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## Kidney Disease Modeling with Organoids and Organs-on-Chips

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### Abstract

Kidney disease is a global health crisis affecting more than 850 million people worldwide. In the United States, annual Medicare expenditures for kidney disease and organ failure exceed \$81 billion. Efforts to develop targeted therapeutics are limited by a poor understanding of the molecular mechanisms underlying human kidney disease onset and progression. Additionally, 90% of drug candidates fail in human clinical trials, often due to toxicity and efficacy not accurately predicted in animal models. The advent of *ex vivo* kidney models, such as those engineered from induced pluripotent stem (iPS) cells and organ-on-a-chip (organ-chip) systems have garnered considerable interest owing to their ability to more accurately model tissue development and patient-specific responses and drug toxicity. This review describes recent advances in developing kidney organoids and organ-chips by harnessing iPS cell biology to model human-specific kidney functions and disease states. We also discuss challenges that must be overcome to realize the potential of organoids and organ-chips as dynamic and functional conduits of the human kidney. Achieving these technological advances could revolutionize personalized medicine applications and therapeutic discovery for kidney disease.

### Keywords

kidney disease; *in vitro* models; organoids; organs-on-chips; stem cells; disease modeling

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#### AUTHOR CONTRIBUTIONS

S.M. and J.H. conceived the idea for this review article. S.M. and R.B. wrote the manuscript. R.B. created the figures and illustrations. R.B. was supervised by S.M. All authors revised and edited the manuscript and approved the article for publication.

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## INTRODUCTION

Each human kidney consists of more than one million nephrons that develop from derivatives of the mesoderm germ layer. Kidney development is shaped by dynamic and highly coordinated cellular and molecular programs that emerge from evolutionarily conserved genetic programs and environmental factors (1). Thus, efforts to uncover the mechanisms of nephrogenesis require the ability to reconstruct and deconstruct tissue architecture. Previous studies employed traditional 2D cell culture models comprising immortalized or primary cells and provided a preliminary understanding of kidney cell biology. Given the complex architecture of the kidneys, simple cell culture models or 2D systems often provide a limited understanding of tissue–tissue interactions and interfaces, fluid dynamics, and biophysical forces in the development and function of the organ and how these processes could be harnessed to build functional models of the organ (2). Studies based on animal models have improved our understanding of physiology and pathophysiology, but species-specific differences continue to limit medical advances and extrapolation to humans. For instance, despite encouraging results in mouse models of polycystic kidney disease (PKD), the mechanistic target of rapamycin (mTOR) inhibitor sirolimus showed albuminuria and peripheral edema in human clinical trials, highlighting potential differences in disease mechanisms between mouse models and humans (3). In other cases, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)-mediated coronavirus disease 2019 (COVID-19) affects human organs (including the kidneys) differently than those in other animals (4–7). Thus, studies that rely solely on animal models can significantly limit understanding of human renal tropism. For instance, the expression of human angiotensin-converting enzyme 2 (ACE2) transgene in mice is nonphysiological, often driven by the nonnative keratin 18 (K18) promoter, and its expression varies significantly when compared with the human complex renin-angiotensin-aldosterone system (RAAS) (8). Transgenic rodent models with human ACE2 exhibited SARS-CoV-2 infectivity and pathogenicity (9), but these rodents have not been shown to be naturally susceptible to the virus or exhibit symptoms typically associated with human infection with the same virus. It remains largely unknown whether the animal models of COVID-19 can closely replicate human responses or aid in antiviral therapy discovery.

Furthermore, many over-the-counter medications, including acetaminophen, aspirin, and proton-pump inhibitors, can cause chronic kidney disease (CKD) (10). Kidney injury leads to progressive deterioration of organ function with irreversible loss of terminally differentiated cells, leading to acute kidney injury (AKI) and CKD (11). For reasons including those outlined above, ex vivo models and testing platforms that are more predictive of human responses are still needed.

Human stem cell–derived organoids and organ-on-a-chip (organ-chip) microphysiological systems (12) have attracted significant interest owing to their ability to model human physiological responses. Over the past few decades, a better understanding of extracellular matrix (ECM) biology and the ability to grow spheroids in suspension cultures have enabled the development of miniaturized tissues and complex multicellular models. Sasai and colleagues (13) reported one of the first organoid studies. The authors employed human embryonic stem cells to demonstrate the intrinsic ability of pluripotent stem cells

to respond to extracellular signals and self-assemble in vitro, mimicking molecular and cellular phenotypes of cortical tissue development. The advent of cellular reprogramming technologies inspired subsequent studies aimed at modeling and illuminating in vivo-like cellular responses (14, 15). Several labs have successfully generated kidney organoids from human induced pluripotent stem (iPS) cells, starting with the pioneering works of Osafune and colleagues (16), Nishinakamura and colleagues (17), Bonventre and colleagues (18), and Little and colleagues (19) (Figure 1). These miniaturized kidney models are typically generated by exposing stem cells and their derivatives to exogenous signaling factors that stimulate specific molecular pathways associated with organ development and tissue patterning, which often promote the generation of multicellular structures resembling native kidney tissues. Organoids also allow for tissue-tissue interactions regulated by biochemical and cell-cell signaling (e.g., stroma-tubule cross talk). The convergence of organoid and single-cell transcriptomic technologies offers unprecedented opportunities to study nephrogenesis and disease mechanisms with relevance to humans (20–22).

Microengineering technologies including organ-chip microphysiological systems are also advancing ex vivo modeling of human tissue structure and function as well as patient-centered disease modeling and drug discovery (23). Organ-chips are microfluidic devices with one or more hollow channels lined with cells and tissues cultured under dynamic fluid flow with defined mechanical stretch and strain profiles. Since the landmark development of a lung-on-a-chip model from the Ingber research group (24), we and others have developed several organ-chip models of the human kidney proximal tubule and glomerulus and demonstrated the ability of these engineered systems to recapitulate tissue development, function, and disease phenotypes (25–31). Some of these kidney-on-a-chip models have also been fluidically coupled to develop body-on-a-chip systems for studying interorgan communication and pharmacokinetic and pharmacodynamics analyses (32–34). Because organ-chips can reconstitute organ-level function and help predict drug responses, they serve as excellent platforms for toxicity screening and drug discovery.

In this review, we describe cell culture models for studying kidney biology and disease, including kidney organoids and organ-chips, and discuss their advantages and drawbacks. We also discuss the limitations of organoids and organ-chips and recent progress in addressing them. Finally, we discuss some specific challenges and future directions for the field of kidney tissue engineering and disease modeling.

## DERIVATION OF KIDNEY CELLS FOR DISEASE MODELING

### Primary and Immortalized Cells

Primary kidney cells derived from biopsy samples or discarded tissues offer some clinical relevance. However, limited availability combined with suboptimal culture conditions and donor-to-donor variability reduce the robustness and scalability of assays requiring personalized or patient-specific samples or large numbers of cells. Additionally, static and long-term in vitro cell culture conditions induce molecular-level changes leading to the loss of specialized cell structures such as podocyte arborizations or foot process interdigitations, the apicobasal polarity of tubular epithelial cells, loss of compartmentalized expression of lineage-specific markers (e.g., nephrin), and dedifferentiation into mesenchymal-

myofibroblast-like cells (35). Hence, extensive genotypic and phenotypic characterizations are required to assess the quality of primary cells in routine culture.

To improve access and availability of primary cells, immortalization techniques are used to transform primary cells to produce larger quantities of cells on demand. Specifically, primary cells isolated from human or other animal tissues are usually immortalized through altered expression of oncogenes such as SV40 T long antigen or human papillomavirus 16 (HPV-16) E6/E7 genes, which endow the transformed cells with an ability to undergo long-term proliferation (36). However, expression of oncogenes and oncoproteins elicits molecular changes resulting in aberrant proliferation of terminally differentiated cells, increased expression of inflammatory cytokines, and limited human physiological relevance. Still, immortalized kidney cells have been widely used for studying human kidney biology due to their relative ease of handling, high proliferation rates, and low cost. When cultured over prolonged periods, however, conditionally immortalized cells develop substantial changes in their genetic and epigenetic profiles, including chromosomal rearrangements and copy number variations (37, 38). Such genetic variations can lead to significant batch-to-batch variability and functional outcomes. After several passages, immortalized cells often lose many of their specialized molecular markers and morphological phenotypes, leading to failure in their ability to recapitulate physiological features of human tissues or molecular mechanisms of disease. For example, conditionally immortalized renal tubule cells lose expression of organic anion transporter 1 (OAT1) and OAT3 expression (39), human kidney 2 (HK-2) immortalized proximal tubule epithelial cells can have some multidrug resistance protein 1 (MDR1) efflux pump expression on their apical side but lose their influx transporter expression and function (40), and immortalized human kidney podocytes have dedifferentiated foot processes and fail to express many of the slit diaphragm proteins necessary for proper formation and function of the blood filtration barrier (41, 42). These limitations have profound effects on the successful establishment of assays for mechanistic studies and therapeutic discovery.

### **Induced Pluripotent Stem Cell–Derived Kidney Cells**

Because of their ability to self-renew indefinitely and differentiate into desired cell types (43), human iPS cells hold tremendous promise for overcoming some of the critical limitations of primary and immortalized cells. Some of the key advantages of iPS cells and their derivatives include the unlimited availability of desired cell types and the ability to generate specific tissue cells from any patient. In the past decade, much attention has been given to refining 2D systems and developing organotypic 3D cultures of greater physiological relevance. Under appropriate signaling conditions, iPS cells can undergo proliferation, differentiation, spontaneous self-assembly, and symmetry-breaking events and can form tissue-like structures with architectural complexity mimicking the organization and cell diversity of a developing kidney (17–19, 44). iPS cells have also been directly differentiated into kidney cells including podocytes (27, 28) and tubular cells (45) and coupled with endothelial cells to generate vascularized kidney chips (28, 31). Currently, several protocols exist for the generation of kidney cells and organoids from human iPS cells. Despite these advances, iPS cell–based disease models remain technically challenging, and methods for the directed differentiation of many kidney cell types are yet to be

established. The yield and efficiency of stem cell differentiation methods often vary between cell lines, producing a large percentage of off-target cells that frequently require cell sorting, symmetry-breaking events, manual dissociation to isolate or enrich for desired cell types (46, 47). For example, a recent study by Humphreys and colleagues (48) compared two kidney organoid protocols for the efficiency of differentiation and observed significant variability. Developing optimized disease models require soluble (growth factor) and biophysical (basement membrane) cues to guide tissue differentiation from iPS cells. These media formulations and ECM molecules have been continuously refined to help induce directed differentiation of cells toward a particular kidney lineage. Additionally, organoids typically produce immature or fetal-like cells that may not be appropriate for modeling nephropathies affecting highly specialized cell types such as the collecting duct (CD) cells or mesangial cells of the nephron. Also, recapitulation of the pathophysiology of certain diseases may require cell–cell interactions (e.g., macula densa cells and glomerulus) that are found in vivo but not found or characterized in existing kidney organoid models (49). Thus, new and improved methods for the derivation of functional or specialized kidney cells and organoids could help to significantly advance the field and provide human-relevant models to illuminate pathogenesis and therapeutic target discovery. Below, we describe some of the organoid differentiation methods, direct reprogramming of kidney lineage cells, and organ-chip models, as well as their advantages and disadvantages.

### Cell Reprogramming and Genome Engineering

Direct lineage reprogramming switches one cell type to another without transitioning through a pluripotent state. Direct reprogramming has the potential to reduce the risk of teratoma formation typically associated with pluripotent cells and their poorly differentiated derivatives (50). Previous studies relied on viral vectors for lineage priming, and these vectors could integrate into the genome with unexpected disruption of random genomic loci. Also, direct lineage conversion efficiency by the ectopic overexpression of transcription factors using viruses has been extremely low. While direct reprogramming is yet to be fully explored, some initial efforts have been made to develop reprogrammed kidney cells from somatic cells. For instance, Little and colleagues (51) reprogrammed HK-2 cells into nephron progenitor-like cells using a cocktail of six transcription factors: *SIX1*, *SIX2*, *OSR1*, *EYA1*, *HOXA11*, and *SNAI2*. Lienkamp and colleagues (52) identified the transcription factors *Emx2*, *Hnf1b*, *Hnf4a*, and *Pax8* as regulators of fibroblast reprogramming to renal tubular epithelial cells. Later, Little and colleagues (53) further modified their protocol to an inducible piggyBac transposon system for direct reprogramming of HK-2 cells into nephron progenitor-like cells using only three transcription factors: *SIX1*, *SNAI2*, and *EYA1*. Note that the transdifferentiation of cells is fundamentally different from developmental lineage differentiation pathways, where a transcription factor necessary for direct reprogramming may not mirror events that naturally occur in human development.

With the advent of several genome editing tools such as CRISPR, prime editing, and base editing (54), patient-specific mutations can be introduced or corrected in iPS cell lines to generate isogenic cells (55). Cell or tissue phenotypes can be studied using these genome-engineered models representing the wild-type and mutant cellular phenotypes. Recent

reports demonstrated the utility of this combinatorial approach for studying cystogenesis in iPS cell–derived organoids from patients with PKD (56), apolipoprotein L1 (*APOLI*) nephropathy in kidney organoids derived from iPS cells with *G1* mutation (57), congenital nephrotic syndrome and fibrosis based on mutations in *nephrin* (58) and *podocin* (44) genes, and drug-induced nephrotoxicity (18, 19, 46, 59–61). Small-molecule-based reprogramming methods have become attractive alternative strategies to guide cell fate decisions, as the approach tends to be nonimmunogenic and cost effective.

Advanced techniques, such as trajectory analysis using single-cell technologies or predictive gene regulatory network identification, are required to optimize transcription factor expression efficiency for cellular reprogramming. Reconstruction of the reprogramming path on the basis of pseudotemporal ordering of the cells that have undergone lineage priming (typically identified from single-cell analyses) can help identify unique transit amplifiers and associated transcription factors (Figures 2 and 3) to help improve cell reprogramming efficiencies.

## MINIATURIZED KIDNEY TISSUES

### Organoids

Kidney organoids are miniaturized kidneys that develop from stem cells or transit amplifying progenitors with the ability to self-organize, spatially restrict lineage in a manner similar to in vivo tissue patterning, and recapitulate some functions of the kidney. Human iPS cells are desirable cell sources for developing organoid models due to their pluripotency or self-renewal and differentiation capacity. In vivo, kidney development requires morphogen gradients to enable maturation of transit-amplifying progenitor cells (such as the intermediate mesoderm) (19). Despite the challenges in faithfully recapitulating in vivo processes, significant progress has been made in generating kidney organoids with multiple cell lineages associated with nephrogenesis (17–19, 46) (Figure 1). However, the stochastic nature of organoid differentiation often leads to reproducibility issues and high levels of cellular heterogeneity that include generation of off-target or nonkidney cell types.

In an initial effort to generate metanephric kidney-like cells, Osafune and colleagues (16) differentiated nephron progenitor cells (NPCs) from human iPS cells by activating canonical WNT, bone morphogenic protein 7 (BMP7), and transforming growth factor  $\beta$  (TGF $\beta$ ) signaling pathways. By using a combination of fibroblast growth factor 2 (FGF2) and retinoic acid, Bonventre and colleagues (45) differentiated NPCs from intermediate mesoderm (IM) cells that self-assembled into tubule-like structures. These early endeavors gave insight into how transit-amplifying cells develop from stem cells and undergo cell–cell interactions to form primitive kidney tissues. In 2014, Nishinakamura and colleagues (17) drew inspiration from mouse kidney development to uncover the roles of multiple molecular factors in the differentiation of the ureteric bud from IM cells. The authors showed that prolonged exposure to WNT signaling enables the differentiation of metanephric mesenchyme from epiblast-like cells instead of the ureteric epithelium. By extending this knowledge, these authors developed metanephric mesenchyme by phasic activation of TGF $\beta$ , BMP4, FGF9, and WNT signaling in iPS cells. Coculture models consisting of

the resulting metanephric mesenchyme-like cells and isolated mouse embryonic spinal cord resulted in 3D organoids with some glomerular-like cell clusters (Figure 1).

Around the same time, Little and colleagues (66) reported a method for synchronous induction of embryonic stem cells into a ureteric bud and the metanephric mesenchyme that self-assemble to form kidney-like tissues. To address some of the challenges associated with off-target cell populations, sporadic differentiation, and the lack of properly formed nephron-like structures in kidney organoids, Little and colleagues (19) modified their protocol to help shift the anterior IM cell population toward a more posterior fate. In utero, posterior IM cells descend late from the primitive streak cells and differentiate into the metanephric mesenchyme that gives rise to all known cells of the nephron (67). By following this principle, the authors first activated WNT signaling in iPS cell-derived presomitic mesoderm for extended periods before inducing nephrogenic fate with FGF9 supplementation. This strategy resulted in differentiated organoids exhibiting interconnected nephron-like tissues consisting of key cell types, including podocytes, tubular cells, and CD-like epithelial cells, as well as endothelial and stromal-like cells. In 2015, Bonventre and colleagues (18) sequentially differentiated iPS cells toward posterior primitive streak cells using WNT signaling followed by activation of TGF $\beta$  signaling to induce posterior IM lineage. Subsequent treatment of the posterior IM cells with exogenous FGF9 enabled formation of bulbous-like renal vesicles that spontaneously self-assembled into kidney organoids.

Intriguingly, many of the abovementioned kidney organoid protocols involve activation of WNT, FGF, and BMP signaling, through either endogenous production by the cells (18) or exogenous supplementation (19) of signaling factors. Regardless of the mode of activation for the implicated signaling pathway, the activity and timing of relevant transcription factors regulate the phenotypic characteristics of the resulting differentiated cell lineages. Thus, specific transcription factors can help establish or maintain cell fate and polarity within developing organoids, including formation of anterior versus posterior IM cells (19) or pretubular aggregates (18) versus metanephric mesenchyme, and therefore guide the formation of specific precursor cells that are primed to develop into respective progenies. Because of the complex and often spontaneous positioning of cells in developing organoids, it is possible that differential or inconsistent placement of cells in different batches of organoids can result in differences in the availability, local concentration, and responses to cell-secreted morphogens, leading to the widely observed variability and reproducibility associated with many organoid differentiation protocols. For instance, both the Takasato and Morizane methods for generating kidney organoids involve the induction of IM from primitive streak cells; however, the Takasato method relies on FGF and WNT activation, while the Morizane method employs primarily TGF $\beta$  pathway activation. Because cells can autonomously secrete factors necessary for their differentiation, it is possible that differentiating cells (e.g., primitive streak cells) endogenously produce morphogens in the reported kidney organoid differentiation protocols to enable cell fate transition to the IM lineage. In such situations, exogenous addition of some signaling factors, such as activin A, may be dispensable in IM differentiation. Interestingly, Freedman and colleagues (46) generated kidney organoids by embedding iPS cells in Matrigel<sup>®</sup> followed by activation of WNT signaling. Although the protocol did include exogenous FGF9 (a widely used

morphogen for organoid cultures), it is possible that the differentiating cells secreted FGF isoforms that helped to produce nephron-like cells and structures. Thus, exogenous addition of signaling factors can be dispensable in some cases, as experimentally demonstrated for IM differentiation from mesoderm cells by the Musah et al. (28) protocol for the guided differentiation of mature kidney podocytes from human iPS cells.

Human kidney organoids have undoubtedly expanded the repertoire of tools and technologies used to study organ development, understand disease mechanisms, and screen for potential therapeutics. Still, there are some limitations to this technology. Notably, kidney organoids have molecular and phenotypic characteristics of immature or fetal-like kidney cells and tissues (68–70), which limit their use for studying postnatal cellular and molecular mechanisms of disease and organ-level development or function. Additionally, the diverse cell types in kidney organoids include off-target populations, and the resulting tissues are often not appropriately organized or patterned. For example, vascular cells in stem cell–derived kidney organoids usually fail to form functional blood vessel networks, limiting their ability to transport blood, nutrients, and waste products. Preliminary efforts to overcome this challenge included transplantation of the engineered organoids into animal models (48, 71, 72). For instance, Montserrat and colleagues (73) adapted the Takasato protocol to generate renal vesicles with WNT, TGF $\beta$ , and FGF9 within 8 days. After an additional 8 days of maintenance, these organoids showed some nascent vascular endothelial cells surrounding renal structures but lacked a vascular circuit. Transplantation of the organoids in the chick chorioallantoic membrane led to the invasion of blood vessels *in vivo*. Nishinakamura and colleagues (71) transplanted kidney organoids and human umbilical vein endothelial cells with agarose rods soaked in vascular endothelial growth factor (VEGF) into mice and observed slit-diaphragm-like structures in the podocytes. Freedman and colleagues (74) showed that exogenous addition of VEGF in developing kidney organoids increased the production of endothelial cells, and others showed that modulating WNT pulse increased the percentage of glomerular-like structures with adjacent vasculature (75). Due to the lack of stable and functional microvascular networks, organoids achieve nutrient and gas exchange primarily through passive diffusion. For these reasons, organoids cannot be propagated long term or beyond a specific size without significant cell death and tissue disintegration. To help address this challenge, Lewis and colleagues (76) developed a strategy to culture kidney organoids under fluid flow in microfluidic devices, which significantly improved the development of vasculature along with formation of tubular- and glomerular-like kidney cells and structures.

Furthermore, kidney organoids often require manual dissociation (mechanical treatment), leading to random symmetry-breaking events (77). Enzymatic dissociation of organoids can result in disrupted cell–cell and cell–matrix interactions necessary for proper tissue formation and patterning. Such mechanical and enzymatic treatments can also induce variability between protocols and batches of experiments, since the extent and nature of cell dissociation in spontaneously formed clusters may be challenging to faithfully reproduce with consistency. When dissociated, the resulting smaller cell clusters can be exposed to different levels of oxygen and nutrient gradients that can induce rapid proliferation and dedifferentiation into off-target cells. Variabilities in organoid cellular composition may also arise from the use of poorly defined animal products, such as Matrigel and fetal bovine

serum, the composition of which varies between product lots and batches (46, 76). Such batch-to-batch variability can have serious implications for mechanistic studies and for drug and biomarker discovery. Given the roles of organoids in the recent significant progress in kidney disease modeling using tissue bioengineering, it is likely that the utility of organoids will expand beyond basic biological studies as new technologies continue to help improve the structure and function of stem cell–derived kidney models and their potential for clinical translation.

### Organs-on-Chips

Organ-chip technology integrates engineering and biological principles to create complex in vivo–like tissue and organ models that employ ECMs, multiple cell types, cell–cell interaction, spatiotemporal patterning, and shape-guided tissue morphogenesis. The availability of robust human cell types, including patient-derived primary cells, immortalized cells, and differentiated iPS cells, have increased the utility and practicality of organ-chips when trying to mimic dynamic cell culture conditions and complex tissue phenotypes with clinical implications (23). In addition to recapitulating in vivo–like tissue architecture, the dynamic cell culture environment provided by organ-chip microfluidic platforms permits fluid circulation or perfusion at desired flow rates to model organ-specific blood flow and shear stress (Figure 2).

The ability of microfluidic devices to precisely model fluid flow is an attractive platform for replicating glomerular and tubular functions of the kidney. Podocytes of the kidney glomerulus are highly sensitive to shear stress, and they dynamically change their actin cytoskeletal network when exposed to pulsatile fluid flow (28). Thus, pressure gradients, fluid flow, and shear stress can impact podocyte biology and function. Although the glomerulus chips do not represent the entire structure and function of the native glomerulus, this simplified engineered system aims to model glomerular function and help uncover the mechanisms of disease, including those arising from genetic mutations (Figure 2). Musah et al. (27, 28) developed a glomerulus-on-a-chip device consisting of two parallel fluidic channels separated by a porous, flexible polydimethylsiloxane (PDMS) membrane. Stem cell–derived podocytes were differentiated in situ in the organ-chip device starting with human iPS cell–derived IM cells. The chip was vascularized by culturing glomerular endothelial cells in a fluidic channel adjacent to the epithelial (podocyte) channel. Cell differentiation and tissue formation on the chip were carried out in the presence of cyclic mechanical strain along with simultaneous fluid flow. The resulting cells developed foot processes and tissue structure mimicking the glomerular capillary wall, where blood filtration occurs.

To model drug-induced nephropathy, the authors perfused the vascular channel with Adriamycin<sup>®</sup>, successfully modeling a drug-induced nephropathy characterized by podocyte foot process effacement, epithelial and endothelial cell damage, and proteinuria. In a follow-up study, the Musah research group (31) developed an isogenic glomerulus-on-a-chip using iPS-derived podocytes and endothelial cells from an individual patient, a powerful approach with implications for personalized medicine and mechanistic studies of kidney disease. In a separate study, Qin and colleagues (78) developed a rat

glomerulus-on-a-chip by seeding primary glomeruli microtissue into solidified Matrigel on a PDMS chip, which resulted in cell migration under high glucose condition (away from the primary rat glomerulus). Their study suggested that glomerular cells can undergo an epithelial-to-mesenchymal transition and tend to move away from the tissue-resident basement membrane under diabetic conditions, leading to increased permeability of the glomerular filtration barrier and proteinuria. In another study, Da Sacco and colleagues (41) cocultured primary human endothelial cells and primary human podocytes separated by a collagen type I gel. When exposed to sera obtained from patients with membranous nephropathy, the organ-chips developed albuminuria similar to the clinical disease phenotype. Intriguingly, the sera-induced proteinuria was significantly reduced upon treatment with  $\alpha$ -melanocortin-stimulating hormone. However, these primary podocytes were not terminally differentiated or polarized, and the cell lineage-specific marker expression was not adequately compartmentalized (41). Future work could build on generating more complex microphysiological systems with other glomerular-resident cells directly differentiated from iPS cells to model a given patient's own response to a drug or sera.

In 2013, Jang et al. (26) developed a proximal tubule-on-a-chip with primary human proximal tubular epithelial cells. Under physiological fluid shear stress ( $0.22 \text{ dyn/cm}^2$ ), the primary cells arranged themselves to form polarized columnar cells that expressed Na/K-ATPase on the basolateral side and aquaporin 1 (AQP1) on the apical side. The tubular cells under dynamic fluid flow conditions demonstrated albumin uptake from the apical side, glucose transport through sodium-glucose cotransporter 2 (SGLT2), and high P-glycoprotein efflux transporter activity-mediated cisplatin injury. This injury response was prevented in the presence of the organic cation transporter (OCT2) inhibitor cimetidine. The results of this earlier research influenced several subsequent studies including those reported by Teo and colleagues (79), Pisignano and colleagues (80), Vaidya and colleagues (29), and Verneti et al. (33) using primary human proximal tubular epithelial cells for the development of functional renal tubules with polarized epithelia and compartmentalized receptor expression. Seminal studies on functional renal tubule development by Masereeuw and colleagues (36) demonstrated secretory clearance of albumin-bound uremic toxins and albumin reabsorption. In another study, Kelly and colleagues' (25) model of the human proximal tubule involved cell-remodeled basement membrane under fluid flow, similar to in vivo observation. The resulting tissue chips also exhibited basolateral solute transport, apical solute uptake, ammonia generation, and calcitriol synthesis via calcidiol hydroxylation. By applying 3D-bioprinting technologies, Lewis and colleagues (81) introduced immortalized proximal tubule epithelial cells in a gelatin-fibrinogen ECM hydrogel, maintaining them for more than 2 months. These organ-chips significantly improved phenotypic and functional properties of the tubular cells under fluid flow and demonstrated dose-dependent nephrotoxicity upon treatment with cyclosporin A. Kidney tubules-on-a-chip have also been used to model cell phenotypes associated with the X-linked monogenetic disorders Lowe syndrome and Dent II disease. Smythee and colleagues (82) generated *OCRL* knockout HK-2 cells and modeled tubulopathies commonly observed in Lowe syndrome. Their diseased organ-chips demonstrated loss of megalin-dependent reabsorption function in the *OCRL* knockout organ-chips due to failure in the endocytosis of receptor-associated protein

(RAP) with a concomitant upregulation of fibrotic ECM genes [collagen type I  $\alpha$ 1 chain (*COL1A1*) and *COL5A1*].

The nephron is composed of at least 24 cell types that play a key role in blood filtration, tubular reabsorption, tubular secretion, electrolyte balance, osmolarity regulation, and immune cell regulation (83). So far, only single compartments on the nephron (particularly glomerulus and proximal tubules) have been successfully modeled in vitro. Key constraints in engineering the remaining nephron components are the limited access to functional cells found in the mature nephron and the lack of directed differentiation protocols to generate the necessary cell types from human iPS cells. Adequate access to human nephron cell sources from kidney biopsies and/or novel stem cell differentiation approaches could enable the generation of different types of organ-chips to model nephron physiology and diseases. In the future, multiple interconnected organ-chip models could allow simultaneous evaluation of pharmacological agents and screen for nephrotoxicity or uncover disease mechanisms (Figures 2 and 3). These interconnected organ-chips can be coupled with mechanical actuation systems to generate nephron compartment-specific shear stresses. The interconnected organ-chips could also enable direct monitoring of glomerular ultrafiltration, tubular acid-base equilibria, ion transport, nutrient and organic compound secretion, and reabsorption and help dissect the molecular determinants of kidney function between a diseased and a healthy state.

Basement membrane proteins are required to facilitate cell adhesion in organ-chips. However, most previous reports used either Matrigel or collagen type I. It will be imperative to use basement membrane proteins that are intrinsic to the cell type of interest (e.g., laminin 521 or laminin 511 for podocytes) for cell adhesion instead of proteins that have extensive batch-to-batch variability or lack in vivo relevance (84). Developing directed differentiation methods will also help minimize cell heterogeneity between patient lines and allow the characterization of genetic and epigenetic factors influencing disease etiology and therapeutic outcomes. Furthermore, the microfluidic chip channel dimensions can be engineered to recapitulate hemodynamic forces and cell-cell communication. From an organ-system standpoint, the complexity of the tissue originates from the dynamic interaction between cell types. The ability to robustly mimic these complex cell interactions could help achieve more in vivo-like responses in the engineered in vitro models (Figure 3).

Given the rapid advancements in single-cell technologies, a more holistic systems approach can be undertaken to develop in-depth understanding of drug activity and mechanisms of action for applications in personalized health care. Devising reproducible protocols for the generation of nephron-chips could facilitate (a) prediction of drug metabolism (85) and deciphering the role of autocrine-paracrine effects; (b) evaluation of pharmacokinetic and pharmacodynamic properties of drugs and biologics with direct relevance to a particular patient or population; (c) generation of human iPS lines from patients of different sexes, ages, and ethnicities to develop better understanding of a given drug's mechanism of action and potential side effects (Figure 3); (d) single-cell data integration to analyze cell fate and injury responses before and after therapeutic treatment (Figure 3); (e) CRISPR genome engineering to introduce or correct renal risk variants for therapeutic discovery (Figure 4);

(*f*) discovery of novel disease biomarkers; and (*g*) analysis of protein–drug and drug–drug interactions (e.g., the interaction between antiretrovirals and tuberculosis drugs).

## MODELING KIDNEY FUNCTION

### Glomerulus

Constituents of the renal corpuscle enable the kidney's filtration function and help maintain acid–base equilibria, blood volume, electrolyte balance, erythropoiesis, and calcium and phosphate metabolism. The glomerulus is located within the renal corpuscle, and it consists of a tuft of capillaries encased by specialized epithelial cells called podocytes. The glomerular filtration barrier comprises tripartite components, including the glomerular endothelial cells, the glomerular basement membrane (GBM), and the slit diaphragms formed by the podocytes. The slit diaphragms formed by interdigitating foot processes from neighboring podocytes impart size- and charge-selective molecular filtration (86). The visceral layer of the glomerulus is lined with podocytes, while the parietal layer is lined with parietal epithelial cells. Parietal epithelial cells may have some stem-like properties that allow them to dedifferentiate into glomerular and tubular cells in response to injury (87). However, the role of parietal epithelial cells in kidney injury response is yet to be fully understood.

The glomerular sieve is primarily permeable to small molecules such as water, urea, glucose, and electrolytes and mostly impermeable to large proteins including albumin. The afferent arteriole carries blood to the glomerulus from the systemic circulation, which further branches into small conduits, delivering blood into filtration capillaries. These capillaries converge to form the efferent arteriole. The renal corpuscle also consists of a juxtaglomerular apparatus with extra-glomerular mesangial and granular stromal cells that maintain tubule–glomerular feedback (88). The mesangial cells provide structural support and immune function to the glomerulus and modulate capillary flow and glomerular ultrafiltration surface area (89, 90). Damage to these cells is often characterized by glomerular sclerosis, which highlights the intimate interaction between the mesangium and the glomerular filtration barrier. The juxtaglomerular apparatus and hormonal activity of the distal tubules help regulate blood pressure via the RAAS (49). The juxtamedullary region also drives erythropoiesis by secreting hypoxia-inducible factor (91).

The glomerular filtration barrier is responsible for the selective molecular filtration function of the kidneys. Molecules cross the glomerular filtration barrier as a result of a combination of factors including size (<70 kDa), charge (imparted by the glycocalyx), and transcapillary pressure (92, 93). This phenomenon has been modeled by groups using organ-chip platforms (27, 28, 31). Multiple proteins, including nephrin, podocin, Wilms tumor 1, and short transient receptor potential channel 6 (TRPC6), work in concert to maintain the glomerular filtration barrier (94). Although glomerulus-on-a-chip platforms provide a dynamic microenvironment with cells differentiated from the same iPS cell source and appropriate shear stresses, the glomerulus also consists of other cell types, such as mesangial cells, pericytes, and fibroblasts. Establishing methods for the directed differentiation of these cell types from stem cells and their subsequent integration into microfluidic organ-chip

platforms could improve modeling of the dynamic microenvironment with in vivo–like 3D complexity.

### Proximal Tubule

The ultrafiltrate from the glomerulus enters the proximal tubule, where much of the kidney's reabsorption function occurs. Using cellular and paracellular transport mechanisms, the proximal tubule reabsorbs the glomerular filtrate, the content of which includes water, NaCl, bicarbonates, glucose, and amino acids (95). The specialized proximal tubule epithelial cells have an extensive surface area on their apical side composed of finger-like projections called the brush border with leaky tight junctions between the cells for maximum fluid reabsorption. Apart from a paracellular route, water reabsorption occurs via AQP1 and AQP2. While AQP1 is expressed on the apical and basolateral side of proximal tubules, AQP2 is predominantly expressed in the CD cells (96). Cellular transport by the tubules is coupled to the sodium concentration gradient established by the basolateral Na<sup>+</sup>/K<sup>+</sup>-ATPase. Solute reabsorption, including glucose and inorganic phosphates, is coupled to the sodium cation gradient by the Na<sup>+</sup>-glucose and Na<sup>+</sup>-phosphate cotransporters (97). Many kidney organoid models have studied the expression of sodium transporters such as Na<sup>+</sup>/K<sup>+</sup>-ATPases (81, 98–100) and Na<sup>+</sup>-Cl<sup>-</sup> (SLC12A3) (101) and Na<sup>+</sup>-Pi (SLC34A3) cotransporters (102). Reinke and colleagues (100) demonstrated sodium intracellular uptake in the organoid kidney tubules, indicating the presence of mature proximal tubule epithelial cells in their organoid. In vivo, urine osmolarity can be used to measure water reabsorption. However, such measurements have not been performed with kidney organoids due to the lack of a tubular organization, CDs, and ureter-like structures.

In vivo, cellular chloride reabsorption occurs through the apical exchange of cellular formate when the luminal chloride concentration is high. Once chloride is in the lumen, protons from Na<sup>+</sup>/H<sup>+</sup> exchange associate with formate anions to produce formic acid. Formic acid passively diffuses across the apical membrane of the proximal tubule, while chloride ions exit basolaterally through the same membrane using the K<sup>+</sup>/Cl<sup>-</sup> cotransporters. In autosomal dominant polycystic kidney disease, altered chloride secretion has been implicated in fluid accumulation inside cysts in the distal tubule and CD. Deciphering a mechanism behind altered chloride trafficking and cyst enlargement could help in therapeutic discovery.

The glomerular filtrate contains a significant amount of glucose, which is reabsorbed by SGLT2 (98, 103). Using a tubule-on-a-chip device, Zheng and colleagues (103) developed the first renal vascular–tubular unit to model the proximity of the tissue–tissue interaction under fluid flow and native ECM components. Using this model system, they measured glucose uptake in a renal vascular–tubular unit and performed biochemical analysis of the effluent. In another study, Kelly and colleagues (25) developed a microfluidic proximal tubule unit and observed polarized expression of AQP1, AQP2, and SGLT2 in their organ-chip model. Their model was metabolically competent by its capability for ammoniogenesis and vitamin D biotransformation. When treated with the SGLT2-specific inhibitor dapagliflozin, glucose uptake was significantly reduced, further validating the power of organ-chip systems to model tubular physiology.

The proximal tubule is also equipped with Na<sup>+</sup>-dependent and -independent transport systems that play a crucial role in amino acid reabsorption. For instance, cystine, lysine, arginine, and ornithine are trafficked through SLC3A1 and SLC7A9 channels (104). Additionally, peptide hormones such as parathyroid hormone and trace amounts of large proteins such as albumin found in the filtrate can be reabsorbed via endocytosis and subsequently degraded in acidified endocytic lysosomes within the cell, depending on H<sup>+</sup>-ATPase and chloride channels in the vacuolar membrane (105, 106). Erdmann and colleagues (82) modeled this phenomenon using an organ-chip platform, where impairment of this machinery led to proteinuria and Dent disease.

Proximal tubules can also uptake proteins via endocytosis mediated by LDL receptor–related protein 2 (LRP2) multiligand receptor complex in a size-dependent manner. This phenomenon has been modeled using organoids (46, 107), where larger dextran molecules localize on the apical side of tubular cells (60, 75, 81) or at the intracellular junctions (19, 46, 75, 81). Interestingly, PKD models generated from kidney organoids failed to endocytose dextran after cystogenesis (75), possibly due to loss of membrane protein machinery required for trafficking. Intriguingly, albumin uptake by the proximal tubule cells in kidney organoids could be accelerated or reduced by hydrogen peroxide (108) or leptin (109), possibly by controlling the expression or availability of LRP2 endocytic receptors.

Proximal tubular cells can also biosynthesize 1,25-dihydroxy vitamin D<sub>3</sub> (calcitriol) by hydroxylating 25-hydroxy vitamin D<sub>3</sub> (calcidiol) with the help of cytochrome P450 27B1 (CYP27B1) (110, 111). To model vitamin D<sub>3</sub> metabolism, Himmelfarb and colleagues (25) successfully recapitulated the conversion of calcidiol to quantifiable levels of bioactive calcitriol within their microphysiological system under fluid flow (0.5 μL/min). Recently, Lewis and colleagues (112) developed an organoid-derived proximal tubule-on-a-chip that exhibited significant upregulation of OATs and organic cation transporters (OCTs) under fluid flow. Perfusion of these chips with cisplatin and aristolochic acid-supplemented media led to significant toxicity to the tubular cells. In vivo, OATs/OCTs are localized on the basolateral side of tubular cells and they function by helping to transport organic compounds (113, 114). OAT-mediated transport is driven by an α-ketoglutarate gradient (115), whereas OCT2 (SLC22A2) functions by electrogenic transport (116). Organic anions such as urate, succinate, ketoacids, and protein-bound β-lactam antibiotics such as penicillins and cephalosporins are transported through OAT1 (SLC22A6) (36). Solute trafficking using these receptors can be blocked by treating tubular cells with cimetidine and probenecid (112). When Lewis and colleagues (112) treated their proximal tubule chips with these drugs, toxicity imparted by cisplatin and aristolochic acid was significantly reduced. In another study, Weber et al. (25) used a microphysiological model of the human kidney proximal tubule to show transport of para-aminohippurate through the basolateral side of the tubular cells via OAT1/3 and apical efflux (MRP2/4) transporters. Thus, several groups have successfully demonstrated the utility of organ-chip models in recapitulating several important biological processes of the proximal tubule.

## Collecting Duct

The CD is the terminal component of the nephron, and it determines the final composition of the urine. The CD consists of cortical and medullary regions. The cortical CD comprises tightly packed epithelia with principal and intercalated cells. Principal cells are heterogeneous and plastic on the basis of metabolic demands (117). They can also exist as principal-intercalated hybrid CD cells (118). Saxena et al. (119) reported that uropathogenic *Escherichia coli* infection can switch principal–intercalated hybrid cells into intercalated type A cells. This finding is consistent with the observation that hybrid cells represent a plastic state and can be polarized toward either lineage depending on the environmental stimulus. Intercalated cells can be further divided into type A and type B cells (120), where the former mediate acid secretion and bicarbonate reabsorption and the latter help in bicarbonate secretion and acid reabsorption. The presence of aldosterone in principal cells causes sodium to enter through the apical-side epithelial Na<sup>+</sup> channels (ENaC) and exit through the Na<sup>+</sup>/K<sup>+</sup>-ATPase located on the basolateral side of the cells. Mutations in ENaC increase sodium recovery from the lumen, leading to hypokalemia (Liddle syndrome). In contrast, intercalated cells help in acid–base secretion. Type A cells have a proton pump on their apical side and an anion exchanger pump on the basolateral side. This mode of subcellular localization is reversed in type B cells. Both type A and type B cells work in concert to maintain acid–base equilibria. Medullary CD cells have AQP2 on the apical side and AQP3/4 on the basolateral side. Inspired by the unique arrangement of CD cells, Bonventre and colleagues (121) devised a strategy to differentiate ureteric bud and CD organoids from iPS cells with high efficiency. The authors showed that their CD organoid–resident principal cells with ENaC (amiloride-sensitive) activity exhibited remarkable plasticity to differentiate into intercalated cells with V-type ATPase (bafilomycin-sensitive) activity only via exogenous expression of forkhead box I1 (FOXI1). Using a Transwell system, the authors also demonstrated that FOXI1-induced electrogenic activity of V-type ATPase was pronounced in the apical part of the Ussing chamber, mimicking the acidification function of type A cells while keeping the basal chamber more alkaline (121). When antidiuretic hormones bind to receptors on the basolateral membrane, intracellular signaling cascades through G protein–coupled receptor family proteins become activated, which further triggers downstream adenylyl cyclase. Expression of adenylyl cyclase increases the cellular level of cAMP (cyclic adenosine monophosphate), which possibly explains the initiation of cystogenesis in CD cells. Bonventre and colleagues' CD organoid model or similar strategies could be used to interrogate some of these questions in the future.

## KIDNEY DISEASE MODELS

Kidney diseases—including acute and chronic kidney injuries—are a global health crisis for which targeted therapies are still needed. The lack of viable drug candidates is partly due to the limitations of existing experimental models (primarily animal models and traditional cell culture vessels), which do not accurately model or predict human biological responses and disease mechanisms. Organoids and organ-chips are advanced in vitro platforms for humanized disease modeling that have the potential to illuminate disease mechanisms and help at multiple stages of the drug-discovery pipeline. Organoids and organ-chips are fast-

evolving technologies that have demonstrated significant ability to mimic physiological maturation and function of cells and tissues in vitro. In this section, we discuss some of the seminal studies on various forms of kidney diseases using organoids and organ-chips.

### Pharmacologically Induced Kidney Diseases

One of the essential functions of the kidney is to eliminate toxins from the blood; hence, kidneys are often the prime target for drug or metabolism-induced toxicity. Drug-induced toxicity can lead to AKI onset and progression to CKD. In addition to chemotherapy drugs, many over-the-counter medications are nephrotoxic. Progress in the development of new drug candidates has declined over the past decade, partly because potential drug leads often fail to translate into approved drugs. Unfortunately, drug-induced toxicity is often detected late due to the lack of appropriate preclinical models. This section briefly highlights some of the drug-induced nephropathies that have been observed clinically and modeled using organoid or organ-chip platforms.

**Glomerular compartment.**—The glomerulus is a ball of capillaries wrapped in highly specialized epithelial cells called podocytes. The podocytes interact with the underlying endothelial cells through the GBM. These cells can be directly injured during prolonged treatment with nonsteroidal anti-inflammatory drugs (NSAIDs) (122). NSAIDs can influence the deposition of complement component 3 (C3) and immunoglobulin G (IgG) on the GBM, leading to membranous nephropathy (partly characterized by thickening of the GBM), reduced glomerular filtration rate (GFR), increased vasoconstriction, and ischemia (10, 123). When exposure to NSAIDs causes such severe side effects, a common clinical option is to withdraw patients from the drug. Intriguingly, coculture of podocytes and endothelial cells in an organ-chip platform with serum from patients clinically diagnosed with membranous nephropathy showed significant albuminuria, which could be alleviated by treatment with  $\alpha$ -melanocortin (41). Musah et al. (28) developed a vascularized kidney glomerulus-on-a-chip platform using human iPS cell–derived podocytes and primary glomerular endothelial cells and demonstrated podocyte effacement and detachment in the presence of Adriamycin. Recently, the Musah research group (31) developed a personalized/isogenic version of the glomerulus-on-a-chip system (where all the cellular components were generated from a specific individual’s stem cells). Adriamycin treatment in these organ-chips reduced podocyte viability and disrupted the vasculature and epithelium, resulting in albuminuria. Little and colleagues (124, 125) also showed dose-dependent reduction of podocyte markers in kidney micro-organoids and organoid glomeruli treated with Adriamycin.

Long-term exposure to steroids and street drugs can lead to podocyte foot process effacement and cell detachment from the GBM (126). Bisphosphonate drugs such as pamidronate, often used to treat osteoporosis, cause focal segmental glomerulosclerosis and glomerulonephritis through cytoskeletal reorganization of podocytes (127). Puromycin taken up by podocytes (via SLC29A4) causes dedifferentiation of cells and disrupts the glomerular filtration barrier ensuing proteinuria, as reported by Da Sacco and colleagues (41).

**Tubular compartment.**—Constituents of the glomerular filtrate and other signaling factors in an injured glomerulus can significantly affect the structure and function of the tubules. For example, chemotherapeutic agents such as gentamicin and vancomycin (18, 46) and immunosuppressive agents such as cyclosporine can cause tubular injury (128) as they enter this compartment of the nephron. Additionally, patients suffering from bipolar disorder are often prescribed lithium-based drugs, which can damage the CD cells (129). Several antiretroviral drugs, including acyclovir, indinavir, foscavir, ganciclovir, and atazanavir, and sulfonamide antibiotics are linked to AKI onset (130). SGLT2 inhibitors for type 2 diabetes mellitus can cause tubular oxidative stress (131). Grainger and colleagues (132) observed a cisplatin-mediated AKI-like phenotype in their organoids with a concomitant increase in *CYP2E1* and *KIMI*. In line with this observation, Kaplan and colleagues (133), Schroeter and colleagues (134), Bonventre and colleagues (18), Vaidya and colleagues (29), Lienkamp and colleagues (52), Freedman and colleagues (74), Little and colleagues (19), and Sander and colleagues (59) also observed destruction of tubule organization in kidney organoids upon treatment with cisplatin in a dose-dependent manner. The cisplatin and doxorubicin injury phenotype was similar in the organoid models. For example, Little and colleagues (19) and Montserrat and colleagues (73) found caspase-3 (CASP3) localization in their tubular cells, whereas Bonventre and colleagues (18, 46), Montserrat and colleagues (73), and Freedman and colleagues (74) observed  $\gamma$ H2A histone family member X ( $\gamma$ H2AX) foci formation and kidney injury molecule 1 (KIM-1) expression in the injured tubules with cisplatin. Interestingly, Sander and colleagues (59) observed C-X-C motif chemokine ligand 8 (CXCL8), KIM-1, and  $\gamma$ H2AX expression predominantly in the stromal cells and some tubules. Consistent with Musah and colleagues' observations (28, 31), doxorubicin injury was mostly localized to the glomerular compartment of the organoid, with reduced expression of podocyte lineage genes (124, 125, 135).

Sometimes, nephrotoxic agents can accumulate within tubular cells when xenobiotics block efflux pumps on the cells. The accumulated nephrotoxic compounds can activate xenobiotics that subsequently exacerbate nephrotoxic effects (136). Biotransformation and accumulation of drugs within the cells can further complicate screening for drug candidates and present difficulties in predicting dosage regimen or drug response. Eaton and colleagues (30) developed an integrated liver–kidney chip system using primary hepatic parenchymal and proximal tubular epithelial cells in an ex vivo organ-chip system. The introduction of aristolochic acid (a carcinogenic compound) in the chip demonstrated its nitroreduction capability followed by sulfate conjugation by the hepatic cells. Upon efflux from the liver cells, this compound was internalized by the tubular cells. Over time, this compound accumulated in the tubular cells and biotransformed into a reactive metabolite, sulfonyloxy-aristolactam, bound to DNA and many proteins, leading to significant toxicity.

Drug-induced toxicities constitute a significant cause of drug attrition, leading to withdrawal from preclinical testing and beyond. Although immature with respect to cell phenotype and tissue function, current organoid models can emulate drug-induced toxicities and facilitate drug discovery. Still, mature organoids, organ-chips, assembloids, or organoids-on-chips are needed to study human-specific toxicity mechanisms.

## Viral Infections and Kidney Disease Progression

Viral infections, including those resulting from human immunodeficiency virus (HIV) and SARS-CoV-2, can have a lasting impact on the kidneys, primarily affecting the glomerulus of the nephrons. While these viral infections are known to have multiorgan tropisms, in the following sections, we highlight some of the key studies that investigated viral infection-associated kidney disease using 2D cell culture systems and organoids.

**HIV-associated nephropathy.**—HIV is a lifelong chronic disease where the virus exists in multiple anatomical reservoirs in vivo, including the kidneys. Although current antiretroviral therapies can suppress viral multiplication, cessation of treatment causes a resurgence of the virus. Patients with HIV have a high risk of AKI and CKD, commonly known as HIV-associated nephropathy (HIVAN), which is a collapsing form of focal segmental glomerulosclerosis characterized by proteinuria, tubule dysfunction, and interstitial inflammation (137). HIV patients with a poor cluster of differentiation 4 (CD4) count can also develop immune complex-mediated glomerulonephritis, including IgA nephropathy, membranous nephropathy, membranoproliferative glomerulonephritis, and a lupus-like proliferative glomerulonephritis. These infections can be exacerbated by hepatitis B and C infections or diabetes. HIV patients who also carry a high-risk *APOL1* mutation have an increased risk for HIVAN (137, 138).

It is believed that kidney cells do not express HIV-1 receptors, including CD4, CCR5, and CXCR4, which are expressed on the surface of CD4<sup>+</sup> T cells. However, the kidney interstitium has many immune cells, including the leukocytes, which express some canonical HIV-1 entry markers. HIV-1 can be transferred through cell-to-cell interactions, as commonly observed in retroviruses. Studies by Klotman and colleagues (139) showed the transfer of viral material from HIV-infected T cells to noninfected tubular cells. The generation of effective virions requires HIV-1 genome integration with the host genome. When the authors cultured infected tubular cells with noninfected T cells, they observed a bidirectional exchange of virions (140). There is a significant trade-off between antiretroviral drugs and kidney disease development. For instance, current retroviral therapies, including tenofovir, can significantly reduce GFR, leading to the discontinuation of the drug to restore kidney function (141, 142). Thus, better drug testing platforms could help identify patient- or population-specific drugs to manage viremia and kidney function. However, current kidney organoid models and organ-chips lack immune cell components and might not accurately model viral uptake and processing. Also, disease mechanisms illuminated using kidney organoids will likely mimic the infectious state of immature/developing tissues and not fully capture the mechanisms in adult tissues. Studies of HIVAN using human kidney organoids or organ-chips are limited. However, the technologies are poised for advancing the field, as recently initiated for COVID-19 (discussed below).

**SARS-CoV-2.**—COVID-19 was a global pandemic caused by SARS-CoV-2. Worldwide, the disease affected more than 750 million people and resulted in nearly 7 million deaths. In critically ill COVID-19 patients, AKI was common, affecting 20–40% of patients admitted to intensive care units. AKI from COVID-19 usually results in poor recovery and a high mortality rate. Accumulating evidence suggests that this poor outcome was even worse in

patients with underlying conditions such as coronary atherosclerotic heart disease, CKD, and congestive heart failure (143). Kidney biopsy samples from SARS-CoV-2-infected patients revealed significant glomerular hypertrophy, foot process retraction, chronic sclerosis, crescentic glomerulonephritis, and fibrosis-like phenotype (5). Autopsy samples also showed SARS-CoV-2 nucleocapsid protein expression in the cytoplasm of proximal tubule cells (4). It is widely recognized that the SARS-CoV-2 virus exhibits multiorgan tropism, affecting the kidneys, heart, and lungs.

Subsequent findings by Freedman and colleagues (144) revealed SARS-CoV-2 infection in the proximal tubule of kidney organoids, which was ameliorated in ACE2<sup>-/-</sup> organoids, suggesting a crucial role for the ACE2 receptor in viral entry. The infected organoids also expressed collagen type I, and aberrant production of this ECM protein is associated with fibrosis. Additionally, the infected renal tubules and podocytes within the kidney organoids showed elevated levels of TGFβ signaling, which probably explains the fibrosis-like phenotype observed in the organoids. In general, kidney organoids possess a substantial number of stromal cells which, when injured, have the propensity to deposit matrices implicated in disease onset and progression. The observation that SARS-CoV-2-infected kidney organoids express COL3A1, platelet derived growth factor receptor α (PDGFRA), and paired-like homeodomain 2 (PITX2) in the stromal cells (4) probably explains the appearance of profibrotic behavior in the cells. Additionally, the enrichment of mitogen-activated protein kinase (MAPK), tumor necrosis factor α (TNF-α), and Janus kinase–signal transducer and activator of transcription (JAK-STAT) pathways in infected podocytes and proximal tubule cells in the resulting organoids highlighted the possibility of fibroblast proliferation, myofibroblast deposition, and elevated inflammatory signaling. Since mesenchymal cells demonstrate fibroblast/myofibroblast-like behavior, it is possible that these cells were highly susceptible to SARS-CoV-2 infection and released a significant number of inflammatory cytokines, exacerbating the heterogeneous sclerosis-like phenotype in the organoids. It is important to note that current methods for generating kidney organoids, including the Takasato protocol, produce cells that are developmentally equivalent to the first or second trimester of gestation (48), and the organoid-resident cells are not fully differentiated or functional. Hence, extrapolation of findings from these underdeveloped tissue models to adults could be difficult. Nonetheless, preliminary research in the field is aiding in the identification of signaling molecules that drive kidney injury in response to COVID-19.

In line with the early studies, Garreta et al. (145) differentiated kidney organoids using a diabetic condition involving oscillatory pulses of normal (5 mM) and high (25 mM) glucose. Remarkably, diabetic organoids with SARS-CoV-2 infections had enhanced inflammatory-related processes [e.g., CXCL family genes, interferon (IFN), TNF-α, interleukin 2 (IL-2) and IL-6, and soluble urokinase plasminogen activator receptor (SuPAR)], profibrotic signaling, cell injury, diabetic-related pathways [e.g., CCAAT enhancer binding protein delta (CEBPD) and STAT3], and upregulation of glycolysis-related processes, which confers substantial risk for AKI (145). Podocytopathies, including collapsing glomerulopathies, glomerulosclerosis, and tubular necrosis, are associated with COVID-19-associated nephropathy, in which innate immunity also plays an essential role (146). Chen and colleagues (147) reported the recruitment of macrophages, natural killer

cells, and T cells along with the deposition of complement C5b-9 in necrotic tubules of severe COVID-19 patients. A significant caveat in organoid protocols is the lack of immune cells. Without the appropriate immunogenic factors, the tubular injury does not mimic cytokine storm (marked production of IL-1 $\beta$ , TNF- $\alpha$ , IL-6, IL-7, IL-8, IL-9, and IL-10)–driven AKI. While Jansen et al. (4) observed viral particles in podocytes and proximal tubular cells, Garreta et al. (145) found them mostly in the proximal tubule cells of kidney organoids. These inconsistencies could be attributed to inherent variability in organoid differentiation, including the starting cell lines, different genotypes, or batch-to-batch variations, or even to differences in spatiotemporal cues and patterning. COVID-19 patients can also experience hypoxemia because of acute respiratory distress (pneumonia), leading to tubulopathies (148). Such respiratory disorders can lead to hemodynamic disturbances in the vasculature, altered vascular permeability, and subsequent glomerular-tuft collapse and podocyte effacement (149). Future work can use directed differentiation methods to generate mature and functional cell types (podocytes, proximal tubule cells, endothelial cells, and lung alveolar cells) and interface them in multiple interconnected organ-chip platforms to model interorgan disease mechanisms in cardiopulmonary and renal tropism of SARS-CoV-2 infection.

Recently, Musah and colleagues (7) set out to investigate the mechanism of SARS-CoV-2 uptake in podocytes. The authors discovered that iPS cell–derived podocytes express many spike-interacting and processing factors that might facilitate SARS-CoV-2 entry into the cells. Although the expression of the viral processing factor transmembrane serine protease 2 (TMPRSS2) was low in podocytes, the cells expressed significant amounts of cathepsin L (CTSL)—an alternate processing factor. Transcriptome analyses and antibody-mediated receptor-blocking experiments revealed that podocytes utilize both ACE2 and basigin (BSG)/CD147 to bind and internalize SARS-CoV-2 viral particles and use CTSL to process the virus. Treatment of human iPS cell–derived podocytes with live SARS-CoV-2 showed viral uptake and plaque formation, which correlated with enhanced viral replication inside podocytes. Phenotypically, the infected podocytes lost their cytoarchitecture, demonstrated mislocalization of lineage-specific proteins, and showed hypertrophy, possibly reflecting the heterogeneous adaptive injury responses. This observation is in line with a report from Penninger and colleagues (150), who showed that human recombinant soluble ACE2 protects human kidney organoids from SARS-CoV-2 infection. Montserrat and colleagues (145) also made similar observations in their diabetic kidney organoid model, paralleling the findings by Musah and colleagues (7).

## CHALLENGES AND FUTURE DIRECTIONS

### Addressing Cell Maturation and Function

Organoids and organ-chips are fundamentally different, yet complementary, techniques to model tissue development, disease phenotypes, and drug pharmacokinetics. While organoid differentiation protocols are based on spontaneous self-organization using soluble and insoluble signaling factors, organ-chips rely on precision-engineered constructs with specific structures and compartments to model tissue–tissue interfaces within a dynamic cell culture environment. Still, realizing the full potential of organoids and organ-chips

relies on advances in and integration with the following: (a) developmental bioengineering, i.e., supplying developmental cues under physiologically relevant conditions to allow stem cells to differentiate into desired cell types (e.g., mesoderm germ layer or IM cells, which serve as precursor cells for the kidney) to mimic kidney development; (b) biomimetic materials, i.e., surface functionalization with proteins usually expressed or secreted by the developing tissue (e.g., laminin 521 for podocytes) or using a generic basement membrane rich in multiple ECM proteins (e.g., Matrigel); and (c) nutrient supply and biophysical microenvironment, i.e., replenishing media supplemented with soluble growth factors to specifically activate or inhibit signaling pathways in the developing tissue (e.g., BMP7 signaling in intermediate mesoderm cells) along with cyclic mechanical strain to mimic in vivo biophysical cues and help maintain the terminally differentiated cell phenotype (e.g., podocytes in a glomerulus-on-a-chip and tubule epithelial cells on a proximal tubule-on-a-chip).

The principle of organ-chips requires researchers to have access to specific cell types capable of modeling a desired organ's physiology and pathophysiology. However, there are several limitations of organ-chips. For instance, researchers do not always have adequate access to desired cells to build organ-chips. Organ-chips also require knowledge of microfabrication techniques and at least some basic knowledge of microfluidics and fluid dynamics. In contrast, organoids intrinsically generate a highly heterogeneous cell mass containing organ-specific cell types and a considerable number of off-target or irrelevant cell types. This challenge arises from the fact that organoid differentiation is complex, often leading to the generation of immature tissues that are developmentally equivalent to first- or second-trimester kidney cells and tissues. This is partly due to chaotic differentiation programs, lack of supporting cells such as pericytes and fibroblasts in some cases, absence of a functional vasculature, and high heterogeneity and inadequate cell–cell signaling. Prolonged in vitro culture of organoids leads to loss of structural integrity, and the cells located at the center of the organoids fail to properly exchange nutrients and gas, leading to pronounced cell death (60).

Understanding the kinetics of molecular exchange during self-assembly and simultaneously mapping cell fate in developing organoids could help minimize random differentiation. Kidney organoids usually have a high percentage of neurons, myocytes, and melanoma cells (48, 72). Hence, supplementing the media with neurogenic or myogenic differentiation inhibitors may increase the proportion of kidney lineage cells in the organoids. Cells often require scaffold proteins or matrices for adhesion and differentiation. With time, these scaffolds are remodeled by the cells. Most differentiation protocols require Geltrex™ or Matrigel, which are poorly characterized. Some protocols require collagen type I as a supporting matrix, which has been implicated in fibrosis (151). Hence, careful selection and design of the ECM are necessary for robust culture and differentiation of organ-specific cell types (e.g., full-length laminin 521 or 511 for kidney podocyte differentiation and culture). Alternatively, synthetic hydrogels of varying stiffnesses, viscoelasticity, and architecture can be synthesized to mimic the native tissue's microenvironment (152–155). Furthermore, differentiating organoids can be coupled with the organ-chip platform to harness the dynamic cell culture environment the technology offers to help form mature or functional organoids- or assembloids-on-chips. These technologies can be combined to enhance in

vivo-like function and disease phenotype. Organoids-on-chips can also help identify optimal culture conditions for in vitro organoid expansion, vascular branching, and long-term propagation (76).

The current tissue-engineered constructs lack sex specificity owing to limited cell sources. Organoids and organ-chips provide a unique opportunity to study sex-based differences and mechanisms contributing to sexual dimorphisms in the kidneys. For instance, McMahon and colleagues (156) observed sex bias in tubular receptor expression of OATs and amino acid transporters in mice. However, these have not been validated in human kidney organoids or organ-chip models. Generating iPS cells from different sexes will enable the development of targeted treatments. Exogenous supplementation of sex hormones in organoids and organ-chips differentiated from cells derived from the opposite sex could reveal disparities in disease progression or risks associated with transgender populations (157). Inclusion of sexual diversity in cell types and tissue models will also provide a foundation for a deeper understanding of the role of diverse ethnic backgrounds in disease progression, for example, uncovering *APOL1* nephropathy and comorbidities in women versus men from similar or distinct ancestries, and help personalize drug discovery and repurposing.

### Structure to Dictate Function

The nephron comprises a biological structure hierarchy that delineates how distinct cells form, organize, and function. However, how these intrinsically complex structures originate, diversify, and segment is still unknown. Conventional murine and transgenic models have been an excellent resource for elucidating mechanisms of kidney development. However, murine models have significant limitations owing to their inherent developmental differences and the timeline, architecture, and function of their kidneys compared with humans (158).

An essential requirement of organ engineering is identifying design principles on the basis of a reductionist analysis of the nephron. Individual functional units of the nephron can be analyzed to determine key cell types and their organization, function, and organ-specific biochemical environment. Advanced model systems such as organ-chips have been utilized to emulate kidney function using only the relevant cell types (23, 25, 28). Such tissue-chips can also integrate nephron function into a coherent, multiscale map that illustrates the complexities of nephron development and function (Figure 2). Since the kidney develops from the mesoderm germ layer and its IM, directed differentiation of nephron cell types from the IM could allow the coupling of multiple parts of the nephron in an organ-chip platform. Microfluidic devices contain multiple fluidic compartments or channels separated by semipermeable membranes. The fluidic channels can be engineered with various dimensions to mimic in vivo tissue niche and geometry. After populating the channels with transit-amplifying IM cells, defined serum-free media can be perfused through the respective channels. Maintaining a microfluidic gradient through perfusion will allow terminal differentiation and tissue-tissue coupling. Simultaneous application of mechanical actuation could promote the differentiation of more mature cell types sensitive to fluid flow-mediated shear stress. Tissue vascularization also plays an essential role in organ maturation. For instance, Musah and colleagues (31) coupled iPS cell-derived podocytes and endothelial cells to recapitulate patient-specific glomerular capillary wall development

and function. Similarly, complementary interactions between tissues (ureteric epithelial and distal nephron) can enable structured tissue assembly with the programmable spatial arrangement (47).

To generate mature and well-organized kidney organoids, guided organization of iPS cells and their differentiated derivatives is necessary. Organoid differentiation can be coupled with gradients of morphogens and inductive cues, diverse substrate geometry of varying stiffnesses, or modulation of gene regulatory networks (e.g., use of CRISPR activation or inhibition) for epigenetic remodeling. The role of biophysical cues and tissue-resident forces has yet to be evaluated in kidney organoids. Hence, combining organoids and organ-chips with orthogonal or synergistic engineering of niche factors, including convoluted channels for tubules, varying the microchannel diameter for different tubular compartments, interfacing cells with a functional vasculature, and varying the fluid flow rate and pressure in separate compartments of the nephron, will help reconstruct high-order, high-fidelity, mesoscale kidney organoids or assembloids. It is conceivable that such systems could recapitulate genetic risk variants and drug-associated nephropathies and replicate in vivo human clinical data.

### Precision Medicine and Patient-Specific Therapeutic Discovery

Antibiotics and immunosuppressive drugs have a profound effect on the kidney tubules. Even standard antiretrovirals and aminoglycoside antibiotics can cause proteinuria and hematuria, leading to AKI (123). It is widely recognized that animal models often fail to predict human nephrotoxicity, which presents a major roadblock to drug development. Many failed clinical trials provided a stark reminder that drug activity varies significantly across populations and species. The failures in drug discovery based on one-for-all clinical trial designs have financially battered the health care system while worsening health care costs and the social burden of AKI/CKD. Additionally, when patients are simultaneously prescribed multiple medications, it becomes difficult to predict outcomes because different medicines can interact with each other, compete for detoxification, or be biotransformed into harmful xenobiotics potentiating toxicity. Thus, the discovery of patient-specific targeted therapies using organoids and organ-chips has gained considerable interest given their potential to address some of these challenges.

The convergence of organoids and organ-chips with single-cell analysis, in situ sequencing, and high-multiplex imaging mass cytometry is providing unprecedented opportunities to study the molecular and cellular mechanisms of tissue development and disease and to advance precision medicine applications. These techniques can also provide positional information about proteins and other biomolecules within organoids. These technologies can be extrapolated to understand the biochemical signature for each cell within patient-specific models under defined environmental or genetic conditions. Generation of reporter cell lines that mark genes associated with kidney development and disease [paired box 2 (*PAX2*) in oligomeganephronia or spalt-like transcription factor 1 (*SALL1*) in renal hypoplasia] or markers that are expressed in the principal or intercalated cells of the CDs would aid in the optimization of differentiation conditions and help unravel novel approaches to combat kidney diseases. The urgent need to develop personalized preclinical models

will become even more critical as pharmaceutical companies move toward personalized therapies. Recently, the US Congress passed the FDA Modernization Act 2.0, which authorizes the use of organ-chips as an alternative to animal models to accelerate drug discovery. As advancements are made to generate functional conduits of the human kidneys, microphysiological systems including organoids and organ-chips could someday become viable therapeutic options for personalized kidney care.

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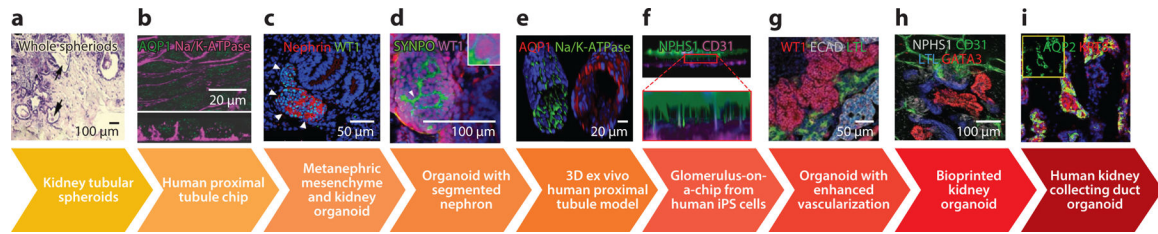
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**Figure 1.**

Timeline of seminal protocols for developing in vitro kidney models (spheroids, tubuloids, organoids, and organs-on-chips) from either primary cells or pluripotent/embryonic stem cells. (a) Dekel and colleagues (159) developed spheroids from primary hKEpCs via self-assembly. The spheroids expressed some renal development and stemness markers and reconstituted the tubular epithelia upon chick chorioallantoic membrane grafting. (b) Ingber and colleagues (26) cultured hKEpCs in a microfluidic system that mimicked critical functions of the kidney proximal tubule. Under physiological fluid flow, the cells underwent polarization and demonstrated primary cilia formation along with albumin transport, glucose reabsorption, and brush border alkaline phosphatase activity mimicking in vivo responses. (c) Later, Taguchi et al. (17) identified cues necessary for preferential induction of kidney metanephric mesenchyme and developed kidney organoids via self-assembly and phasic WNT stimulation. These organoids contained nephron-like structures, including glomeruli with podocytes, renal tubules with proximal and distal regions, and lumina. (d) Using human iPS cells, Morizane et al. (18), Takasato et al. (19), and Freedman et al. (46) identified cues necessary for the induction of nephron-like structures with cell populations showing some characteristics of proximal tubules, podocytes, and endothelium. (e) In 2016, Himmelfarb and colleagues (25) developed a 3D microphysiological kidney proximal tubule with proximal tubular epithelial cells that demonstrated basolateral solute transport, apical solute uptake, and intracellular enzymatic function in a physiologically relevant manner. These cells exhibited polarized expression of AQP1, AQP2, and SGLT2 and could be maintained for more than 28 days. This model also demonstrated elevated glucose reabsorption, ammoniogenesis, and vitamin D biotransformation. (f) Musah et al. (27, 28) developed the first method for mature podocyte differentiation from human iPS cells and generated a vascularized human kidney glomerulus chip that recapitulated glomerular tissue–tissue interfaces and the kidney’s selective molecular filtration of albumin and inulin. These chips also mimicked Adriamycin<sup>®</sup>-induced albuminuria and podocyte injury. (g) In 2019, Garreta et al. (73) modified the Takasato protocol to differentiate kidney organoids from human iPS cells. Exposure to the 3D microenvironment led to strong transcriptional congruence with second-trimester human fetal kidneys. At the same time, Homan et al. (76) developed an organoid-chip platform and demonstrated that organoids cultured under fluid flow had more mature proximal and distal compartments with enhanced cellular polarity (tubules), enlarged glomerulus, and adult gene expression. (h) In the same year, Lawlor et al. (135) developed an extrusion-based 3D cellular bioprinting method to efficiently generate kidney tissue sheets in a 6- or 96-well plate with a higher nephron number per organoid. The resulting tissues enabled aminoglycoside toxicity testing. (i) Shi et al. (121) sequentially balanced the MEK/ERK pathway to develop collecting duct organoids. Their organoid-resident principal cells exhibited remarkable plasticity to toggle between principal

and intercalated cells via exogenous expression of FOXI1. Abbreviations: AQP, aquaporin; FOX, forkhead box; hKEpC, human kidney epithelial cell; iPS, induced pluripotent stem; MEK/ERK, mitogen-activated protein kinase kinase/extracellular signal-regulated kinase; SGLT, sodium-glucose cotransporter.

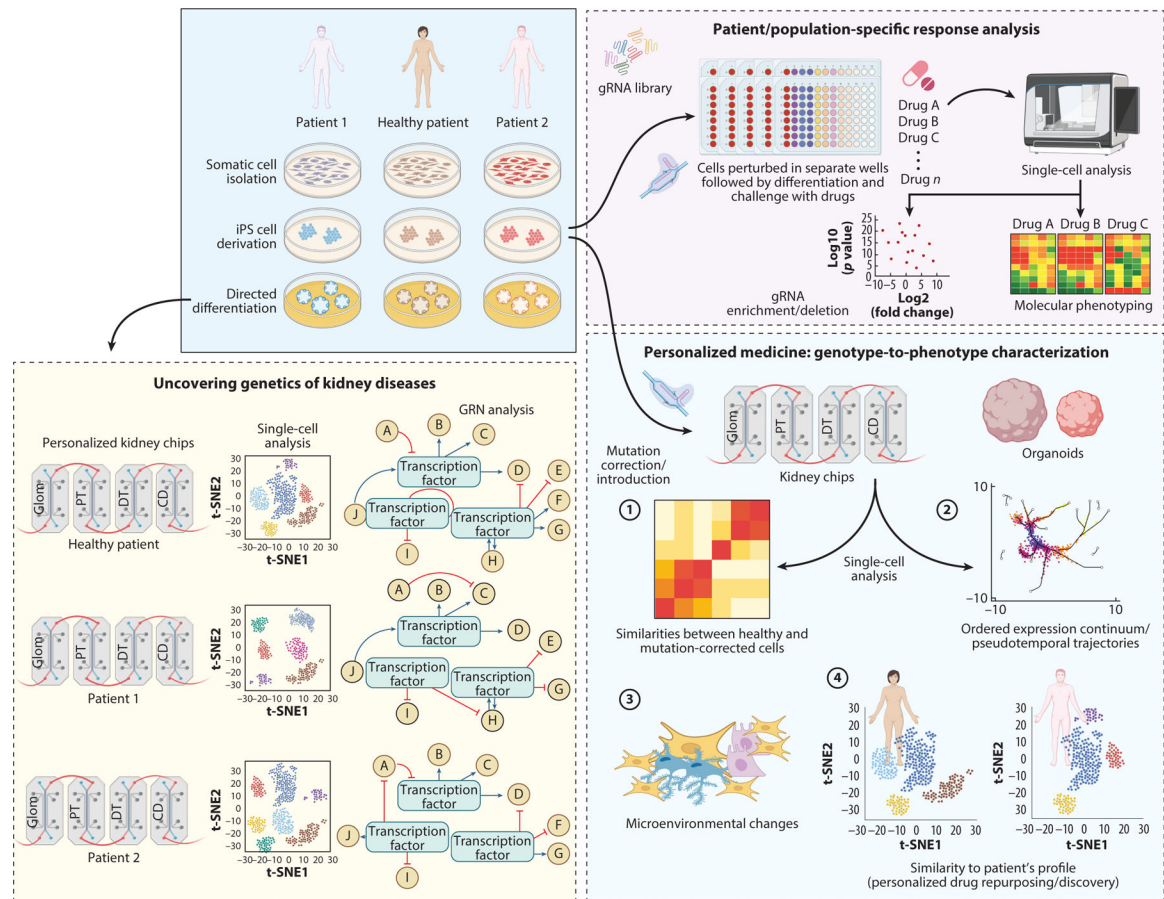
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**Figure 3.**

Advances in organ-on-a-chip systems enable high-fidelity, patient-specific models of the kidney with the potential for studying different therapeutic strategies for translational efficacy, safety testing, uncovering mechanisms of genetic diseases, and personalized medicine. For instance, somatic cells can be isolated from healthy and diseased individuals, followed by iPS cell generation and directed differentiation, which require stepwise germ-layer specification (mesoderm for the derivation of kidney tissue lineages), followed by induction and maturation by culturing with specific growth and signaling factors to obtain the nephron-specific cell types. These cells can be interfaced with endothelial cells generated from the same parent iPS cell line to engineer patient-specific organ-chip platforms. High-throughput single-cell analysis can be carried out on samples generated with the organ-chips to help uncover novel cell states, biased lineage trajectories in the presence of mutations, and GRNs for mechanistic insights. Differentiated cell types can be subjected to CRISPR screen experiments to study pathogenicity of specific gene variants using patient-derived models. Single-cell protein and RNA profiles could reveal the regulation of genes and programs involved in pathogenesis. Distinct RNA profiles across disease and healthy chips could highlight the regulation of key signaling pathways whose genetic perturbation confers an altered molecular phenotype. Diseased chips or organoids can be corrected using CRISPR or prime editing genome engineering methods to examine whether correcting specific genetic mutations can return the cell or tissue phenotype and

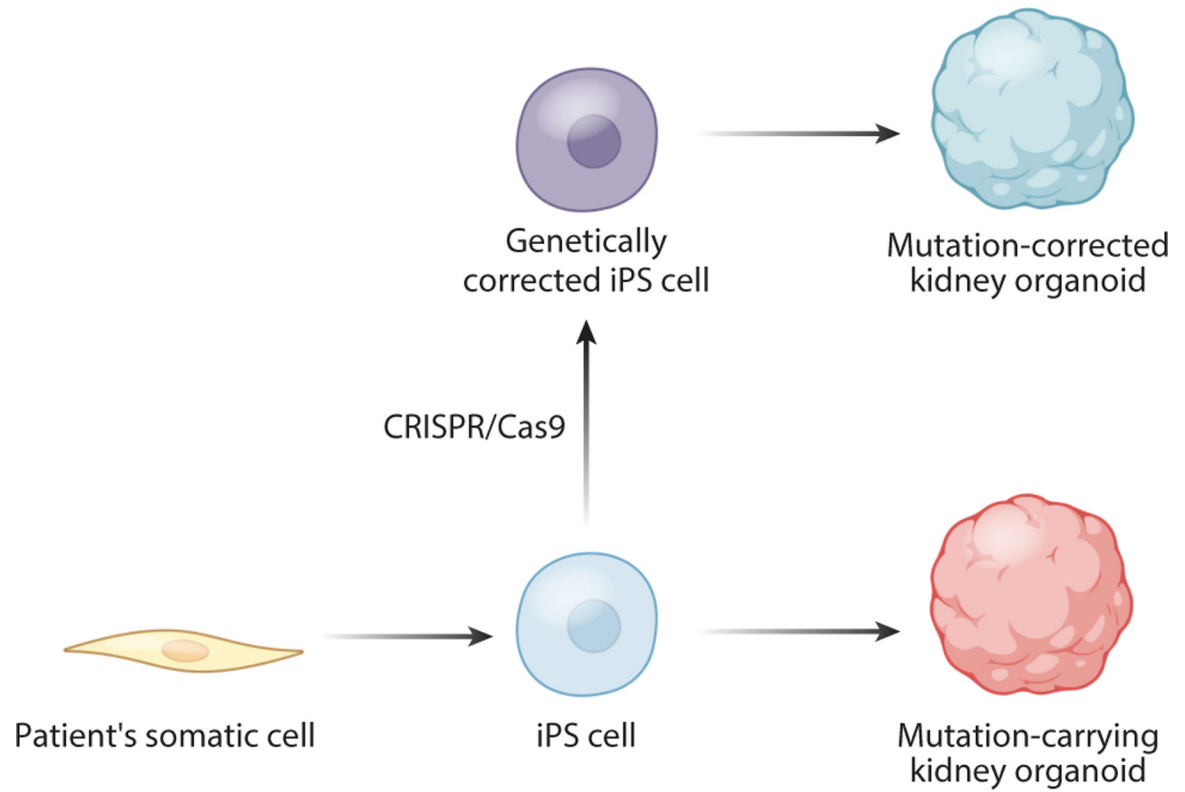
function to the healthy state. Abbreviations: CD, collecting duct; DT, distal tubule; Glom, glomerulus; GRN, gene regulatory network; gRNA, guide RNA; iPS, induced pluripotent stem; PT, proximal tubule; t-SNE, t-distributed stochastic neighbor embedding.

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**Figure 4.**

Overview of the strategy for generating isogenic systems for personalized disease modeling applications. Genetic mutations can be corrected and compared with healthy controls for similarity profiling between healthy and corrected cell states. Abbreviations: Cas9, CRISPR-associated protein 9; CRISPR, clustered regularly interspaced palindromic repeats; iPS, induced pluripotent stem.