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Kidney organoid research: current status and applications

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Organoids are being widely introduced as novel research models in multiple research fields. Human-induced pluripotent stem cells-derived kidney organoids became an indispensable tool to study human kidney development, model various diseases and infections leading to kidney damage, and offer a new route towards better drug development and validation, personalized drug screening, and regenerative medicine. In this review, we provide an update of the most recent developments in kidney organoid induction: their main goals, advantages, and shortcomings. We further discuss their current applications in providing modeling and treatment avenues to various kidney injuries, their use in genome-wide screening of kidney diseases, and the cell interactions occurring in these kidney structures.

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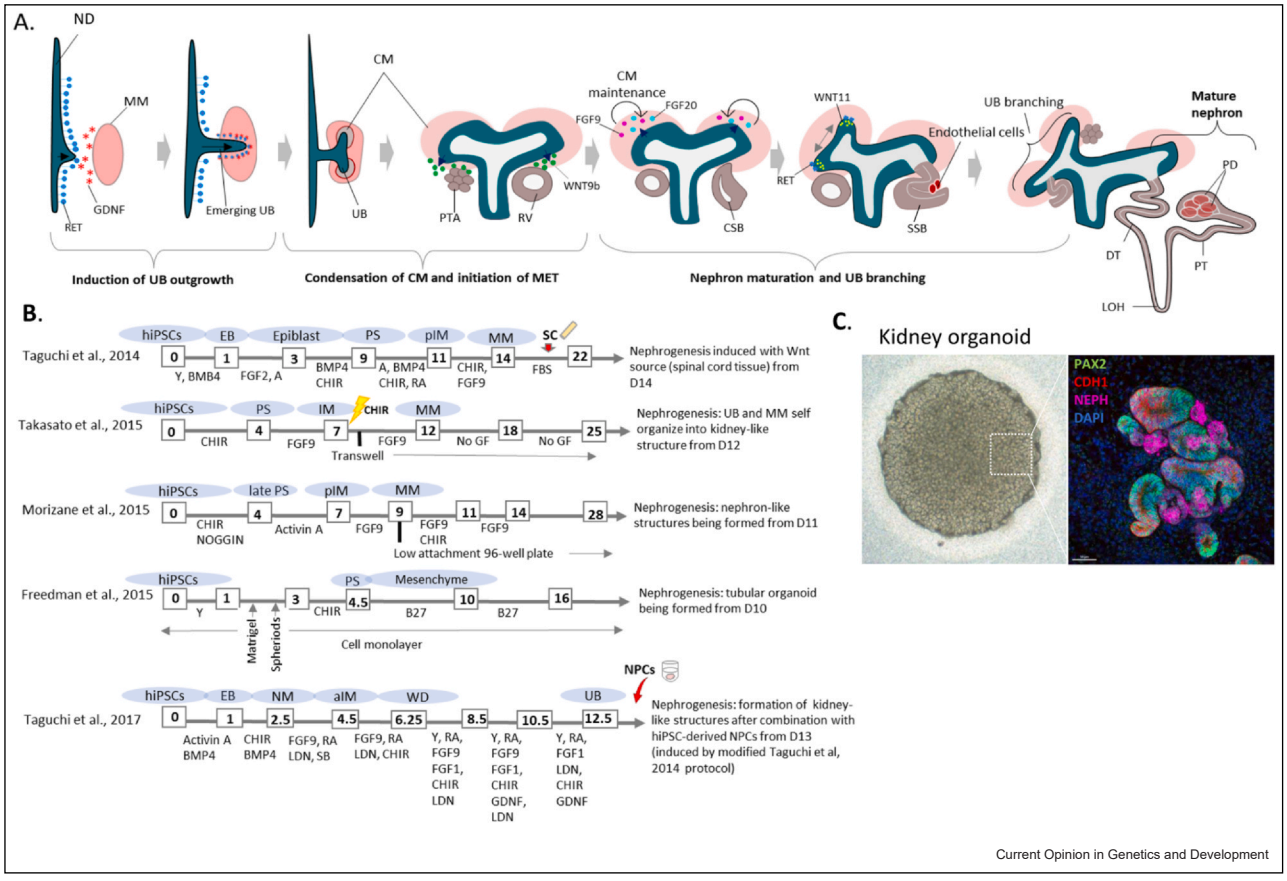
Introduction

The complex process of kidney development starts from the reciprocal interaction of two embryonic cell populations: ureteric bud (UB) and metanephric mesenchyme (MM) [1] (Figure 1a). Six2$^+$ nephron progenitors (NPs) in the MM give rise to all segments of the matured nephron [2], while the UB undergoes multiple branching events, eventually forming the collecting duct (CD) and ureter. Both cell populations emerge from intermediate mesoderm (IM), however, they have distinct origins related to the timing they entered through the primitive streak (PS). The UB is derived from the Wolffian/nephric duct (ND) and originates from earlier 'anterior IM', whereas the MM originates from the posterior-most fraction of nephric mesenchyme that is laid down later during axis elongation ('posterior IM') [3]. During kidney organogenesis, UB outgrowth from the ND is initiated by glial cell line-derived neurotrophic factor (GDNF) secreted from the MM cells, while Wnt9b secretion from the UB triggers nephrogenesis in Six2$^+$ NPs. These NPs surround the UB and are often referred to as the cap mesenchyme (CM) [4] (Figure 1a). Controlled by GDNF–Ret (GDNF–receptor) interaction, the emerged UB will undergo repeated branching events at its tip region(s): Ret-expressing UB cells respond to MM-secreted GDNF by upregulating Ret and Wnt11, thereby positively regulating branching morphogenesis and increasing GDNF expression in the MM [5,6]. The CM requires the activation of fibroblast growth factor (FGF) signaling for its maintenance: FGF9 and FGF20 are produced by MM cells and maintain the stemness of Six2$^+$ NPCs within the CM [7]. When stimulated by Wnt9b, NPCs are driven into a nephrogenesis cascade, forming initially pretubular aggregates that organize into polarized renal vesicles *via* mesenchymal-to-epithelial transition, and consecutively transit through comma-shaped body and S-shaped body (SSB) structures to ultimately form capillary loop nephron stages [8] (Figure 1a). Matured nephrons then further develop through the elongation and segmentation of their distal and proximal tubules and establish the loop of Henle [9]. Developing nephrons connect to the CD network during SSB formation *via* a distinct spot at the most distal edge of the SSB [8] (Figure 1a).

In the last decade, the process of nephrogenesis has been reproduced using human-induced pluripotent stem cells (hiPSCs) differentiation towards kidney organoids. Several pioneers in kidney organoid research succeeded in generating nephron-like structures *in vitro*: the protocols of Takasato et al. [10–12], Taguchi et al. [13,14], Freedman et al. [15] and Morizane et al. [16] therefore form the core induction protocols. Optimization of differentiation and new culture techniques further diversified the applications of kidney organoids, from studying kidney development towards disease modeling through the introduction of specific mutations in hiPSCs and the analysis of patient-derived kidney organoids.

Figure 1



Early nephron development *in vitro* and core protocols for directed differentiation of hiPSCs to organoids. **(a)** Nephron formation mechanism. **(b)** Core kidney organoid induction protocols. **(c)** Kidney organoid induced by the Takasato et al., 2015 protocol at day 14 of differentiation. PAX2, Paired box gene 2 (early nephron marker); CDH1, Cadherin 1 (DT marker); NEPH, Nephrin (PD marker). A, Activin A; B27, B27 medium; CHIR, CHIR99021; CSB, comma-shaped body; DT, distal tubule; EB, embryoid body; FBS, fetal bovine serum; GF, growth factors; pIM, posterior intermediate mesoderm; aIM, anterior intermediate mesoderm; LDN, LDN193189; LOH, loop of Henle; NM, nephric mesenchyme; NPC, nephron progenitor cells; PD, podocytes; PT, proximal tubule; PTA, pretubular aggregates; RA, retinoic acid; RV, renal vesicle; SB, SB431542G; SC, spinal cord; WD, Wolffian duct; Y, Y27632.

In this review, we will focus on the most recent progress made in kidney organoid generation, including novel approaches in induction techniques (Table 1), the practical application of kidney organoids in their current state (Table 2), and discuss the future potential and remaining challenges that need to be addressed to expand their use towards regenerative medicine applications.

Novel approaches in human-induced pluripotent stem cells-derived kidney organoids induction

Initially, kidney organoid protocols either induced an MM cell population [13,15,16] or a combination of UB and MM cell populations [11,12] from pluripotent stem cells, that could then be further differentiated into nephrons (usually in the presence of a source of Wnt signaling) or nephrons and CD, respectively (Figure 1b,c). Taguchi et al. [14] offered a route to generate MM and UB cell populations separately, only to combine them at a later timepoint to induce nephrogenesis (Figure 1b). As the forementioned core protocols have been modified to serve different purposes and new ones introduced [17,18], a review by Little and Combes [17] nicely summarizes current kidney organoid induction protocols. Due to space limitations, however, we will focus on the most recent adaptations and their applications (Table 1).

A serious drawback in current organoid induction protocols remains insufficient lineage specification and immaturity of generated kidney cell types. Specifically, during initial induction, the parameters to separately generate metanephric NPs, mesonephric NPs, and any other mesenchymal lineages remains to be fully understood. To address this — and illustrating the complexity of interactions between different mesenchymal cell populations — Tsujimoto et al. [19], introduced a modular differentiation system in which mesonephric and

Table 1

Novel approaches in hiPSC-derived organoids induction.

Protocol reference	Novel approach	Potential advantage/application	Core differentiation protocol
Tsujimoto et al., 2020 [19]	Differentiated hiPSCs through CDX1$^+$ epiblast-like cells into multiple mesoderm lineages: IM, PAM and LPM. Generated mesonephros and metanephros from epiblast-like cells.	Recreated nephrogenic niches from separately induced metanephric NP-like and UB-like cells, that were able to form SSB-like structures, tubules, glomeruli, and CD *in vitro* and further vascularized *in vivo.*	MM: new protocol. UB induction: modified from Mae et al., 2018.
Uchimura et al., 2020 [20]	Generated human kidney organoids with CD by separately combining differentiated MM- and UB-like progenitors. Used aldosterone and vasopressin to improve the maturation of CD cells.	Revealed the role of Notch in setting the ratio of principal and intercalating cells in the UB. Created an organoid model to study PC and IC development and interconversion, and cystogenesis in the CD.	MM until day 7: Takasato et al., 2016. From day 8: newly developed protocol.
Lawlor et al., 2021 [22]	Applied extrusion-based 3D cellular bioprinting to deliver rapid and high throughput generation of kidney organoids with highly reproducible cell number and viability.	Automated extrusion-based bioprinting deliver improvements in throughput, quality control, scale, and structure. Can be used for drug testing.	Takasato et al., 2015 and 2016
Fujimoto et al., 2020 [26]	Replaced mouse NPCs with human iPSC-derived NPCs.	Demonstrated the possibility to regenerate human kidneys from hiPSC-derived NPCs.	Taguchi et al., 2014, 2017
Homan et al., 2019 [27]	Introduced a millifluidic culture system for organoid culture on a chip. Showed that physical and environmental cues are involved in organoid development and vascularization.	Revealed how low-level induced FSS leads to enhanced formation of vascular network in organoids.	Morizane et al., 2017
Mae et al., 2020 [29]	Created UB organoids with tubular lumens and repeat branching morphogenesis. Established a method for expansion of single iUB cells into tip colonies that can form iUB organoids with differentiation potential.	iUB organoids were used to model multicystic dysplastic kidney.	Modified from Mae et al., 2018.
Howden et al., 2021 [30]	Showed the distal nephron can adopt the features of ureteric epithelium, able to trigger nephron development from NPs in the absence of a WNT signal.	Generated UE could be passaged and cryopreserved, as well as further differentiated into ureteric stalk cells. Method used to model ARPKD in PKHD^{null} UE stalk.	Takasato et al., 2016
Zeng et al., 2021 [31]	Developed 3D hPSCs-derived induced UB organoid that mimics branching morphogenesis and develop into mature CD.	Genetically modified UB organoids can model congenital anomalies of kidney and urinary tract. Provided enhanced understanding of development, regeneration, and diseases of the CD system.	New protocol.
Tanigawa et al., 2022 [34]	Induced dorsal SPs from mouse ESCs and combined mouse ESC-derived NPs, UB and SPs to generate kidney organoid.	Generated organoid showed improved UB branching and appropriate localization of NPs at the tips of UB, organoid structure resembled kidney *in vivo*.	NP, UB and SP (up to day 6.5): Taguchi et al., 2014, 2017; SP from day 6.5: new protocol
Little and Combes, 2021 [17]	Review that summarizes all available kidney organoid induction protocols of the last five years.		

iUB- UB induced from hPSCs; LPM, lateral plate mesoderm; PAM, paraxial mesoderm.

metanephric mesoderm-like cells, as well as paraxial and lateral plate mesoderm-like cells were separately generated from hiPSCs *via* a CDX1$^+$ epiblast-like intermediate state. The metanephric NPC fraction was then combined with separately induced UB cells to generate glomeruli, interconnected renal tubules and CD fates [19] (see Table 1). Despite a lack in higher-order kidney maturation, the authors were able to obtain a robust CDX2$^+$ 'late' PS cell population using an ALK5 (TGF-β receptor) inhibitor in combination with relatively low levels of CHIR99021 (a GSK-3β inhibitor commonly used to stimulate Wnt signaling during kidney organoid induction). With fewer cytotoxic effect compared to higher CHIR99021 doses, used in many of the core induction protocols, this argues for a reinvestigation on how the level and timing of Wnt, as well as the onset of Smad2/3-related signaling, affects PS cell population induction, interplay, and selection.

A study by Uchimura et al. compared simultaneous induction of MM and UB progenitors (Takasato et al. [12]) to the separate induction of MM (Takasato protocol until day 7) and UB (unique protocol) after which these populations were dissociated and mixed (from day 7). The authors noted that separate induction, followed by the combination of MM and UB, significantly reduced off-target cell populations and improved maturity of renal tubules and CDs [20]. These data highlight that

Table 2

Kidney organoid application in recent studies.

	References	Target for application	Summary
Disease models	Low et al., 2019 [41]	ARPKD model (PKHD1)	ARPKD hiPSC-derived kidney organoids showed drastic cystogenesis upon upregulation of cAMP compared to those derived from gene-corrected ARPKD hiPSCs, enabling successful drug testing *in vitro*.
	Shimizu et al., 2020 [42]	ADPKD model (PKD1)	hiPSCs-derived kidney organoids differentiated from gene-edited PKD1-mutant as well as ADPKD patient-derived hiPSCs can reproduce renal cysts. Model shows a predictive validity for drug screening.
	Kuraoka et al., 2020 [43]	ADPKD model (PKD1)	Cysts formed in UB organoids derived from iPSCs with homozygous deleted PKD1, as well as in UB generated from heterozygous mutant hiPSCs and from a patient with ADPKD.
	Mae et al., 2020 [29]	MCDK model	hiPSCs-derived HNF1b$^{+/-}$ UB organoids showed loss of apicobasal polarity and reduction of the expression of MCDK-associated genes.
	Kim et al., 2021 [45]	Fabry disease model	Gene-edited, hiPSC-derived GLA-mutant kidney organoids showed significant deformation of nephron compartments, increased oxidative stress and apoptosis, and decreased expression of podocyte and tubular markers. Treatment with recombinant human α-Gal resulted in amelioration of the pathological phenotype.
	Ohmori et al., 2021 [47]	Congenital NS model	Two different NPHS1^{-} mutations were examined using kidney organoid from patients hiPSCs. Study revealed that slit diaphragm-associated proteins NEPHRIN and PODOCYN were significantly affected.
	Jansen et al., 2022 [49]	Idiopathic NS model	The authors successfully modeled podocyte pathophysiology associated with congenital NS as well as idiopathic NS in patients using a hybrid organoid induction protocol for the generation of well-matured podocytes resembling adult podocytes.
	Majmundar et al., 2021 [48]	NOS1AP NS model	Kidney organoid with the patient's splice variant of NOS1AP revealed malformed glomeruli with increased apoptosis; consistent with a mouse model.
	Liu et al., 2020 [44]	APOL1 nephropathy model	Single-cell transcriptomic profiling of human genome-edited kidney organoids revealed APOL1 risk variants and provided a novel approach for studying the pathophysiology of APOL1 nephropathy.
	Helms et al., 2021 [50]	SARS-CoV-2 model	SARS-CoV-2 viral variants α, β, γ, κ, and δ were able to replicate in organoids. Gene expression patterns in the infected organoids reflect proteomic signatures of COVID-19 in the urine of critically ill patients. Infection was ameliorated in genome-edited ACE2$^{-/-}$ organoids and blocked *via* treatment with *de novo* designed spike binder peptides.
	Jansen et al., 2022 [53]	SARS-CoV-2 model	SARS-CoV-2 could directly infect kidney cells and subsequently cause fibrosis *via* the activation of multiple profibrotic signaling pathways leading to the progression of AKI or COVID-19-associated CKD.
Drug/Toxicity Screening	Dvela-Levit et al., 2019 [54]	Validation of the drug BRD4780 for MKD treatment	Confirmed the effectiveness of small molecule BRD4780 for treatment MKD. BRD4780 could clear mutated mucin protein from kidney organoid cells and similarly reverse the proteinopathy as seen in a mouse model.
	Monteil et al., 2020 [55]	ACE2 protein application for SARS-CoV-2 treatment	Confirmed the expression of ACE2 receptors on kidney organoid. Proved the effectiveness of human recombinant soluble (hrsACE2) as an antiviral treatment.
	Wysocki et al., 2021 [56]	ACE2 protein application for SARS-CoV-2 treatment	Verified the effectivity of short variant of hrsACE2 protein using hiPSC-derived kidney organoids.
	Digby et al., 2020 [57]	Cisplatin Toxicity	Evaluated the injury response in low-dose, cisplatin-treated organoids. Validated the use of human kidney organoids to model cisplatin-induced injury, with the potential to identify new AKI biomarkers and develop better therapies.
	Kim et al., 2021 [58]	Tacrolimus toxicity	Validated a kidney organoid model for tacrolimus toxicity by showing its strong nephrotoxic effect on the viability, size, and tubule polarity of kidney organoids. Revealed a critical role of autophagy in Tacrolimus-associated nephrotoxicity.

Table 2 (*continued*)

	References	Target for application	Summary
	Gulieva et al., 2021 [59]	Cryoprotectant toxicity	Used kidney organoids to estimate the toxicity level for different cryoprotectants. Highlighted the potential of using kidney organoids as a model system for investigating cryopreservation strategies.
	Lawrence et al., 2022 [60]	Toxicity screening assay	Used kidney organoid to identify a marker of oxidative stress-HMOX1. Established HMOX1-reporter renal organoid as an accurate nephrotoxicity screening assay.
CRISPR/Cas9 Genome Screening	Ungricht et al., 2022 [71]	Functional database for genetic regulation of kidney development and function	Created a functional dataset based on a genome-wide loss-of-function screen in hiPSC-derived kidney organoids. Improved MM induction and provided novel candidates of disease-related genes. Their created dataset highlights different ways of communication between heterogeneous cell populations in complex tissues.
	Shamshirgaran et al., 2021 [70]	Rapid target validation In disease modeling	Used an iCas9 hiPSC line and Cas9-mediated gene editing to introduce mutations in PKD1 and PKD2 genes and confirmed the ADPKD-associated phenotype. Demonstrated the versatile use of genetically engineered kidney organoids for rapid target validation in the context of disease modeling.
Functionality assay	Shankar et al., 2021 [62]	Functional renin	Investigated the regulation of renin production and identified the renin-producing cells using hiPSC-derived kidney organoids. Revealed the endocrine function of human kidney organoids, an important feature for regenerative medicine in the context of the endocrine system.
	Shankar et al., 2021 [63]	Vitamin D metabolism	Confirmed vitamin D metabolism in hiPSC-derived kidney organoids. Evaluated the expression of genes involved in vitamin D metabolism and examined the regulation of vitamin D–related enzymes. Provided evidence that organoids partially recapitulate renal hormonal systems.
	Gupta et al., 2022 [66]	Kidney injury and repair model	Revealed that the homologous recombination is a critical mediator of tubular repair, and a prolonged tubular injury weakens the DNA-damage response, leading to incomplete repair and, therefore, irreversible kidney destructions.

accuracy of the induced nephric cell populations as well as the timing in combination of MM and UB might be crucial to improving the morphology and functionality of *in vitro*-derived kidney-like structures. Especially, to proceed to higher-order kidney organoids, protocols should aim to mimic embryonic development accurately enough as to induce the right cell populations — including previously overlooked populations such as stroma, blood vessels or immune cells — at the right time and in the right confirmation (also see [17]).

Recently, the combination of cell culture with 3D-bioprinting or microfabrication techniques has gained traction as a novel promising approach in organoid studies [21–24]. To overcome speed and scale limitations during kidney organoid induction, Lawlor et al. introduced an automated extrusion-based 3D-bioprinting technique that can rapidly generate large numbers of highly reproducible kidney organoids in size and cellular composition. Most importantly, these bioprinted organoids showed less heterogeneity compared to manually generated organoids, and revealed interesting differences when printed in certain shapes. Improved maturation of the proximal tubular epithelium was observed in bioprinted lines of cells ~12 mm long, but not when the cells were deposited as single dots, highlighting the importance of shape, scale and geometry during *in vitro* kidney differentiation. In addition, this method was successfully used to perform drug-induced toxicity studies [22]. Together with a study by Czerniecki et al. introducing an automated high-throughput screening system for kidney organoid cultivation [25], both studies not only set a successful example of combining cell biology and bioengineering, but offer a route to reduce kidney organoid variability. A feature that will aid reproducibility and inter-experimental comparison. In another innovative adaptation, Fujimoto et al. replaced murine $Six2^{+}$ NPCs with hiPSC-derived NPCs using xenogeneic transplantation to the mouse kidney. Even though hiPSC-derived nephron development did not progress beyond the renal vesicle stage, this work nicely demonstrates the possibility of using human kidney progenitors for regenerative purposes [26].

A fundamental feature of a functioning kidney is their intricate vascularization; a feature that many research groups have extensively studied, including in kidney organoids. Despite significant improvement of organoid

vascularization when transplanted under the kidney capsule of donor animals, *in vitro* vascularization remains the next big challenge. Homan et al. introduced a millifluidic chip culture system studying the environmental cues needed for proper development and optimized vascularization [27]. When kidney organoids were partially embedded in a gelatin-fibrin matrix and continuous fluid flow was applied through the chip, the flow-induced fluid shear stress (FSS) lead to enhanced formation of vascular network. Quantitative analysis further showed a fivefold increase in vessel percent area, and 10-fold increase in average vessel length when cultured under high FSS compared to static or low FSS conditions [27]. Thus, the importance of physical forces for glomerular angiogenesis cannot be understated. Moreover, we anticipate further technical improvements in flow-chip design, overall organoid health — as well patterning and size — are bound to increase.

Ureteric bud and collecting duct organoids

While kidney organoids attract significant attention as a model for nephron-associated injuries and diseases, their application as a model for CD malfunctions remains relatively obscure. In the last two years, several research groups presented new approaches in UB and CD organoid induction and illustrated their practical application. Based on the protocol for UB induction of Mae et al. [28], Tsujimoto et al. functionally tested their NPCs by combining them with hiPSCs-derived UB. This generated improved UB structures and CDs, likely due to the interaction with a more specific, metanephric-like NPC population [19]. Later that year, Mae et al. presented induced UB organoids with a lumen structure and repeat branching morphogenesis, and used these organoids to model multicystic dysplastic kidney (see below). Moreover, the authors established a method for the expansion of a single UB cell into a tip colony [29], highlighting their protocol robustness and similarity between *in vitro*-generated single UB cells.

Highlighting the importance of UB maturation signals, Uchimura et al. showed that the hormones aldosterone and arginine vasopressin (AVP) are critical for promoting differentiation of CD cell types, including both principal cells (PCs) and intercalated cells (ICs) [20]. In a study demonstrating the plasticity of kidney epithelium cells, Howden et al. unveiled the ability of distal nephron cells to adopt a ureteric epithelium (UE) phenotype [30]. Furthermore, cells in these induced UE cultures could be passaged and cryopreserved. More importantly, induced UE cells could provide the necessary stimuli for hiPSC-derived NPs to form segmented nephrons in the absence of Wnt signal. The induced UE could also develop a ureteric stalk identity that was accelerated in the presence of aldosterone and vasopressin, and was used to model polycystic kidney disease (PKD) in $PKHD^{null}$ UE stalk [30].

Another novel protocol for UB induction was introduced by Zeng et al., in which 3D UB organoids could be developed from mouse embryo, human fetal kidney, human embryonic stem cells (ESCs) and hiPSCs. These generated UB organoids were easy to expand into hundreds of homogenious UB structures, mimicked branching morphogenesis and develop into mature CD [31]. The authors went on to show that CRISPR/Cas-induced *Ret/RET* knock out (KO) in mouse or human cells lead to the loss of branching morphogenesis. Although this study illustrated branching morphology of the CD, further optimization of this protocol will be crucial to resemble the comparably high branching capacity of CD reconstituted from mouse embryonic kidneys (Table 1). Taken together, these recent studies have provided new insight in the generation of UB/CD organoids, their maturation, branching morphogenesis and the potency of the cells within them. Future steps will have to recreate an optimal microenvironment that allows for reliable branching at the UB tips whilst simultaneously preventing budding and inducing further patterning of the CD fraction.

Role of stromal cells

Aside from the NPs and UB, a third important group of cells — epithelial parenchymal cells, called stroma — are widely present in the developing kidney. Previous studies have argued for the role of kidney-specific stroma, often overlooked during organoid formation [32,33]. Stromal progenitors (SPs) surround the MM and navigate nephrogenesis by producing GDNF, as well as FAT4, required to suppress the proliferation of NPs. Tanigawa et al. recently identified three stromal cell populations in the embryonic kidney — dorsal, intermediate, and ventral SPs — and provided evidence for the role of FGF9 and BMP4 in their establishment. The authors then combined independently induced mouse ESC-derived NPs, UB with SPs to generate kidney organoids. Resulting organoids showed robust UB branching surrounded by $Six2^{+}$ NPs. Moreover, UB cells with top-stalk identity were shown to express Ret and Wnt7b, resembling embryonic kidneys *in vivo* [34]. This impressive study has highlighted the significant role of the renal stroma in the navigation of nephrogenesis and leads the way for recreating similar SP populations from hiPSC.

Conclusion

Summarizing the progress in kidney organoid induction, improving the maturation and vascularization of kidney organoids as well as integrating bioengineering assays like bioprinting, microchips or automated high-throughput screening systems are steadily providing promising outcomes. We note that current protocols for kidney organoid induction are moving towards more accurate modular formats, separately differentiating major kidney progenitors like NPs UB and SP with

subsequent combination of those cell populations into one organoid. This approach is more time-, labor-, and cost-consuming, however, at this time it might allow for improved morphology and functionality. With advances in creating microenvironments during hiPSC differentiation however (e.g. using microfluidics to create signaling gradients, or microchambers on chips), simultaneous induction of kidney populations could be revisited. With several kidney organoid applications already proving their worth (see below), kidney organoid research is eagerly anticipating future breakthroughs in 3D culture methods that could support bigger, healthier and more matured organoids.

Current state of kidney organoid applications

The ability to generate kidney organoids from hiPSCs transformed the field of kidney-oriented research. Higher-order nephron and CD structures have made kidney organoids a convenient model to study a wide spectrum of kidney injuries *in vitro*, perform toxicity screening, use in personalized drug screening, and to dissect poorly understood mechanisms of kidney morphogenesis and function. Even though hiPSC-derived kidney organoids cannot yet compete with genuine human kidney tissues, recent studies have highlighted the valuable impact this model has had in the understanding and etiology of chronic kidney disease (CKD), infectious diseases and injuries. Moreover, the combination of gene-editing tools with organoid induction has opened the possibility to perform genome-wide screening to identify previously unknown aspects of kidney development and study genetic traits of pathological kidney processes where patient samples have been insufficient or inaccessible.

Kidney disease modeling

CKD is a serious health problem worldwide [35,36] The global toll of CKD is steadily rising due to population growth, aging and lifestyle preferences [37–39]. More than 25% of patients have a hereditary predisposition for CKD [35] and more than 200 gene mutations are predicted to be associated with CKD [36]. In recent years, several research groups have actively used kidney organoid models to address various other kidney abnormalities, including CKD.

Polycystic kidney disease is the main genetic cause of CKD. PKD causes excessive cystogenesis in the kidney, severely damaging tissues and ultimately leading to kidney disfunction. Soon after their introduction kidney organoids become a convenient model for PKD studies. Cruz et al. showed an important role of the microenvironment in the progression of PKD, where adhesion cues, extracellular matrix microenvironment or cAMP levels can significantly affect cystogenesis [40]. Czerniecki et al. further revealed an unexpected role of myosin in PKD progression; when non-muscle Myosin II is inhibited, it causes accelerated cyst formation and growth [25]. In the following years, other studies of PKD organoid models followed. In the study of authosomal recessive PKD (ARPKD), Low et al. used patient hiPSC-derived kidney organoids to show that compound heterozygous mutations in the *Pkhd1* locus are responsible for the progression of ARPKD, and correction of *Pkhd1* locus can rescue the ARPKD phenotype. [41]. In autosomal dominant PKD (ADPKD) organoid models, Shimizu et al. and Kuraoka et al. used patient-derived and gene-edited hiPSCs to reproduce PKD1 mutations-associated renal cyst formation in renal tubules [42] and UB structures [43], respectively.

Gene-edited organoid models have been used to model other kidney diseases as well. A study on fetal multicystic dysplastic kidney disease (MCDK) was introduced by Mae et al., using hiPSC-derived HNF1b$^{+/-}$ UB organoids [29]. Interestingly, MCDK features could be reproduced in these organoids, such as altered apical-basal polarity and reduced expression of MCDK-associated genes. A kidney organoid model of Apolipoprotein L1 (APOL1)-mediated nephropathy was introduced by Liu et al., in which single cells of genome-edited kidney organoids were transcriptomically profiled. Two allele variants of APOL1 (both associated with heightened risk of developing CKD) were studied on the effect they had on the transcriptomic profiles of various kidney cell types, thus providing a novel approach for studying the pathophysiology of nephropathy at the single-cell level [44]. Fabry disease X-linked lysosomal storage disease is caused by a mutation in the galactosidase alpha (GLA) gene, and was modeled by Kim et al., using hiPSC-derived GLA^{KO} kidney organoids [45]. GLA^{KO} organoids showed significant deformation in nephron compartments, increased oxidative stress, apoptosis, and decreased expression of podocyte and tubular markers. Similar to the treatment of Fabry disease patients, this pathological phenotype could be partially rescued by enzyme replacement therapy with recombinant human α-galactosidase A [45].

Nephrotic syndrome (NS) is another frequent kidney disease, caused by mutations in Nephrin1 (*NPHS1*) or Podocin (NPHS2) genes. It is characterized by severe proteinuria due to the impairment of the podocytes' slit diaphragm, where NEPHRIN and PODOCIN proteins are needed for its function. Tanigawa et al. confirmed that NS patient-derived kidney organoids can reproduce NS symptoms, such as impaired NEPHRIN localization and altered slit diaphragm formation. Moreover, correction of the congenital *NPHS1* point mutation in this patient-derived iPSC line could rescue the phenotype [46]. Using patient hiPSC-derived kidney organoids, Ohmori et al. studied two different mutations in NPHS1 and showed that NEPHRIN and PODOCYN were

significantly impaired or undetectable in mutant organoids [47] (Table 2). Majmundar et al. revealed a novel monogenic cause of NS, due to a rare splice variant of the Nitric oxide synthase 1 adaptor protein (NOS1AP) gene. A direct comparison between animal and kidney organoid NOS1AP models further showed affected organoids have podocyte abnormalities similar to those observed in the mouse model [48]. More recently, Jansen et al. claimed to produce a podocyte population that resembled more matured podocytes *in vivo*, using a hybrid organoid induction protocol (based on Takasato et al. [11] and Uchimura et al. [20]). Patient-derived organoids carrying a heterozygous Podocin (*NPHS2*) mutation showed low NPHS2 expression and impaired NPHS1 localization, that could be restored after genetic correction. These rescued organoids further showed increased activity of endothelial growth factors and transcription factors controlling podocyte physiology [49]. Taken together, these recent studies reliably recapitulate disease phenotype, strongly supporting their use in studying kidney diseases, such as NS and other monogenic diseases. We anticipate that with improved organoid maturation, also aspects of late-onset disease, or aging could be modeled *in vitro*.

Another important milestone has been the use of organoids as a model for studying the effects of viral infections of the kidney. Helms et al. used hiPSC-derived kidney organoids to model the effect of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) on kidney cells [50]. SARS-CoV-2 viral variants α, β, γ, κ, and δ were able to replicate in organoids. Moreover, the gene expression patterns in the infected organoids reflected the proteomic signatures of COVID-19 in the urine of critically ill patients. Since ACE2 is the main receptor for SARS-CoV-2 infection [51,52], infection was ameliorated in genome-edited ACE2$^{-/-}$ organoids and blocked *via* treatment with *de novo* designed spike binder peptides (Table 2). In another COVID-19-associated kidney injury study, Jansen et al. showed that SARS-CoV-2 can directly infect kidney cells and subsequently cause fibrosis *via* the activation of multiple profibrotic signaling pathways. Interestingly, this study could form an explanation for the development of CKD in post-COVID-19 patients, as well as Covid-19-associated acute kidney injury (AKI) [53].

Drug and toxicity screening

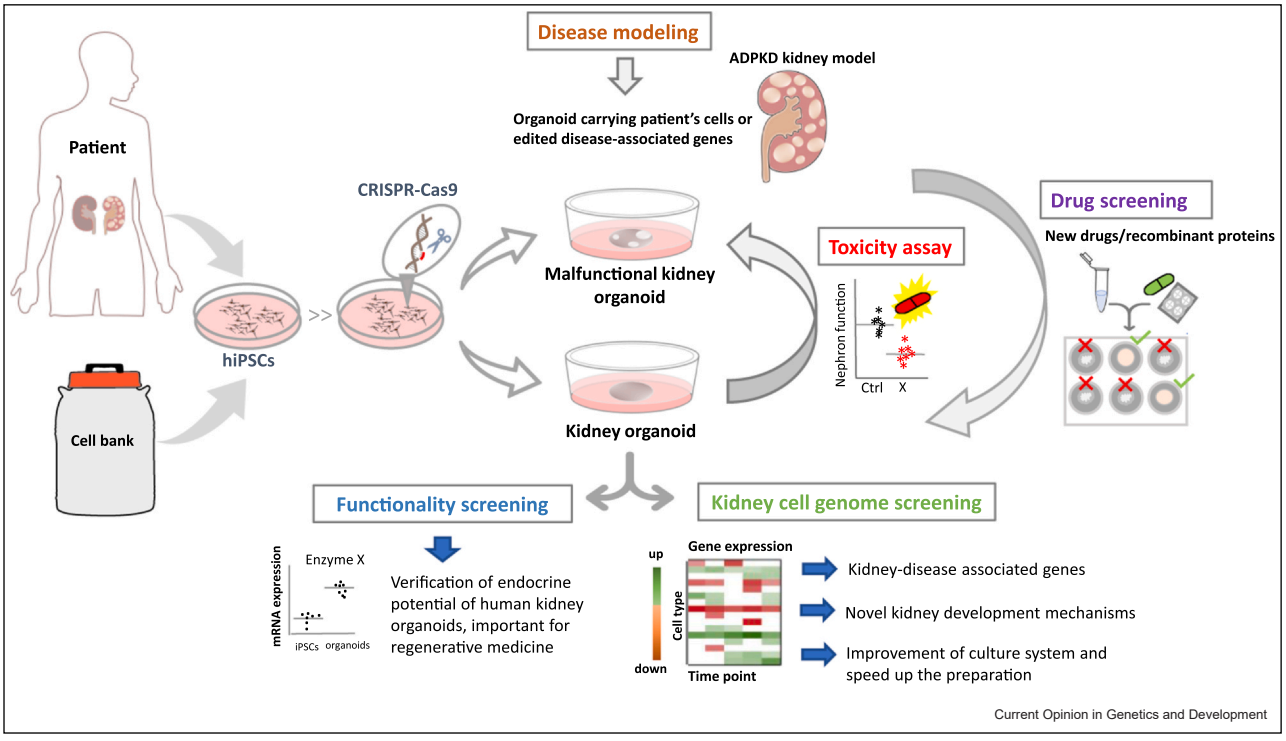
Application of organoids for drug and toxicity screening can solve two problems at once: reduce the usage of test animals and increase the reproducibility of screening results under relatively easier controllable experimental conditions. Kidney organoids have been aptly used by Dvela-Levitt et al., to validate BRD4780, a small molecule used to treat Mucin 1 kidney disease (MKD), caused by a mutation in the MUC1 gene [54]. The authors showed that BRD4780 could clear pathological mucin proteins from kidney organoid cells and reverse proteinopathy. In addition, these kidney organoids responded to BRD4780 treatment in a similar way to a mouse model [54]. Addressing the current COVID-19 pandemic, Monteil et al. and Wysocki et al., both reported the ability of SARS-CoV-2 to infect kidney organoid *via* ACE2 receptors expressed on kidney organoid cells. They further confirmed the effectiveness of human recombinant soluble ACE2 (hrsACE2) as an anti-viral treatment [55] and verified the effectiveness of a short variant of hrsACE2 protein in the treatment of SARS-COV-2-associated kidney injury [56], respectively. Interestingly, a novel albumin-binding domain fused with the ACE2 receptor (ACE2 1–618-ABD) could neutralize SARS-CoV-2 infectivity in human kidney organoids (Table 2).

In parallel with drug effectiveness screenings, kidney organoids have been proven useful for nephrotoxicity assays. Digby et al. used a kidney organoid model to evaluate a cisplatin-induced kidney injury response and identified new biomarkers for AKI [57], whereas Kim et al. looked at the nephrotoxicity in kidney organoids caused by the immunosuppressive agent tacrolimus, a drug commonly used in organ transplantation [58]. The severe toxic effect of tacrolimus on viability, size, and tubulogenic polarity was shown in kidney organoids and revealed a critical role of autophagy in tacrolimus-associated nephrotoxicity. A study carried by Gulieva et al. used kidney organoids to evaluate the toxicity level for different cryoprotectants and addressed the potential of kidney organoids in cryopreservation studies [59] (Table 2). A new creative idea for drug-induced toxicity screening was presented by Lawrence et al. The authors identified the toxic stress marker, HMOX1, that is activated in kidney organoids during oxidative stress response. Organoids derived from a HMOX1-mCherry-reporter hiPSC line could accurately respond to a variety of toxicants in a dose-dependent manner, as the intensity of m-Cherry fluorophore expression was indicative of the amount of stress [60]. This potential ground-breaking screening tool will need to be further validated with a higher number of toxic compounds. Accurate stress-sensing reporter organoids will become very useful in re-evaluating the nephrotoxicity of known and new drugs, as well as providing avenues for large-scale nephrotoxicity screens of compounds present in our everyday environment.

Functionality assay

In addition to blood filtration and reabsorption of water, electrolytes and small molecules from the primitive urine, the kidney also plays an essential role in endocrine regulation (e.g. renin production, vitamin D metabolism, erythropoietin secretion) [61]. In this regard, Shankar et al. demonstrated that the hormone renin was secreted by stromal cells in kidney organoids and that its

Figure 2



Summarized scheme of kidney organoid applications in their current state.

release was increased upon stimulation with forskolin [62] The authors further showed that kidney organoids can metabolize vitamin D into its active form — a feature required for *in vivo* bone mineralization — by enzymes expressed in the proximal tubule cells [63] (Table 2).

Previous animal studies have shown the intrinsic ability of kidney cells to undergo repair after injury; a process controlled by BMP, Wnt and Notch signaling [64,65]. To address this, Gupta et al. recently studied kidney injury and repair in hiPSCs-derived kidney organoids and revealed an intrinsic mechanism of tubular repair [66]. In cisplatin-injured proximal tubules, the authors could show reactivation of proliferation and increased DNA repair. The homology-directed repair (HDR) component, Fanconi anemia complementation group D2 (FANCD2), was found upregulated threefold in tubules shortly after injury. After the repair process was complete (~7 days after cisplatin-induced injury), cells reduced their HDR activity and exited the cell cycle. Furthermore, loss of HDR activity led to the incomplete kidney repair [66].

Thus, with the current genetic and imaging tools available, kidney organoids are set to complement animal models in our understanding of kidney function. More importantly, hiPSC-derived organoids offer us the biological tool to study human tissues during homeostasis, environmental fluctuations, injury, and repair.

Genome-wide screening

More and more studies are using CRISPR/Cas9-edited hiPSCs for kidney organoid induction. While the results are promising, creating and validating gene-edited cell lines remains a time-consuming process [67]. To mitigate this, two research groups applied a new approach in kidney organoid loss-of-function screening by using a doxycycline-inducible Cas9 (iCas9) system integrated into the AAVS1 locus [68,69]. Shamshirgaran et al. directly differentiated gene-edited hiPSCs into kidney organoids, omitting the time-consuming step of clonal line generation, and obtained over 80% editing efficiency in the generation of PKD1 and PKD2 knockout cell populations to recreate their ADPKD model [70]. Ungricht et al. created a functional dataset based on a genome-wide loss-of-function screening in kidney organoids using an iCas9 system. Genetic knockouts were induced at different timepoints during organoid differentiation, from hiPSC to day 14 of culture, thereby covering gene function at different stages of differentiation [71]. Their cell differentiation strategy forms an excellent example on how to gain new insight into the developmental processes that shape early

nephrogenesis, and how to improve modeling and predict the onset of genetic kidney diseases.

Conclusion

From these broad applications conducted over the last three years, we can observe the tremendous impact kidney organoids have had as a research model, and proven to be a robust tool for cell biology and medicine. Specifically, we would like to highlight functionality assays, such as (multi-colored) fluorescent reporter organoids in combination with high-throughput screening, as an underexplored direction in organoid applications. Reporter organoids could be reporting on a wide array from cell identity to function and stress. Moreover, with the year-on-year increase of various chemicals in our environment, kidney organoids could form a cheap and effective way to test potentially harmful compounds, especially those that affect the unborn fetus. Furthermore, research related to the COVID-19 pandemic has shown that viral infections can be studied in organoids, where it might be too difficult to study patients, potentially offering new ways to identify harmful or obscured effects in kidney cells.

Discussion

Since their introduction, the interest in human kidney organoids has continuously grown. In the past, experimental animals have been used extensively to reveal important genetic and molecular factors required for kidney development and function. Currently, experimental animals are still widely used due to the inaccessibility and ethics surrounding human (embryonic) tissues, yet within less than a decade since their introduction, kidney organoids now form a strong contestant set to replace many of them. Moreover, they will be highly valuable in the study of interspecies differences. In addition, multiple studies have now verified kidney organoids as a robust model to study kidney disease and injury, and highlighted their potential in drug and toxicity screening, including personalized screening using patient-derived iPSCs (Figure 2). The combination of kidney organoids with biotechnological techniques, such as (inducible) genome-editing and 3D bioprinting, has further elevated the model as an easy, sufficient and cost-effective way to study the human kidney. Improved protocols for kidney organoid induction are continuously being introduced to challenge the unknowns. Despite visible progress in kidney organoid study, however, several critical milestones remain to be achieved to create a fully functional *in vitro* kidney model. As proper development of these kidney structures ensures their correct function, vascularization, innervation, and cell maturation form some of the next challenges in the field [72,73]. These obstacles can only be partially improved upon after transplantation to host animals, but not to a sufficient degree [74] and innovations in 3D organ culture will be needed to provide more mature kidney tissues *in vitro*. Interexperimental variability [75] and frequent contamination with off-target cell populations [73] will also need to be addressed in the future. Taken together, the human kidney organoid model has grown to become the valuable research tool it is today, already providing us with many practical applications highlighted in this review. However, there is still a long way ahead before reaching their final purpose: creating functional tissues for regenerative medicine applications and ultimately kidney transplantation.

Conflict of interest statement

Nothing declared

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