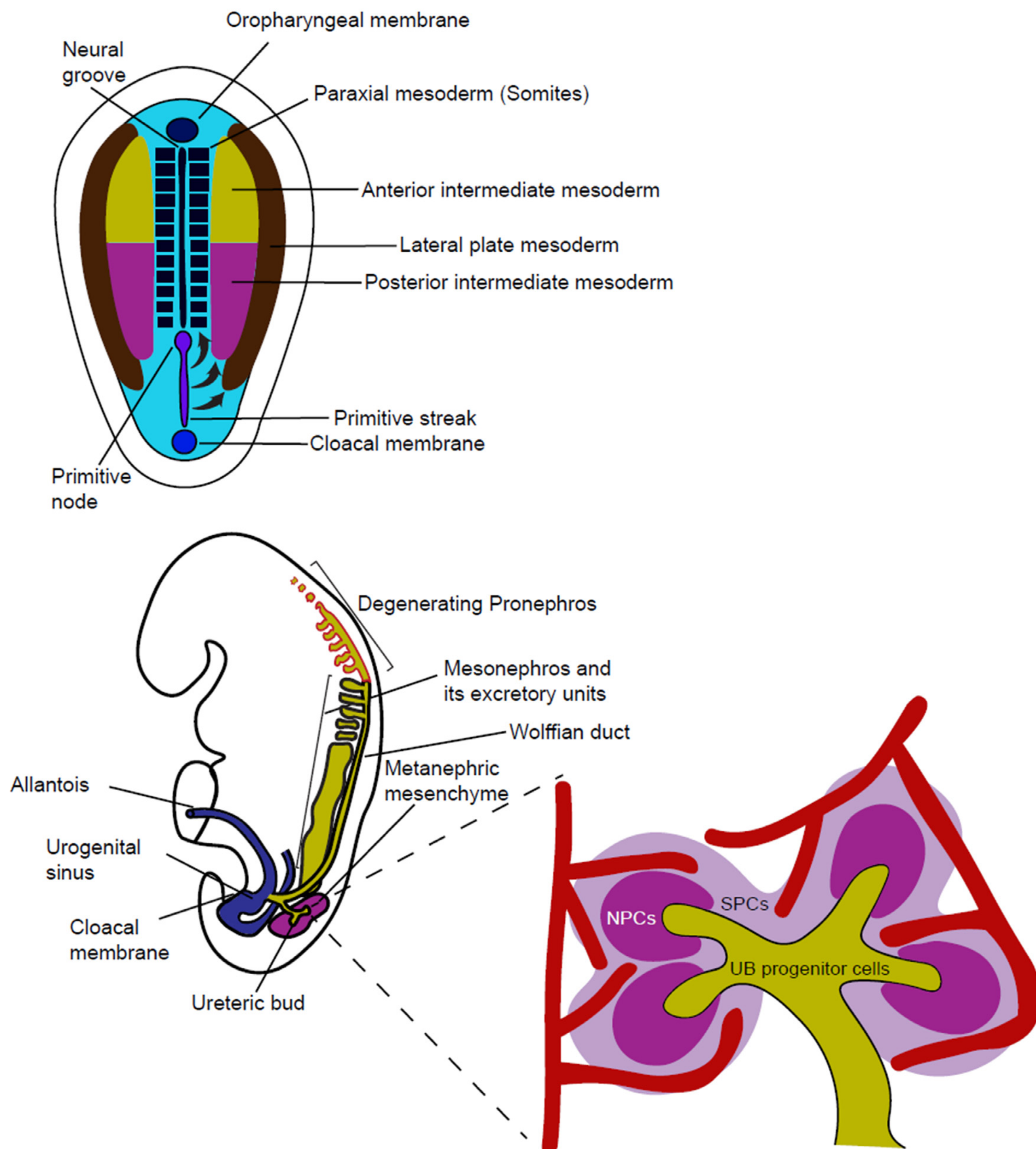

Fig 2. The embryological development of kidney at different stages. The upper schema shows the formation of paraxial, intermediate, and lateral plate mesodermal layers, derived from the primitive streak as a result of cellular immigration along its craniocaudal axis. The lower illustration reveals the embryological stages of pronephros, mesonephros, and metanephros. Pronephros, mesonephros, and the ureteric bud (UB) are originated from the anterior intermediate mesoderm (aIM) whereas metanephric mesenchyme (MM) is derived from the posterior intermediate mesoderm (pIM). The lower right schema reflects the microstructure of MM- and UB-derived progenitor cells.

kidney. Three-dimensional (3D) culture technologies enabled embryonic and adult stem cells to differentiate into kidney tissues by potentiating their self-organizing capacity to form multi-cellular tissues. Organoids derived from hPSCs serve as a novel tool for disease studies. In addition, organoids produced in 3D culture can be used as organ building blocks as they have similar basic structural and functional features exhibited by

their native counterparts in the human body.[20,43] In this section, we summarize the differentiation protocols of kidney organoids.

Taguchi et al. achieved a milestone in kidney developmental research by elucidating the distinct origins of ureteric and metanephric lineages.[92] Nephron progenitor cells from both mouse embryonic stem cells (ESCs) and human induced pluripotent stem cells (iPSCs) were generated by directing their differentiation towards pIM-derived MM cells, which subsequently commenced their maturation into nephron epithelia when cocultured with mouse embryonic spinal cords. First, OCT3/4$^+$ hPSCs were treated with BMP4 followed by activin A and FGF2 to shift their pluripotent status to the epiblast stage. Then, the differentiation was initiated by BMP4 and a glycogen synthase kinase 3 (GSK-3) inhibitor, CHIR99021 (CHIR) to induce T$^+$CDX2$^+$TBX6$^+$HOX11$^+$ posterior nascent mesoderm.[7] The subsequent treatment of activin A, BMP-4, retinoic acid, and a moderate dose of CHIR progressed the differentiation into OSR1$^+$WT1$^+$HOX11$^+$ pIM cells. They then applied FGF9 and a low dose of CHIR to induce SIX2$^+$PAX2$^+$SALL1$^+$ MM cells. Finally, these MM cells were co-cultured with mouse embryonic spinal cords to induce tubular and podocyte epithelial cells.

Morizane et al. developed a rapid and efficient protocol with defined components, which generated SIX2$^+$SALL1$^+$WT1$^+$PAX2$^+$ NPCs with ~90% efficiency from both human embryonic stem cells and iPSCs within 8 to 9 days (Fig 3).[75] Furthermore, these NPCs were converted into PAX8$^+$LHX1$^+$ renal vesicles, which had the potential to self-organize into their *in vivo* counterparts; nephron structures including NPHS1$^+$PODXL$^+$ glomerular podocytes, LTL$^+$CDH2$^+$ proximal tubules, CDH1$^+$UMOD (Uromodulin)$^+$ loops of Henle, and CDH1$^+$BRN1$^+$ distal tubules in an organized and continuous manner.[74,75] The differentiation protocol was arranged according to two findings that the egression timing of mesodermal progenitors from the primitive streak determines the anterior-posterior patterning of the mesoderm,[27] and the mesodermal precursors from the posterior primitive streak transform into lateral plate mesoderm instead of intermediate mesoderm.[58] Posterior mesoderm progenitors leave the primitive streak later than the precursor cells of the anterior mesoderm indicating that the posterior intermediate mesoderm cells originate from the late-stage primitive streak. Based on this embryologic specification, high-dose and long-term CHIR treatment was applied together with the BMP4 inhibitor that suppressed the differentiation of lateral plate mesoderm components. During *in vivo* nephrogenesis, Activin A (TGF-β superfamily member) activates Hoxd11 expression, which stimulates the formation of pIM-derived metanephros.[38,45,71] Thus, after the removal of CHIR, activin A treatment was applied between days 4 and 7 to induce OSR1$^+$WT1$^+$HOXD11$^+$ pIM cells which lacked aIM markers, PAX2 and LHX1.[75,92] Next, SIX2$^+$SALL1$^+$WT1$^+$PAX2$^+$ NPCs were generated when pIM cells were treated with FGF9 for 1 or 2 days. Previous studies have shown that UB-derived Fgf9 and Fgf20 are necessary to maintain the stemness and survival of cap mesenchyme in mice,[6] while UB-derived Wnt9b signaling supports the mesenchymal-epithelial transition of MM to nephron epithelial cells.[17] In addition, the induction of Fgf8 and Fgf9 by Wnt9b signaling was also reported,[16,63] referring to the interaction between Fgf9 and Wnt signaling during the reciprocal induction of MM and UB. To mimic the MET in murine NPCs as a result of this reciprocal induction by the UB-derived Wnt9b and Fgf9 signaling,[17] hPSC-derived NPCs were further exposed to a low-dose CHIR pulse together with the ongoing FGF9 following their transfer to 3D suspension culture and brief centrifugation in the ultra-low-attachment plates.[45] After the CHIR pulse, NPCs were transformed to PAX8$^+$LHX1$^+$LAM$^+$ renal vesicles by day 14 of the differentiation, which is consistent with the previous finding that Wnt signaling is abrogated by Dkk1, a key downstream effector of *Lim1 (Lhx1)* gene during the renal vesicle formation.[81] Finally, these renal vesicles spontaneously completed their MET in the absence of any additional factors by forming elongated epithelial nephron structures composed of NPHS1$^+$PODXL$^+$ glomerular podocytes, LTL$^+$AQP1 (Aquaporin-1)$^+$ proximal tubules, CDH1$^+$AQP1$^+$ descending loops of Henle, CDH1$^+$UMOD$^+$ thick ascending loops of Henle, CDH1$^+$UMOD$^-$ distal tubules, and CDH1$^+$AQP2 (Aquaporin-2)$^+$ connecting tubules.[74,75] The common main points of the differentiation protocols by Taguchi and Morizane included the induction of the late primitive streak or posterior nascent mesoderm with long-term WNT activation by CHIR and modified BMP4 signaling (further activation of BMP4 by Taguchi protocol, suppression of excessive BMP4 signaling by Morizane protocol), the differentiation of late primitive streak to pIM with Activin A, the maturation of MM by FGF-9, and the induction of MET in NPCs through transient activation of WNT signaling by either pulse CHIR treatment (Morizane) or coculture with embryonic spinal cords (Taguchi).[75,92]

Although most researchers have performed suspension cell culture for organoid differentiation in low-attachment wells, Freedman et al. used a Matrigel sandwich method to generate kidney organoids in the 3D microenvironment via cavitated epiblast formation from hPSCs. Before starting directed differentiation,

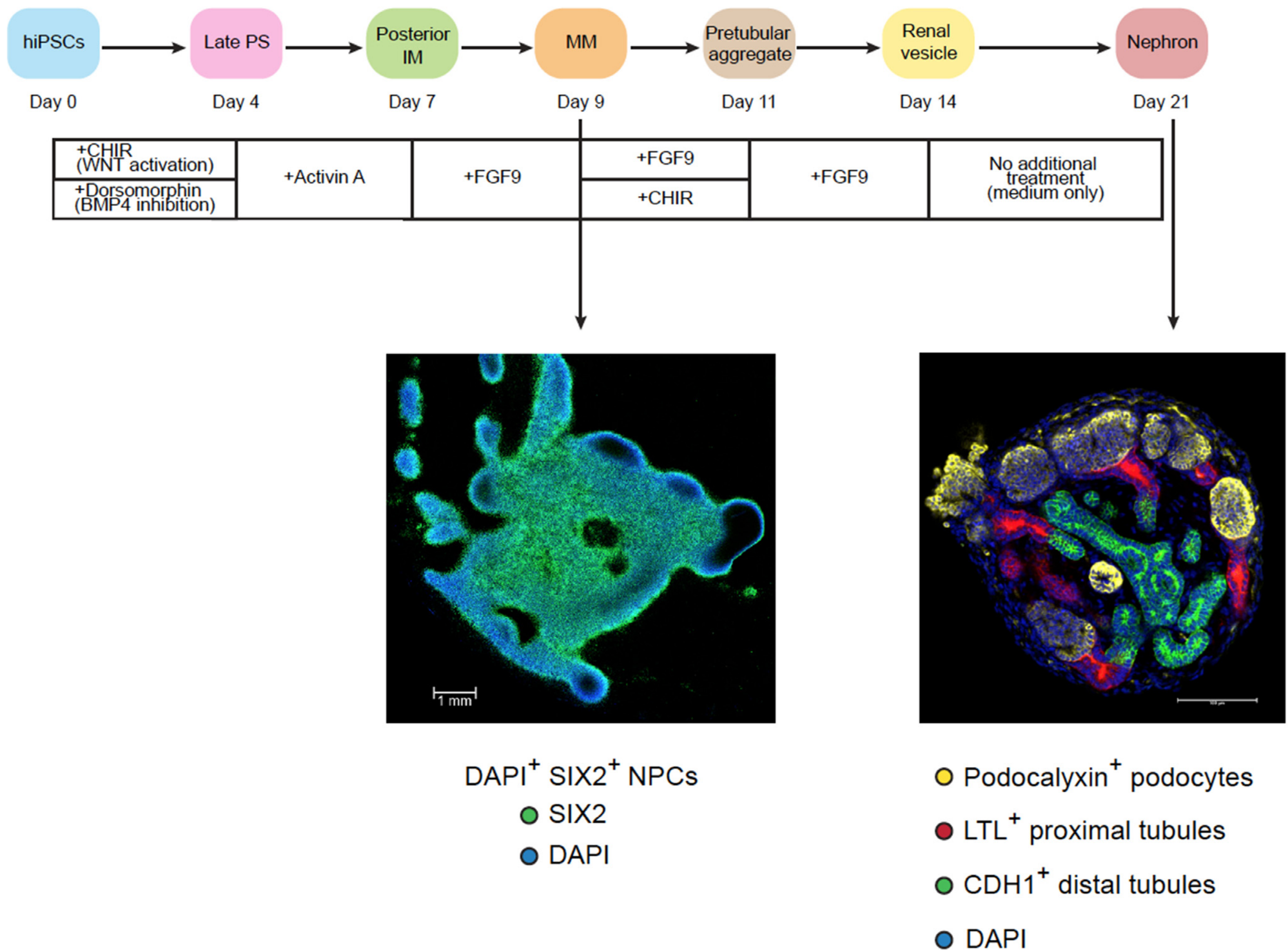

Fig 3. Directed differentiation of kidney organoids by the Morizane protocol. The timescale of this protocol indicates the temporal stages of the organoid development and the temporal supplementation of the differentiation factors. The lower left image (scale bar, 1 mm) by confocal microscopy shows DAPI$^+$SIX2$^+$ nephron progenitor cells (NPCs) at Day 9 while the lower right image (scale bar, 100 μm) clarifies the development of DAPI$^+$Podocalyxin$^+$LTL$^+$CDH1$^+$ nephron organoids at Day 21. PS: primitive streak. IM: intermediate mesoderm. MM: metanephric mesenchyme.

hPSCs were sandwiched between two layers of Matrigel, forming epiblast spheroids that contained a hollow lumen surrounded by monolayer epithelia. These epiblast spheroids were first treated with CHIR for 1.5 days and subsequently exposed to B27-supplemented media by a two-step protocol. After day 10, spheroids were transformed into convoluted tubular organoids which contained PODXL$^+$WT1$^+$SYNPO$^+$ podocyte-like cell aggregates, LTL$^+$ proximal and CDH1$^+$ distal tubular structures, and CD31$^+$vWF$^+$ endothelial compartments.[33]

Takasato et al. was first to report the synchronous induction of the nephrons and collecting ducts from aIM progenitors, together forming a self-organizing kidney structure.[93] They modified the duration of initial CHIR treatment to co-induce both nephron and collecting duct lineages.[94] By using CHIR for 4 days, the optimal duration for the induction of MM together with UB, and subsequent application of FGF-9 for 3 days, they simultaneously induced MM- and UB-derived components including WT1$^+$NPHS1$^+$ glomeruli, LTL$^+$CDH1$^-$ early proximal tubule (day 11), LTL$^+$CDH1$^+$ maturing proximal tubule (day 18), GATA3$^-$LTL$^-$CDH1$^+$ early distal tubule, GATA3$^+$CDH1$^+$ collecting duct.[94] Although GATA3 was used to distinguish between distal tubules and collecting

ducts, later studies indicated that GATA3 can also be expressed by the distal tubules and connecting segments of the nephrons,[22] suggesting the necessity of different protocols to specifically induce UB lineages. Additionally, the same group reported that human kidney organoid-derived glomeruli (OrgGloms) cultured in 3D exhibited proper apicobasal polarization and high expression of podocyte-specific genes such as *NPHS1, NPHS2*, and *PODXL* in addition to their rich content of glomerular basement membrane matrices when compared to conditionally immortalized human podocytes cultured in 2D.[48,94] These polarized podocytes of OrgGloms expressed PODXL at the apical surface and NPHS1 and NEPH1 proteins on the basal side which are critical for the filtration function of the kidney.[56,90] These pioneering studies of kidney organoid differentiation opened new avenues to investigate kidney development and diseases, followed by numerous studies that utilized kidney organoids for disease models.

Disease models using kidney organoids. There are many challenges in using two-dimensional (2D) cell cultures and animal models for drug screening and disease modeling.[43] Conventional monolayer cell cultures lack the tissue complexity and 3D biochemical and biomechanical microenvironments present in human organs.[30] Although murine models are widely used to explore the pathophysiological mechanisms of the genetic or autoimmune and/or inflammatory diseases for the development of novel therapeutic agents prior to clinical studies in patients, there has been a growing concern for the translation of animal studies to humans. The published literature, which compared the differential gene expression profiles of mice and humans via various microarrays during the acute inflammatory responses, has shown that even though different etiologies of acute inflammatory disorders produce very similar genomic responses among different patients, these gene expression signatures in the patients are not correlated with the corresponding profiles in murine models.[61] Furthermore, there have been growing ethical concerns for animal experiments due to the importance of animal welfare and rights, gradually limiting animal experimentation.[2] Therefore, human kidney organoids are of great interest for disease modeling and drug screening owing to their potential to minimize these animal studies.

(1) Kidney organoid models for tubular injury and fibrosis

Drug-induced nephrotoxicity, frequently documented among patients in the intensive care unit (ICU), has remained challenging during ongoing treatment for their primary diseases, causing acute kidney injury (AKI) and CKD progression.[100] For this critical problem, human kidney organoids were tested for nephrotoxicity assays using gentamycin and cisplatin.[75] Gentamicin is a commonly used antibiotic which causes proximal tubular injury, while cisplatin, a chemotherapeutic agent used for a wide range of cancers, also induces dose-dependent proximal tubular damage.[75,105] Both drugs particularly damaged the proximal tubules of organoids as evidenced by the expression of kidney injury molecule-1 (KIM-1) on the luminal surface of LTL^{+} proximal tubules.[102] Interestingly, podocytes did not exhibit injury phenotypes as suggested by the absence of a DNA damage marker, γH2AX, when low doses of these drugs were used. This early work suggested that kidney organoids express drug transporters in a segment-specific manner whereby cell type-specific responses to varied drugs can be tested in the organoid model.

Gupta et al. recently developed an organoid model of kidney injury and repair by applying repetitive cisplatin exposure. This study demonstrated that kidney organoids arose from co-induced nephron and stromal progenitor cells, which led to the generation of segmented nephrons admixed with stromal cells. Given the presence of discernible kidney compartments, the authors histologically monitored intrinsic repair (reversible injury) vs incomplete repair (irreversible injury) throughout a protocol of repeated cisplatin exposure, analogous to cycles of chemotherapy.[44,88] Following an initial 24 hours exposure to low-dose cisplatin, proximal tubules specifically manifest injury markers of DNA damage and KIM1, as previously reported.[75] Knowing that injured tubular epithelial cells compensate by proliferative expansion to preserve kidney architecture during intrinsic repair,[57] the authors demonstrated the induction of intrinsic repair phenotypes of proliferation and dedifferentiation, as evidenced by the reactivation of kidney developmental genes, *SOX9* and *PAX2*. Meanwhile, the DNA damage response triggered the elevation of homology-directed repair (HDR) protein, Fanconi anemia complementation group D2 (FANCD2). In a longitudinal study after the initial injury, the resolution of DNA damage by FANCD2 activation during the intrinsic repair phase terminated the proliferative phenotype with preservation of tubular structures reflecting the reversibility of the injury consistent with completed intrinsic repair. However, when the acute cisplatin injury was repeated biweekly for 5 treatments, incomplete repair phenotypes of tubular atrophy, myofibroblast activation, and interstitial fibrosis signified irreversible injury.[44] Loss of FANCD2 in DNA damaged tubules correlated with the transition from intrinsic to incomplete repair in kidney organoid samples. Mammalian single cell

transcriptomic datasets of ischemic, obstructive, and immune-mediated kidney injury were interrogated to confirm the generalizability of tubular FANCD2 correlating with intrinsic repair, whereas human fibrotic biopsy samples suggested that tubular FANCD2 inversely correlated with incomplete repair. To extend beyond a correlation, a small molecule inhibitor that upregulates HDR was found to rescue FANCD2 in injured tubules, partially reversing tubular atrophy, myofibroblast activation, and interstitial fibrosis.[44] It was concluded that HDR is a mechanism underpinning intrinsic repair following tubular injury in this organoid model developed in the absence of the potential pro and anti-fibrotic effects of the immune system. Of note, the single cell analysis in this study suggested the myofibroblast transition from mesenchymal fibroblast populations in kidney organoids, which is consistent with a recent study in human kidney samples.[64] In addition, another hierarchical single-cell transcriptional profiling model, generated through the multiple single cell RNA-seq datasets of human fetal kidney (HFK), has reclassified the component cellular identities, such as stromal and NPC-like cells within the published kidney organoid datasets.[108] As the HFK dataset grouped stromal cells into 4 subpopulations such as stromal progenitor cells (SPC), cortical stroma (CS), medullary stroma (MS), and mesangial cells (MesS), the organoid stroma was mainly composed of cortical stromal cells that are the origin of myofibroblasts.

(2) Kidney organoids under the inflammatory condition

During acute kidney injury, repair, and fibrosis, immune and/or inflammatory responses play an important role in modulating intrinsic and incomplete repair. Lemos et al. used kidney organoids as a fibrosis model by exposing them to an inflammatory cytokine, IL-1β.[67] Kidney organoids treated with IL-1β developed tubulointerstitial fibrosis with αSMA^{+} myofibroblast activation and collagen deposition. IL-1β signaling through the IL-1 receptor-associated kinase 4 (IRAK4) promoted the upregulation of *MYC* downstream target genes, containing cell cycle regulators and glycolytic-proliferative transcriptional signature, which eventually led to the proliferation of kidney stromal cells (SCs) and their transdifferentiation into myofibroblasts, and the deposition of fibrotic matrices in kidney organoids.

The investigation of the immune system in laboratory mouse strains is significantly limited by the minimum genetic diversity and predominance of an immature immune system that resembles neonatal immune reaction in organs such as the kidney.[12,49] Therefore, human organoids could better reproduce the microenvironment of the native kidney with its complex reciprocal interaction between T and B lymphocytes and antigen-presenting cells (APCs), that involves antigen presentation and simultaneous expression of costimulatory molecules to activate antigen-specific effector T and B lymphocytes during adaptive immune response.[109] Kidney organoids may allow us to better understand the pathophysiology of immune-mediated kidney diseases such as auto-immune glomerulonephritis and enable drug testing for novel therapeutic strategies. A recent example is the investigation of high-risk apolipoprotein-1 (APOL1) variant genes in kidney disease, which is a leading cause of kidney disease in African Americans.[39] Using different combinations of APOL1 variants generated by CRISPR/Cas9 genome editing, Chun et al. demonstrated the role of interferon-gamma signaling that drove lipid accumulation and podocyte toxicity in kidney organoids with high-risk APOL1 variants, which could be reversed by inhibition of diacylglycerol O-acyltransferases 2.[19]

The kidneys are often involved in systemic autoimmune and autoinflammatory diseases with consequent deposition of antibody, complement, and/or immune complexes in glomeruli. Several studies pointed out the contribution of non-immune cells of the kidney in such immune-mediated glomerulonephritis. Interestingly, podocytes can function as antigen-presenting cells to induce CD4+ T cells and also CD8+ T cells via cross presentation during both *in vitro* and *in vivo* settings, while proximal tubular epithelial cells activate the proliferation and cytokine production of CD4+ T cells when cocultured together.[14,40] Furthermore, the presence of co-inhibitory receptors such as PD-L1 in kidney tubules suggests their potential role in actively controlling the immune response.[47,91] The unique contribution of immune and non-immune cells to kidney injury and fibrosis in the organoid models deserves further investigation to clarify the complex interactions of these cells during immune-mediated nephritis. Further, autoimmune and/or autoinflammatory diseases that involve the interaction between epithelia and immune cells could be studied using kidney organoids in combination with genome editing technologies that can modify the genes of interests.[5] The effects of immunosuppressive modalities such as regulatory T cells or CTLA-4 immunoglobulin could also be tested in organoid models[97] for the future applications.

(3) Kidney organoids for hereditary kidney diseases with tubulopathy

Adult-onset autosomal dominant polycystic kidney disease (PKD), the most common form of hereditary renal disorder with an incidence of approximately

1:1000 live births, is characterized by the formation of renal cysts derived from tubular epithelial cells, leading to the progressive loss of renal function in enlarged kidneys.[54] This life-threatening disorder is caused by the mutations in *PKD1* or *PKD2* genes encoding polycystin 1 (PC1) and polycystin 2 (PC2) proteins, which form a heterodimeric complex to function in primary cilia.[10] Freedman et al. introduced a PKD model by using gene-edited human kidney organoids.[33] Following the generation of *PKD*-mutated hPSCs by CRISPR/Cas9 genome editing, they demonstrated the formation and expansion of cyst-like structures in the tubular organoids differentiated from *PKD1* or *PKD2*-mutants. Although ADPKD cysts arise pre-dominantly from the collecting duct, the cysts in this study were localized in the LTL^+ tubules.[33] In 2017, they found that PC1 protein was responsible for the adhesion of tubular epithelial cell compartment to the underlying extracellular matrix (ECM) microenvironment, which was required for the maintenance of the tubular shape and integrity. In addition, PC1 participated in the remodeling of ECM microenvironment, which eventually limited the cyst formation.[25] Therefore, it was shown that removal of adherent ECM components further promoted the cystogenesis of PKD-mutant/PC1-deficient organoids, especially in early stages of the disease. Furthermore, cAMP signaling was shown to induce the enlargement of cyst-like structures, contributing to the pathogenesis of PKD.

Nephronophthisis (NPHP)-related ciliopathy is a progressive fibrocystic kidney disease characterized by shrunken or normal-sized fibrotic kidneys with small cysts at the corticomedullary junction in contrast to PKD presented with huge cysts.[13] Histological findings are characterized by the disruption of the tubular basement membrane, tubular atrophy and/or dilatation, and interstitial fibrosis, eventually resulting in end-stage kidney disease.[32] Little et al. demonstrated the underlying pathology of NPHP in patient kidney organoids by comparing the organoids of an identified NPHP proband (PR) patient with its isogenic control organoids derived from their gene-corrected (GC) iPSCs. By using CRISPR/Cas9 technology, the GC iPSCs were originally obtained from the same NPHP PR patient with compound-heterozygous mutations detected in the *IFT140* gene.[32] When kidney organoids derived from both PR and GC iPSCs were compared, PR organoid tubules exhibited shortened and club-shaped primary cilia, whose phenotype was rescued by the gene correction. Consistent with the downregulation of genes implicated in apicobasal polarity, cell-cell junctions, and dynein motor assembly, spheroid cultures demonstrated the apicobasal polarization defect in PR organoid tubules unlike GC organoids. However, the cystic kidney phenotype was not seen in this study, that might be due to the limitations of static organoid models. A recent study reported that cystic tubular cells derived from ADPKD patients performed basoapical fluidic pumping in the opposite direction compared to normal tubular cells, revealing the importance of hydraulic pressure in ADPKD pathophysiology.[18] Therefore, novel approaches, which can simulate *in vivo* fluidic microenvironment, such as organoids-on-chip approaches can be used in the future to investigate the cyst formation in the ciliopathic organoids in the presence of fluidic shear stress.[43,55] Nevertheless, this work showed an approach to correct patient mutations by CRISPR/Cas9 for generation of an isogenic control, which validated the ciliary pathogenesis in the PR organoids.[32]

(4) Kidney organoid models for glomerulopathy

A glomerulus, a filtration segment of each nephron, comprises fenestrated endothelia, mesangial cells, podocytes, and parietal epithelial cells.[48,87] Podocytes, anatomically defined as glomerular visceral epithelial cells, form the glomerular filtration barrier with their foot processes (FPs) and the capillary endothelial cells.[31,48,87] FPs create interdigitating structures with the adjacent podocytes, forming slit diagrams that prevent the leakage of plasma proteins from the circulating blood together with the glomerular basement membrane (GBM) (Fig 1).[28,31,42,48] Podocyte injury and its structural alterations (eg, foot process effacement) cause nephrotic syndrome that manifests massive proteinuria, severe edema, dyslipidemia, leading to progressive loss of kidney function.[31,48,106] In 2018, Little et al. isolated organoid-derived glomeruli (OrgGloms) expressing *mTagBFP2* fluorescent reporter gene in their developing podocytes, and performed toxicity screening using doxorubicin (Adriamycin), a chemotherapy medication, at various concentrations.[48] Doxorubicin induced loss of BFP2 signal in OrgGloms, the fragmentation and destruction of podocytes in a dose dependent manner.

The podocyte unique structures are supported by slit diaphragms proteins including nephrin, podocin, and synaptopodin.[31] Wilms tumor-1 (*WT1*), earliest podocyte marker during kidney development, is identified as one of the upstream transcription factors which regulate these podocyte genes.[28,75] To reveal the epigenetic transcriptional reprogramming by *WT1*, Ettou et al. performed genome-wide analysis of *WT1* DNA binding and evaluated podocyte gene expression during the course of Adriamycin (ADR)-mediated injury in both murine models and human organoids. They demonstrated that *WT1* is responsible for the maintenance of open chromatin at podocyte genes for their active

transcription.[31] Although ADR-induced injury initially promoted an enhanced open chromatin state at *WT1* target genes such as *NPHS2* (Podocin) and *SYNPO* (Synaptopodin), the chromatin status was subsequently converted from active to repressive, downregulating podocyte genes. Hence, this study suggested that *WT1* is the key transcription factor for podocyte survival during injury and the transcriptional reprogramming of podocyte gene expression can be a novel therapeutic target for CKD.[31] These two studies demonstrate that 3D human kidney organoids can be utilized to study podocyte injury induced by toxicants.

Notably, Little et al. also showed the application of kidney organoids to model the congenital nephrotic syndrome (CNS) caused by the mutations in *NPHS1* (nephrin) and *NPHS2* (podocin).[48,99] Scanning electron microscopy findings of the OrgGloms derived from the CNS patient carrying *NPHS1* gene mutations pointed out the large hypertrophied podocyte bodies, while quantitative western blot analysis confirmed the significant reduction in both nephrin and podocin proteins in the patient derived-OrgGloms compared to the control group. A similar study using hiPSC-derived kidney organoids from a patient with *NPHS1* missense mutation demonstrated the formation of impaired slit diaphragm (SD) in the organoid podocytes.[95] These *NPHS1* mutant podocytes of the organoids were deprived of "pre-SD domain" structure, which was defined as the transit state of rod-like Nephrin protein shifting between the lateral and basal sides of podocytes. Genetic correction of this point mutation restored the nephrin expression, its phosphorylation, and the formation of lateral and basal nephrin^{+} pre-SD domains resulting in the generation of proper SD.[95] Both of these organoid studies modeling CNS revealed different aspects of the structural disruption caused by the nephrin mutation.

One study focused on another cell type of glomeruli, parietal epithelial cells (PEC) (Fig 1). Nishinakamura et al. demonstrated PAX2 is indispensable for morphological maturation of PEC using kidney organoids and transcription activator-like effector nucleases (TALENs). Interestingly, PAX2 was found to be dispensable for *in vitro* nephron formation from human iPSCs.[62] Not only human nephron progenitors (NPs) were formed from *PAX2*-depleted hiPSCs, but, also they were able to become epithelialized to form glomerular and renal tubular structures. However, glomerular PECs remained to be columnar epithelia, lacking the ability to transform into the squamous morphology. Hence, they confirmed that human PAX2 protein is not required for the mesenchymal-to-epithelial transition of nephron progenitors to develop normal glomeruli and renal tubules, but indispensable for the normal differentiation of PECs.[62]

Research work discussed above demonstrate the utility of human kidney organoids for a variety of genetic kidney diseases (ADPKD, nephronophthisis-related ciliopathies, congenital nephrotic syndrome), developmental kidney disorders (PAX2 deficiency), drug-induced glomerular or tubular toxicity, and inflammatory cytokine-mediated renal injury. Patient-derived organoids can also be used for personalized drug screening to assess the therapeutic efficacy and side effects as preclinical trials. Although kidney organoids exhibit most of the structural elements of native kidney *in vitro*,[74,75] the formation of vascular network is limited in the static culture of organoids.[55,75] Organoids generated by Freedman et al. contained CD31 and von Willebrand factor expressing endothelial cords while organoids by Takasato et al. developed CD31^{+}KDR^{+}SOX17^{+} endothelial network.[33,94] However, these studies have not observed mature vascularized glomeruli, yet. Only after subcapsular transplantation of the organoids into mice, host-derived vascularization induced glomerular and peritubular neo-vasculogenesis.[9] Development of vascularized kidney organoids would improve the organoid disease models for translational studies.

Optimizing kidney organoid models. Kidney organoids offer a novel platform for drug screening, disease modeling, and regenerative tissue engineering, since they contain key cell types that self-assemble into the desired nephron-like subunit microarchitectures.[75,93] However, kidney organoids differentiated under static culture conditions lack the microvasculature needed to facilitate both filtration and reabsorption *in vitro*.[55,66] Recently,[55] Homan and Gupta *et al.* demonstrated that subjecting kidney organoids to flow during their differentiation leads to a substantial upregulation in their endothelial progenitor population, which promotes the formation of a pervasive microvascular network and enhances organoid maturation. Specifically, under flow, the vascular invasion of podocyte-rich clusters is observed along with primitive foot process formation and an upregulation in key glomerular markers.[55] Concomitantly, vascular wrapping of proximal tubular segments is enhanced under flow that leads to improved polarity, ciliation, and upregulation of key proximal tubule markers.[55] The importance of flow during kidney organoid differentiation was also highlighted by a recent study from Lee et al. who demonstrated the improved sensitivity to a nephrotoxicant in kidney organoids cultured under flow.[66] However, to date, the ability to address and perfuse the organoid microvasculature from larger vessels has yet to be demonstrated.

Creating kidney organoids with a perfusable vasculature *in vivo* can be achieved by implantation into mice;[8,4,9,69,85,98,112] or chick chorioallantoic membranes,[37] where host vascular integration ensues leading to both organoid maturation and/or rudimentary filtration. Organ-on-chip methods may allow organoids to mimic these native processes *in vitro* through vasculature recruitment from a nearby endothelialized channel or integration with prepatterned vasculature.[116] For example, Nashimoto et al. demonstrated that placing tumor spheroids between endothelialized channels induced vasculature sprouting and fusing leading to perfusable microvasculature between the tumor spheroids and channels.[77] While Zhang et al. 3D printed a vascular scaffold that was subsequently seeded with cardiomyocytes to produce vascularized cardiac tissue.[117] While these are encouraging *in vitro* demonstrations, neither of these approaches have been extended to kidney organoids. Moreover, we posit that vasculature recruitment may offer a better approach, since it mimics *in vivo* vascularization of kidney organoids.[116]

Once perfusable, vascularized kidney organoids are obtained *in vitro*, the next crucial step will be the introduction and integration of a collecting duct system that allows for primitive filtrate removal. Current differentiation protocols that yield MM-derived cell populations lack UB-derived collecting ducts.[22,75,93] However, when embryonic MM and UB populations are combined *in vitro* via core-shell assembly, one observes the formation of a hierarchal collecting duct system.[36,101] As an example, Tanigawa and Tanaka et al. have demonstrated this for murine iPSC-derived kidney organoids by combining induced MM, UB and stromal populations.[96] The assembly of murine ESC-derived NPs, UBs, and stromal progenitors resulted in the generation of organ-like kidney structure, which comprise nephrons with multiple types of stromal cells accumulated around branching UBs.[96] However, to our knowledge, this has yet to be achieved in human kidney organoids.

The ability to achieve filtrate drainage in kidney organoids *in vitro* is important for two reasons: (1) absent this capability, filtrate accumulation will lead to hydronephrosis in vascularized organoids,[115] and (2) filtrate collection is essential for functional assessment. At present, fluorescent probes can be used to study filtration and transporter processes,[33,8,59] while micropuncture techniques enable filtrate composition to be assessed in individual tubules.[15,103] To generate kidney organoids with an open collecting duct or ureter, one can draw inspiration from kidney development and *in vitro* vasculature recruitment models. We hypothesize that such organoids might be generated by embedding them within an extracellular matrix *in vitro* that contains an open nephric duct, produced for example by bioprinting, from which a UB arises and invades a surrounding MM.

Engineering kidney tissues. By further advancing kidney organoids into functional models, one can unlock their potential as building blocks for engineering kidney tissue for therapeutic use. Several 3D bioprinting methods enable the fabrication of human tissues using organoids or multicellular spheroids as building blocks.[3,26,41,65,89] For example, Lawlor and Vanslambrouck et al. created two-dimensional kidney tissue sheets composed of closely printed lines of IM cells that fuse together and further develop over time. The resulting kidney tissue demonstrated correctly segmented nephrons, including proximal tubules that exhibited albumin uptake.[65] Another promising method to generating vascularized human tissues, known as sacrificial writing in functional tissue (SWIFT), was recently developed by Skylar-Scott and Uzel et al.[89] This method involves printing branched vascular networks within a living tissue matrix comprised of densely cellular, organoid building blocks. By embedding a perfusable vasculature within these tissues, one can overcome the nutrient diffusion limitation that gives rise to core necrosis and fibrosis in organoids with diameters above $\sim$500 μm, resulting in a large viable tissue.[89,116] Since SWIFT biomanufacturing is broadly applicable, this method would enable the construction of vascularized kidney tissues using organoids as organ building blocks. In the future, the large scale production of the vascularized kidney tissues from hundreds of thousands of organoids within these engineered tissues will ultimately enable the generation of engineered organ-like structures of kidney containing 1 M+ nephrons akin to human kidneys.[11,78]

***Kidney organoids* to overcome post-transplant immune rejection.** Kidney transplantation is currently the optimal treatment for ESKD patients; however, rejection of the allogeneic kidney transplants by the host immune system is a major obstacle, leading to graft loss.[50,83] Rejection is caused by an immune response against the transplanted organ, driven by the recognition of the transplanted kidney tissue antigens as foreign bodies by the host immune system. Immune rejection of the transplanted graft is classified as hyperacute, acute, and chronic rejection, based on the histopathological features and the time frame of the rejection process following the transplantation.[1] In the course of xenotransplantation from pigs or nonhuman primates to humans, both hyperacute and acute humoral or acute cellular rejection would be evoked by the circulating antibodies, complement, neutrophils, natural killer (NK) cells, T cells, and macrophages.[70]

Hyperacute rejection (HAR) is characterized by extensive thrombotic microangiopathy (TMA),[1,70] mediated by multiple factors including pre-existing IgM/IgG alloantibodies of the recipient to the porcine carbohydrate epitopes (Galα1−3Galβ1−4GlcNAc: α1,3Gal epitope), complement activation, and the hypercoagulable state.[35,114] Recently, 2 studies attempted to overcome the hyperacute rejection by knocking-out α-1,3-galactosyltransferase enzyme and modifying multiple genes associated with complement, coagulant, and immune regulation in the setting of porcine kidney xenotransplantation to brain-dead human recipients.[72,80] However, it is still unclear if those gene editing can prevent TMA in humans for the long-term. In contrast, this hyperacute rejection does not constitute an obstacle for the possible transplantation of human iPSC-derived kidney organoids as human kidney tissues do not express α1,3Gal epitope.

Acute and chronic rejection involve damage to both the graft parenchyma and blood vessels, mediated by alloreactive T cells and alloantibodies against the human leukocyte antigens (HLAs) expressed by the donor organs and/or donor APCs.[1,52,104] HLAs are the predominant target of the immune system based on its great heterogeneity and polymorphism between individuals.[73] The degree of HLA mismatch between donor and recipient correlates with the graft survival as the presence of higher mismatches is associated with a significant higher risk of graft failure.[107] With the future advancement of 3D-biofabricated kidney structures derived from HLA-edited hiPSCs via CRISPR/Cas9 technology, it is conceivable to generate immune-tolerant kidney tissues with manipulated HLA molecules, which enhance the compatibility between donors and recipients, thereby reducing the risk of acute and/or chronic rejection responses. This novel approach may also dampen the need and dosage of current immunosuppressive agents such as calcineurin inhibitors (cyclosporine, tacrolimus), mammalian target of rapamycin (*mTOR*) inhibitors, and T cell-depleting induction therapy.[23,79] Furthermore, enhancing the expression of coinhibitory receptors on kidney cells may provide an additional protection against the immune response to suppress rejection,[82] which might translate into the ultimate personalized therapeutic strategy in transplantation.

Recently, Cowan et al. generated hypoimmunogenic hPSCs using the CRISPR/Cas9 system by ablation of HLA-A/B/C and HLA class II, and overexpression of inhibitory signals such as PD-L1, HLA-G, and CD47 to suppress T cell, NK cell responses, and macrophage engulfment.[50] However, overexpression of checkpoint molecules like PD-L1 bears the risk of malignancy by promoting tumor escape from the immune surveillance.[51] In another study, Hotta et al. developed immune compatible iPSCs by preserving HLA-C, inhibitory for NK cells, in the absence of HLA-A/-B and CIITA [major histocompatibility complex (MHC) class II transactivator essential for the transcription of class II HLA genes].[113] Consequently, 12 HLA-edited (HLA-A- and -B-deleted and HLA-C-retained) iPSC lines could serve as immunologically compatible iPSCs for nearly >90% of the world population,[113] thereby enabling the generation of universal kidney organoids as building blocks, which can give rise to immune-compatible organotypic kidney structures in the future. Overall, the development of these broadly compatible kidney structures may allow a larger scale and cost-effective therapeutic strategy as an alternative to the generation of patient-specific kidney organoids.

Current limitations of kidney organoid models. Kidney organoids offer a tremendous opportunity for the future medical applications, such as generation of immune compatible bioengineered kidneys for the growing demand from CKD patients. However, this unique technology comes with its own restrictions. When a previous study compared the maturation status of organoids directly differentiated via the Morizane and Takasato protocols by using the single-cell transcriptomics of fetal and adult kidney cells, they demonstrated that the organoids by both protocols gave rise to mostly immature cell types at early differentiation stages (~26 days) with respect to their adult counterparts.[110] Even though the epithelial cell types of the adult kidney and kidney organoids correlated well, organoid-derived cells tended to express developmental genes. Efforts need to be made to extend the organoid culture for further maturation. In addition, the single-cell transcriptomics suggested the protocol differences. Morizane protocol appears to induce more matured nephron epithelia than Takasato protocol according to Wu's study. Although it is difficult to have a definitive conclusion due to batch-to-batch variations, further improvements on the current protocols should also be considered. Furthermore, it is essential to improve the current methodology for the evaluation of functional assessment of kidney organoids, which are building blocks for the in vitro bioengineered kidneys.[84] Therefore, the organoids from established protocols should be tested to confirm the recapitulation of normal kidney functions such as glomerular filtration, tubular reabsorption/secretion, and production of erythropoietin and angiotensin II hormones.

CONCLUSION

The kidney functions as a critical organ maintaining the homeostasis of body fluid and electrolyte status by effectively removing the metabolic waste products. Acute kidney injury or progressive chronic kidney

disease may result in renal insufficiency with serious systemic complications in the patients, who eventually need to undergo renal replacement therapies such as dialysis and transplantation, thereby leading to a high socioeconomical burden in the healthcare system. Conventional research techniques remain insufficient to fully recapitulate the kidney physiology and pathogenesis underlying the complex kidney diseases. Thus, the development of directed differentiation protocols, which simulate *in vivo* nephrogenesis, enabled the generation of *in vitro* 3D kidney organoids from hPSCs. Even though the application of patient iPSC-derived kidney organoids advanced our understanding of the kidney maturation, injury, and repair, we still need to facilitate the maturation of glomerular vascularization and incorporate collecting duct system into organoids by modifying the current organoid protocol with additional novel techniques such as 3D-bioprinting and organ-on-a-chip. The ultimate technology of organoids-on-chip may provide the better translational utility for disease modeling and drug screening, potentially replacing the animal models in the future.

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