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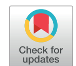
Engineering physiological environments to advance kidney organoid models from human pluripotent stem cells



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Abstract

During embryogenesis, the mammalian kidney arises because of reciprocal interactions between the ureteric bud (UB) and the metanephric mesenchyme (MM), driving UB branching and nephron induction. These morphogenetic processes involve a series of cellular rearrangements that are tightly controlled by gene regulatory networks and signaling cascades. Here, we discuss how kidney developmental studies have informed the definition of procedures to obtain kidney organoids from human pluripotent stem cells (hPSCs). Moreover, bioengineering techniques have emerged as potential solutions to externally impose controlled microenvironments for organoid generation from hPSCs. Next, we summarize some of these advances with major focus On recent works merging hPSC-derived kidney organoids (hPSC-kidney organoids) with organ-on-chip to develop robust models for drug discovery and disease modeling applications. We foresee that, in the near future, coupling of different organoid models through bioengineering approaches will help advancing to recreate organ-to-organ crosstalk to increase our understanding on kidney disease progression in the human context and search for new therapeutics.

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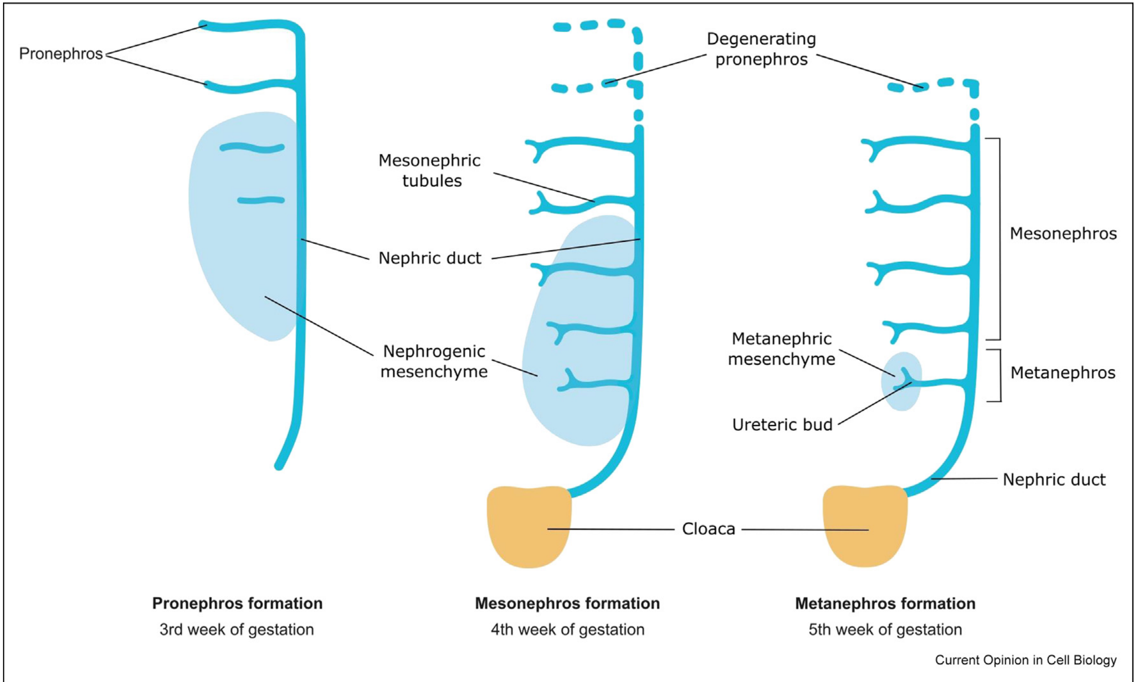
Introduction

Kidney is the most important organ for water homeostasis and waste excretion in our body. It performs central physiological functions for homeostasis: it filters the metabolic waste out of the circulation, regulates body fluid balances, and acts as an immune regulator and modulator of cardiovascular physiology [1]. The adult human kidney comprises over thirty different cell types and more than one million of intricately segmented and patterned compartmentalized epithelial structures, the so-called nephrons, which are the functional units of the kidney [2,3]. Each nephron is differentiated into different regions, mainly the Bowman's capsule that encloses the glomerulus, and the renal tubule, having both different anatomical features and physiological roles. In mammals, the permanent or metanephric kidney is the last of three excretory organs (pronephros, mesonephros, and metanephros) to form (Figure 1) [4]. During embryonic development, the mammalian kidney originates from the intermediate mesoderm (IM) that is formed from a structure called primitive streak (PS) [4]. The anterior IM (AIM) further elongates generating the nephric duct (ND) that forms the ureteric bud (UB), whereas the posterior IM (PIM) differentiates to form the metanephric mesenchyme (MM) [5]. Reciprocal interactions between the UB and the MM result in nephron induction. In this process, a subset of cells within the mesenchyme amalgamates as condensates (Cap Mesenchyme-CM) acquiring an epithelial phenotype through mesenchymal–epithelial transition (MET). While UB branches, nephrons are induced at each UB tip. Branching events lead to the formation of the collecting system (including collecting ducts, renal pelvis, ureter, and bladder trigone). At the same time, the epithelial cells formed upon MET self-organize into

Figure 1



Urogenital system development in humans, culminating in the formation of the metanephric mesenchyme and ureteric bud. Following the development and subsequent regression of the pronephros, the mesonephros emerges caudally, giving rise to mesonephric tubules. These tubules, readily irrigated with blood flow, temporally contribute to urine production and drainage into the nephric duct, ultimately reaching the cloaca. Nevertheless, the mesonephric structures eventually degenerate upon the formation of the metanephros, which comprises the metanephric mesenchyme and ureteric bud.

polarized epithelial spheres (renal vesicle-RV), transitioning into a comma-shape body, and then an S-shape body, a process called nephron patterning (briefly described in Box 1. *Early mammalian kidney developmental events*) [4]. Key molecular pathways mediating the formation of IM and subsequent interactions between the MM and the UB have been identified taking advantage of different animal models being the mouse the gold standard to understand metanephric kidney development [6]. For example, nephron progenitor cell (NPC) renewal and differentiation are regulated by WNT9b/β-catenin signaling, which is controlled by signals from the UB and cortical stroma. Conversely, GDNF/RET/glial cell line-derived neurotrophic factor family receptor α1 (GFRα1) signaling is crucial during primary bud formation and UB branching [5,6].

Since the discovery of hPSC, including embryonic stem cells (ESCs) [7] and induced pluripotent stem cells (iPSCs) [8], both sources have been essential to increase our knowledge of the cellular processes and signaling pathways guiding tissue differentiation and morphogenesis. Exploiting the intrinsic capacity of hPSCs to self-organize in response to renal inductive signals, together with the application of organotypic three-dimensional (3D) culture conditions have resulted in procedures to generate kidney organoids which nowadays are envisioned as powerful tools for disease modeling, drug screening, drug discovery, and personalized medicine.

As hPSC-organoid technology opens new perspectives for research in the fields of biomedicine and tissue engineering, several shortcomings related to their limited matureness and life span still preclude the full realization of these in vitro models for disease modeling and regenerative applications. In this review, we will evaluate these findings and provide an overview on the impact of bioengineering advances to transform hPSC-kidney organoid research in the next years.

Mirroring embryonic cellular interactions for kidney organoid generation and maturation

Early studies for the generation of cells from the renal lineage took advantage of mouse PSCs to mirror the biochemical cues driving kidney development in the mouse embryo [9–13]. Later, other approaches did take advantage of 2D monolayer cultures from both human and mouse PSCs to derive renal progenitor cells that showed renal differentiation potential making use of in vitro kidney re-aggregation assay [14] or by in vivo transplantation [15]. These first studies revealed a role for Wnt, activin, bone morphogenetic protein (BMP),

Box 1. Early mammalian kidney developmental events.

During mammalian kidney morphogenesis the UB experiences repetitive branching into the MM while nephron progenitor cells (NPCs) in the condensed MM undergo a process of mesenchymal to epithelial transition (MET) to generate the segmented nephrons [13]. GDNF released by the MM binds to its receptors RET and GFRa1 in the UB initiating a signaling cascade regulating UB branching via different signaling pathways including Ras/Erk MAP kinase, PLCy-Ca+ and PI3K-Akt pathways [6]. Wnt9b secreted by the UB induces the expression of first-stage nephrogenic markers including Pax8, Lhx1, Wnt4 and Fgf8 in the MM [5]. Wnt4 activation induces MET in the NPCs with the formation of renal vesicle (RV) epithelial aggregates next to the UB. RVs acquire then a proximal-distal (P-D) polarity, where the RV pole that lies near the ureter is distal and the RV pole facing the kidney medulla is proximal. The distinct nephron segment fates -the glomerulus, the proximal tubule, the intermediate tubule and the distal tubule-specify along this P-D axis. Morphologically, polarized RVs transition towards the comma-shaped body (CB). Cells at the most distal pole of the CB fuse to the UB tip to form the "connecting segment" that connects the nephron with the collecting duct. The CB extends to form the S-shaped body (SB) in which the distal and medial segments further differentiate to form the distal convoluted tubule and the loop of Henle respectively, and the proximal segment containing the podocyte cell layer [5].

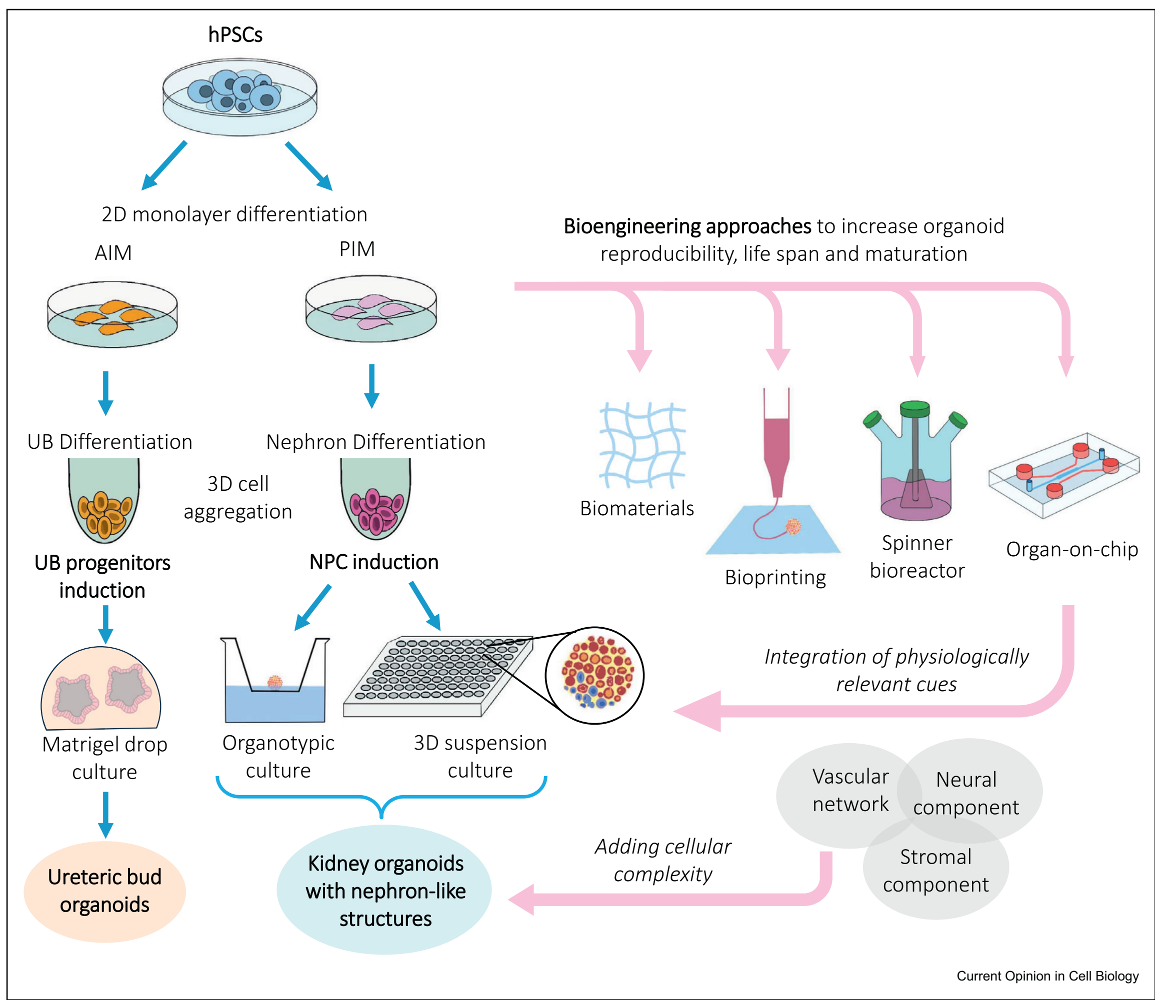
fibroblast growth factor (FGF), and retinoic acid signaling in the generation of renal cells from PSCs [9–15]. Building upon these findings, subsequent works did show the possibility to generate hPSC-kidney organoids mimicking the human embryonic kidney in terms of cellular composition at the transcriptomic level which also showed the capacity to respond to nephrotoxic insults [16–24].

Within these last five years, advances in the analysis of gene expression by single-cell transcriptomics [25,26] coupled with a detailed knowledge from cell-fate studies tracing the progeny of intermediate mesoderm in mice [13,27] have led to a better understanding of kidney morphogenesis. In this regard, recent works profited from bulk RNA seq or single-cell RNA sequencing analysis (scRNAseq) in the time course of hPSC-kidney organoids generation to reveal cellular composition and the degree of differentiation compared to developing human kidneys [28–31]. Collectively, these and other studies highlighted variability between organoids when derived from different hPSC lines [28,29,31], or inter-experimental variability [30], and revealed the presence of variable percentages of off-target populations [31]. More recently, it has been suggested that such variability accounts from the original cell source used for hPSC-kidney organoid generation [32].

In general, methods describing hPSC-kidney organoid generation take advantage of a step-wise approach in which first, hPSCs are committed into IM-like cells and then into NPCs within 7 to 10 days and then exposed to 3D culture conditions that sustain the formation of self-organizing organoids with nephron-like structures in a total time period of 16 to 30 days of differentiation (Figure 2). Usually, these methodologies employ the Wnt signaling small molecule agonist CHIR99021 (CHIR), a glycogen synthase kinase 3β (GSK3β) inhibitor, followed by FGF9 signaling to differentiate hPSCs into IM-like cells and then NPCs. Next, to induce MET, cell aggregates are exposed to a short treatment of CHIR (a general agonist of Wnt signaling), which results in the formation of RV-like structures, the precursors of the final nephron-like structures. Importantly, this single epithelialization induction differs from the process of kidney formation in vivo, where cells at numerous stages of differentiation co-exist within the organ as recently described by the McMahon laboratory [33,34]. Another important consideration raised by experts in the field is that most of the procedures developed so far may better model mesonephric rather than metanephric nephrogenesis [35] potentially contributing to poor patterning and maturation within the resulting nephron-like structures. In addition, poor maintenance of NPCs identity during hPSC-kidney organoid differentiation in vitro could limit nephron patterning and differentiation. Thus, studies defining new differentiation procedures sustaining the maintenance and expansion of NPCs with nephrogenic potential will be key to enhance and control nephron segmentation as recently shown by Vanslambrouck and colleagues [35]. In the same manner, to promote cell-to-cell interactions, researchers have started to assemble different hPSC derivatives generating complex organoid systems. For example, the Nishinakamura group showed for the first time that the manual assembly of hPSC-derived UBs and NPCs with mouse-isolated stromal cells results in the generation of higher-order kidney organoids mimicking the organotypic architecture of the embryonic kidney [36]. Overall, current progress in the development of faithful kidney organoid models from hPSCs has opened new venues to study early events of kidney development in the human context (Box 2. *How to study human kidney development with kidney organoids*) [37]. Recently, methodologies for the generation of UB organoids from hPSCs have been established [38–41]. In the future, a thorough understanding of the spatiotemporal signaling cues and cellular interactions reciprocally driving nephron induction and UB branching will be necessary for the establishment of new methodologies to generate kidney organoid models with functional nephron-like structures connected to a branched collecting duct system.

In addition to biochemical signaling, mechanical forces generated and sensed by cells have a fundamental role in guiding embryonic development, tissue patterning, and morphogenesis. How these mechanical and chemical gradients can be controlled in vitro to guide self-organizing kidney organoids and how this might

Figure 2



Combining hPSC-kidney organoid technology and engineering approaches to improve current kidney organoid models. hPSCs can be guided to differentiate in 2D culture into AIM committed cells or PIM committed cells that will give rise to UB progenitors or NPCs, respectively. Then, 3D cell spheroids are generated by self-aggregation of UB progenitors or NPCs. Usually, hPSC-NPC spheroids can be further differentiated under organotypic culture conditions using the transwell system or as floating spheroids using v-bottom multi-well plates, giving rise to kidney organoids with segmented nephron-like structures. Following a different strategy, hPSC-UB spheroids are embedded in Matrigel drops and further differentiated upon exposure to specific UB inductive signals, leading to the formation of branched UB organoids. The convergence of hPSC-organoid technology with bioengineering approaches such as biomaterial design, 3D bioprinting, microfabrication or organ-on chip will benefit the development of novel methodologies to create more robust kidney organoid models with improved tissue functionalities.

impact cell fate decisions and functionality represents a current challenge when thinking of developing next-generation kidney organoid models. In this regard, recent hPSC-kidney organoid methodologies have incorporated the use of biomaterials, bioreactors, or 3D bioprinting approaches to provide physical cues that can improve organoid differentiation, growth, and long-term culture (Figure 2) [42]. For example, hydrogels derived from porcine decellularized kidney ECM have been proved to enhance vascularization and differentiation of hPSC-kidney organoids [43]. In the future, it is expected that novel hydrogel systems with tunable stress-relaxation characteristics that can be functionalized with tissue-specific ECM cues will be developed to guide organoid self-organization and differentiation efficiently. Another approach to guide kidney organoid self-organization consists of the use of microwell systems to facilitate the generation of highly uniform cell aggregates (Figure 2) [44]. Importantly, it is known that cells can sense topographical cues which modulate cell shape, cellular interactions, and cell-exerted forces that in turn mediate changes in cell proliferation and differentiation. In the future, it will be also interesting to engineer kidney tissue-relevant morphologies using soft hydrogels and viscoelastic materials to enhance hPSC differentiation into kidney organoids.

Box 2. How to study human kidney development with kidney organoids.

For decades, the generation of genetic mouse models have been essential for the identification of genes and signaling pathways involved in the induction and branching of the UB and nephron formation in mice [85–87]. Conversely, our understanding of these developmental processes (i.e, nephron induction and patterning, UB branching) in the human context remains sparse. Recently, the combination of technologies such as scRNAseq and advanced imaging techniques to study human kidney samples from 14 to 17 weeks of gestation has provided new insights into the human nephrogenic program [26]. In this study, authors related transcriptomic data to the developing nephron morphology and could predict cell-type specific functional gene networks [26]. Current hPSC-kidney organoid models have shown to autonomously recapitulate nephron patterning events and respond to signaling pathways key during nephron epithelization and patterning such as Wnt/β-catenin and Notch signaling pathways [20,66]. Both hPSC-kidney and hPSC-UB organoid models have started to show their potentiality as human model systems to study and decipher the precise role of genes in human kidney development and disease. For example, when PAX2-null human NPCs were generated from human iPSCs via transcription activator-like effector nucleases (TALENs), PAX2 was shown to be dispensable for NPCs generation from human iPSCs [88]. In another study, CRISPR/Cas9 system was employed to knock out (KO) Ret/RET in mouse and human UB organoids, proving the critical role of GDNF signaling in UB progenitor commitment and survival and UB branching induction in hPSC-UB organoids [41]. In the future, it is expected that the field will benefit from the refinement of better hPSC-kidney organoid models along with the application of genome editing approaches to provide new mechanistic insights on how genetic networks, signaling pathways and biophysical cues orchestrate human kidney morphogenetic events during health and disease.

Recapitulating kidney function on a chip

In the past few years, microfabrication and 3D printing technologies have been applied to design new milli- and micro-scale devices allowing cell culture and maintenance of hPSC-derived organoid culture in dynamic conditions, including liver-on-a-chip [45–47], heart-on-a-chip [48–51], retina-on-a-chip [52,53], and kidney-on-a-chip [54–57] among others (Box 3. *Organ-on-a-chip technology: traversing the old barriers of static cultures*). Current progress on hPSC-derived kidney-on-chip models for vascularization, nephrotoxicity, and disease modeling are discussed below (Table 1).

Fluid flow for improving vascularization

Biophysical cues, like fluid shear stress, tension, and compression, are crucial for the formation of vascular networks [58]. Organoids fail to develop physiologically relevant perfusable vascularization which in turn represents one of the major limitations for organoid maturation and transplantation approaches. Microfluidic devices have been used to generate self-organizing vascular networks [59–61] or to apply fluid flow showing that shear stress induced the formation of endothelial cell networks within hPSCs-kidney organoids [62]. Other strategies to promote organoid vascularization have made use of microfluidics to apply shear stress to hPSC-kidney organoids in the presence of vascular endothelial growth factor (VEGF) [63], or the co-culture with human umbilical vein endothelial cells (HUVECs) [64]. Besides these efforts, however, the generation of vascularized nephron-like structures with functional filtration capacity in hPSC-kidney organoids still remains a major challenge in the field. In addition, while several recent studies have already shown the impact of biophysical and biochemical cues provided by fluid flow on the maturation of hPSC-kidney organoids [62–64], means to develop a sufficiently dense network of small-scale vessels to perfuse large de-novo tissues is still missing. In this line, Grebenyuk and colleagues have recently developed a 3D printing approach to generate large 3D culture platforms perfused with synthetic capillary-like vessels providing constant supply of nutrients and oxygen to neural and liver organoids [65]. Perfused neural organoids eventually displayed an

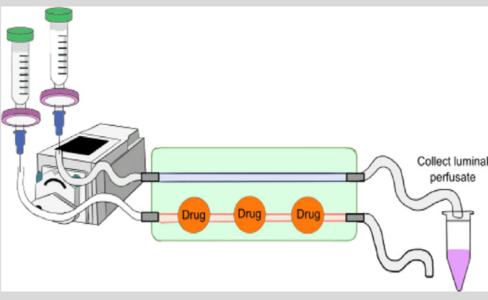
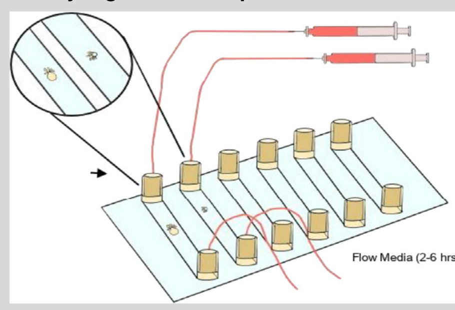
Box 3. Organ-on-a-chip technology: traversing the old barriers of static cultures.

Organ-on-a-chip are micrometer-scaled perfusion devices which can mimic physiological tissue microenvironments and introduce a new component of fluid flow. Following a reductionist approach, the target organ is simplified to its most basic functional elements like structural organization of different cell-types, physical microenvironments, and biochemical interactions between different structural components [89]. Based on these parameters, a simplified organ-on-a-chip model is designed to replicate these features in vitro. The model usually consists of one or more chambers to hold cells and microchannels connecting the chambers with inlet and outlet tubing connections for medium perfusion. The micrometer scaled channels constructed with photolithography or soft-lithography techniques allow for a laminar flow which gives the ultimate advantage of manipulating fluids with ease and having a more precise control over generating stable biochemical gradients which are otherwise missing in static cultures [90]. In the last decade, several organ-on-a-chip models have been developed which emulate specific organ construction and physiological interfaces [89]. Recently, the application of organ-on-chip technology to hPSC-derived organoids holds enormous promise for advancing in the development of faithful physiological-relevant models for drug development in the human context [91]. Importantly, microfluidic devices can be engineered to incorporate mechanical forces recapitulating those of the in vivo milieu (i.e., blood pressure, lung pressure or peristaltic pressure among others) which have an impact on organ development and functioning. Furthermore, optical, chemical or electrical sensors can be introduced to monitor tissue functional activity in real time. For example, in a recent discovery by Cai and colleagues, hiPSC-derived flattened spinal organoids were used to model opioid-induced tolerance and hyperalgesia. The adequacy of this platform was demonstrated upon the use of electrodes to record the organoid's neural activity, which diminished upon opioid agonist treatment, while capsaicin administration (to model nociception) increased the mean firing rate of the organoids [92].

Table 1

Summary of recent hPSC-derived kidney-on-chip models. Different organoid-on-chip approaches have been recently used to create controlled microenvironments to improve kidney organoid vascularization, study nephrotoxicity and model relevant kidney disease phenotypes.

	Chip fabrication and characteristics	Application	Major outcomes	Reference
Kidney organoid-on-chip	A culturing chamber fabricated by 3D printing is used to culture hPSC-kidney organoids on a gelatin and fibrin ECM layer. Controlled fluid shear stress is applied such that fluid flows above the organoids. The device is covered by an acrylic lid.	Enhanced kidney organoid vascularization	High fluid shear stress significantly enhances vasculature and maturity in tubular and glomerular compartments in kidney organoids	Homan KA et al. Nat Methods. 16(3):255–262 (2019) [62].
Kidney organoid-on-chip	The microfluidic chip contains three microfluidic channels which reach the culturing chamber through a porous membrane. Human umbilical vein endothelial cells (HUVECs) are grown in static culture in these channels and later kidney organoids are cultured on top of these HUVECs under flow.	Enhanced kidney organoid vascularization	Human umbilical vein endothelial cells grown in the channels of the chip migrate and proliferate inside the organoid tissue when flow is introduced. They interconnect with endogenous endothelial tissue and form structures with open lumen resembling vessels.	Bas-Cristóbal Menéndez A et al. Sci Rep. 12(1):20699 (2022).
Glomeruli-on-chip	The device is designed with 2 opposed, parallel microchannels separated by Laminin-511-coated porous PDMS membrane with 2 hollow chambers on either sides of these microchannels to simulate cyclic pulsations with the help of vacuum generation.	Adryamycin-induced podocyte injury model	hPSC-derived podocytes recapitulate natural tissue–tissue interface of glomerulus and cause differential clearance of albumin and inulin on co-culture with human glomerular endothelial cells.	Musah, S. et al. Nat. Biomed. Eng. 1, 0069 (2017) [77].

Tubule-on-chip 	The device consists of 2 cylindrical channels which are fully embedded in gelatin-fibrinogen ECM. In one channel, epithelial cells isolated from kidney organoids are seeded and perfusion is applied through the second channel which mimics basolateral drug uptake.	Injury models/ Drug discovery	Higher expression of drug transporters like OCT1, OAT1 and OAT3 are observed along with increased drug uptake assessing the levels of nephrotoxins like Cisplatin and Aristolochic acid.	Aceves JO et al. Sci Rep. 12(1):14997 (2022) [56].
Kidney organoid-on-chip 	The device has 6 culture channels with an inlet and an outlet each. Kidney organoids grown in static culture are pipetted in these channels which are already covered with Reduced Growth Factor Geltrex and cultured under different fluidic regimes.	Disease modeling	Flow, volume and solute concentrations act as positive regulators for cyst expansion in ADPKD organoid models. Cyst-expansion is driven by glucose transport into the lumens of outwards-facing epithelia indicating it occurs via absorptive rather than secretory pathway.	Li SR et al. Nat Commun. 23; 13(1):7918 (2022) [57].

accelerated lineage commitment, while the perfusion of liver organoids correlated with higher expression levels of hepatocyte-specific markers. Additionally, the perfusion and subsequent clearance of determined compounds validated the use of perfused liver organoids as model platforms for drug metabolism. Undoubtedly, the application of such a promising technology in the kidney organoid field could result in the obtention of more complex hPSC-kidney organoids in terms of vascularization and maturation, which could be then used as platforms to interrogate glomerular filtration, drug clearance, or nephrotoxicity assessment.

Kidney organoid on chip for nephrotoxicity, drug screening, and physiology

Current kidney organoid models have already proved their utility as in vitro platforms for drug-induced toxicity screens, exhibiting specific damage responses in both tubular [18−21,66] and glomerular structures [22−24,67,68]. Remarkably, Gupta and colleagues showed that it is possible to model kidney injury and repair in hPSC-kidney organoids, identifying a potential therapeutic candidate to hinder the onset and development of chronic kidney disease [69]. Recently, a proximal tubule epithelial cell (OPTEC)-on-a-chip has been developed to culture proximal tubular epithelial cells isolated from hPSC-kidney organoids further challenging the cells to physiologic luminal shear stress to enhance polarization and promote the expression of proximal-tubule specific functional markers [56].

Kidney organoid on chip for disease modelling

hPSC-kidney organoids have served as very useful platforms to model various renal disease-related phenotypes [70−75]. In a recent work by Morizane's laboratory the authors have established mechanosensitive signals as key drivers of cystogenesis in polycystic kidney and hepatic disease 1 (*PKHD1*) mutant kidney organoids (*PKHD1*−/−) derived from gene-edited hiPSCs, showing the induction of cysts in distal tubules, a hallmark feature of autosomal recessive polycystic kidney disease (ARPKD) in humans, and identifying the important role of mTOR pathway in this process [76]. Another study establishing the role of fluid shear stress in promoting cyst expansion in an autosomal dominant polycystic kidney disease (ADPKD)-on-a-chip model was recently shown by Freedman's laboratory [57]. In the next years, it is expected that hPSC-kidney organoid models will benefit from organ-on-chip technology to create physiologically relevant tissue models for studying complex tissue−tissue crosstalk during health and disease (Figure 3).

Future perspectives

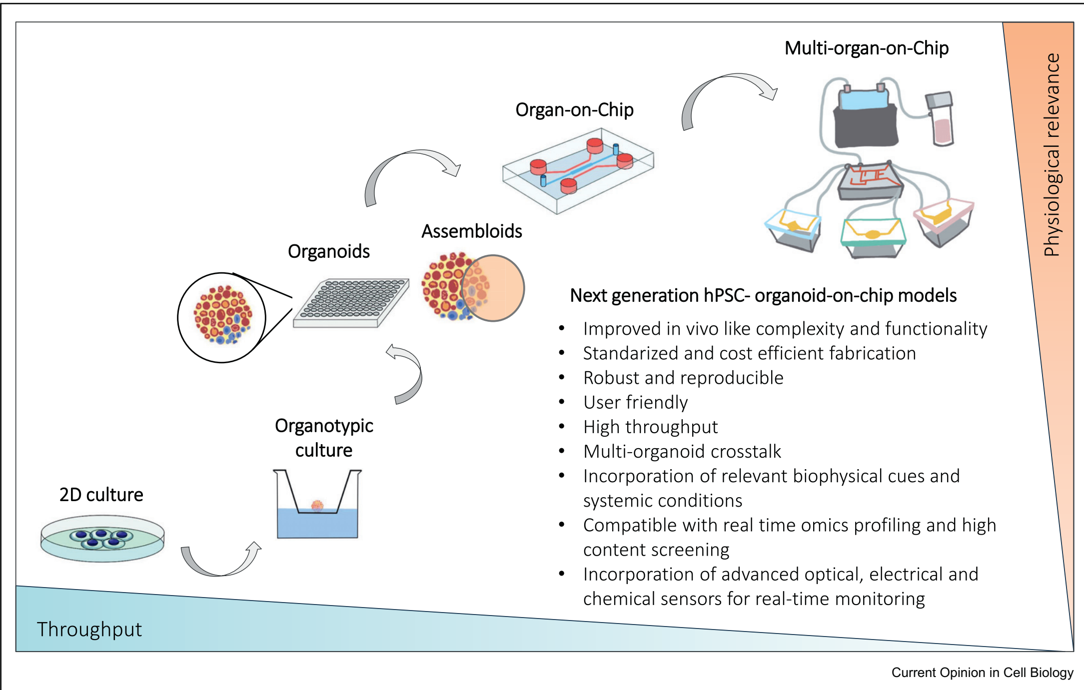
Taken together, within this last decade, accumulative findings have started to define different procedures for the recapitulation of early stages of kidney development using hPSCs. In sum, hPSC-kidney organoids have supported research, modeling human kidney development and disease with yet important challenges ahead related to their immatureness and short life span.

Within the next years, it is expected that our understanding of human kidney development will help to externally control the generation of hPSC-kidney organoids. To that, it will be necessary to properly identify cell culture conditions promoting the generation of expandable hPSC-CM-like cells and test the renal differentiation potential of these cells. Other challenges are related to the identification of the proper interactions between hPSC-CM-like cells together with hPSC-derived-UB and -stromal cells to support and control nephrogenesis in vitro. In the same line, transcriptomic data sets have also been used to reconstruct lineage trajectories to promote maturation of desired kidney cell types or inhibit differentiation of undesired off-target cell types [31]. Thus, it is expected that the convergence of scRNAseq and kidney organoid technology will provide crucial information on how to externally guide kidney differentiation to finally exploit these cell culture platforms for regenerative medicine applications as well as to model renal disease in vitro.

The application of organ-like physiological environments using micro-engineered devices represents another powerful strategy to harness organoid differentiation and disease modeling. To date, most of the kidney organoid-on-a-chip models derived from hPSCs have been used for drug discovery and personalized medicine applications [55−57,76,77]. In this regard, commercially available platforms compatible with high content imaging techniques have been recently applied to emulate glomeruli on chip and tubule on chip using primary cells from adult human kidney tissue [78−80]. However, drug toxicities normally affect both the glomerulus and proximal tubule thus it will be of extreme importance to count with next-generation microphysiological systems incorporating all components of the functional nephron. In this line, a recent work defined a microfluidic chip incorporating the glomerulus and proximal convoluted tubule interfaces emulating filtration, reabsorption, and secretion functions [81]. Apart from such advances, in the future, it will be necessary to develop live functional assays allowing real-time monitoring of hPSC-kidney organoid responses. For example, reporter cell lines for specific renal solute transporters or injury biomarkers will be useful for real-time monitoring and high content analysis of organoid responses [82]. Similarly, biosensors for monitoring secreted metabolites or proteins could be incorporated into organ-on-chip devices for in situ monitoring of organoid functions [83,84].

Hopefully, the next years will be crucial for the development of new hPSC-kidney organoid platforms with

Figure 3



Throughput potential and physiological relevance of current hPSC-kidney culture platforms. The bidimensional culture of hPSC-derived renal cell types, although rudimentary, has been fundamental for the development of more refined models. For instance, the organotypic 3D culture of cells has allowed the mimicry of a liquid–air interface, enhancing the culture complexity. Later, through the generation of organoids or assembloids, more faithful organ models were generated. However, organoid culture on chips displayed several advantages, such as the simulation of shear stresses associated with fluid flow, mimicking the in vivo setup. The inclusion of several organoid-on-a-chip in a single device allowed the generation of multi-organ-on-a-chip, which is currently the most sophisticated humanized model. The increase in complexity comes with a reduction in throughput. Future models aim at increasing throughput while also providing new advantages, including reproducibility, or user friendliness.

enhanced matureness and reproducibility. The application of bioengineering approaches will allow to increase our capacities to monitor and control cellular self-organization, tissue patterning, and differentiation toward the generation of kidney organoid models with increased cellular and functional complexity while ensuring high reproducibility. All these advances together with current efforts in generating new organ-on-chip systems that allow controlled exposure of cells to biochemical and biophysical signals, along with the possibility of recapitulating multi-organ interactions, will have a strong impact on transitioning from bench to bedside using hiPSC-derived kidney organoids.

Glossary

Intermediate mesoderm

A region of the embryonic mesoderm extending anterior–posterior between the paraxial, or somitic, mesoderm and the lateral plate mesoderm.

Pronephros

Primitive embryonic kidney structure, arising at the fourth week of human gestation, and degenerating shortly after. Despite its transient nature, the pronephros is fundamental for the generation of the urinary system, enabling the subsequent development of more advanced kidney structures, the mesonephros and metanephros.

Mesonephros

Transient excretory system, caudally adjacent to the pronephros, which constitutes the first functional excretory system eventually replaced by the metanephros.

Metanephros

Phase of kidney development during the fifth week of gestation in which UB start to develop into collecting duct system. Eventually, the metanephros undergoes a series of complex changes, ultimately establishing the permanent, functional kidney.

Collecting duct system

Tubular network that receives and concentrates urine from the distal convoluted tubule of nephrons and exudes it into the renal pelvis for elimination.

Ureteric bud
An epithelial tube that arises from the nephric duct during embryonic development. Upon a series of branching events and close interaction with the MM, the UB generates a collecting duct system connected to nephrons.

Metanephric mesenchyme
The region of posterior intermediate mesoderm adjacent to the nephric ducts from where the epithelial cells of the kidney emerge upon kidney induction.

Cap mesenchyme
Cells that, upon epithelialization, sequentially form the pretubular aggregate (PA), renal vesicle (RV), C-, and S-shaped bodies, which finally evolve into the mature nephron.

Kidney induction
The development of kidney involves the reciprocal interaction between two cell progenitor populations, the MM and the UB. The MM (containing nephron progenitor cells, NPCs) induces the budding and branching of the UB (mainly via GDNF), while the UB induce MET of the MM to form the nephrons (mainly via Wnts).

Renal pelvis
Funnel-shaped cavity within the kidney, readily converging to the ureter, responsible for urine drainage.

Nephron
The kidney's functional unit, consisting of a blood filtration segment, the glomerulus; and a urine processing compartment, the renal tubules.

Bladder trigone
Triangular-shaped region of the bladder, delineating both ureteric orifices and the urethral meatus.

Pretubular aggregate
Mesenchymal cell subpopulation within the cap mesenchyme which, following mesenchymal-to-epithelial transition, gives rise to the epithelial cells lining the renal vesicle.

Renal vesicle
The first polarized epithelial derivative of the induced MM that is found abutting the branching tips of the UB.

Nephron progenitor cells
Population of self-renewing cells able to give rise to all the cell types of the kidney. They are involved in the homeostasis and repair of the adult kidney, hence their therapeutic potential for treatment of kidney failure.

Human pluripotent stem cell
Stem cell that has the potential to differentiate into any of the three germ layers: endoderm, mesoderm, or ectoderm.

Human embryonic stem cells
Pluripotent stem cells derived from the inner cell mass of a blastocyst, an early-stage pre-implantation embryo.

Human induced pluripotent stem cells
Type of pluripotent stem cell that can be generated in vitro from a somatic cell.

In vitro kidney re-aggregation assay
Method that was first described by Auerbach and Grobstein [93,94], that combined mouse embryonic MM tissue with mouse embryonic UB in vitro, resulting in the generation of both nephrons and UB structures. Recently, to assess the integration and renal differentiation capacity of hPSC-derived kidney progenitors, the field has evaluated their ability to form kidney chimeric structures in vitro by performing re-aggregation assays with mouse embryonic kidney cells.

Embryoid bodies
Three-dimensional aggregates of pluripotent stem cells.

CRISPR/Cas9
It is a prokaryotic immune system that confers resistance to foreign genetic elements such as those present within plasmids and phages and provides a form of acquired immunity. This system has been widely used by geneticists and medical researchers as a promising gene editing technique that enables the removal, addition, or alteration of targeted DNA sequences within the genome sequence.

Single-cell RNA sequencing
Technique which provides the expression profiles of individual cells with optimized next-generation sequencing technologies, providing a higher resolution of intercellular differences.

Reporter cell line
Engineered cell line in which a specific gene has been labeled with a reporter gene in order to measure or identify its expression levels or to be used as selectable markers.

Perfusable vascularization
Engineered blood vessel network capable of enduring blood flow, recreating in vitro the vasculature found in living organisms. Perfusable vascularization thereby enhances cultured tissue survival by facilitating the transport of nutrients and oxygen and allowing waste removal.

Assembloid
3D culture systems arising from the aggregation of multiple organoids, forming architecturally and functionally relevant culture models.

Organotypic culture
Set of 3D cell culture methods attempting to recreate the spatial distribution and functionality of an organ. These culture systems facilitate ECM deposition as well as 3D cell—cell and cell—matrix communication.

Biochemical cues
Chemical molecules present in the culture microenvironment which impact cell protein adhesion mechanisms to the substrate's surface and affect overall cell behavior. These cues involve various factors, such as ECM proteins, growth factors or signaling molecules.

Biophysical cues
Physico-mechanical properties of the cellular microenvironment, which modulate cell adhesion and cellular response. These cues include, for instance, fluid flow, mechanical stimuli, or surface topography, among others.

Microengineered device
Compact device that integrates microfluidic channels with electronic components, operating at the microscale level. By virtue of its miniature size, this device delivers enhanced performance and accuracy, while offering supplementary benefits, including portability and low cost.

Microfluidics
The science that studies fluid flow through micro-sized channels. By using a combination of valves and pumps, fluids may be precisely directed through channels and compartments, ultimately recapitulating complex fluid behaviors.

Fluid flow
Movement of liquids or gases upon exposure to pressure gradients. It may be described as laminar (smooth and parallel) or turbulent (chaotic).

PDMS
Polydimethylsiloxane (PDMS) is an optically clear, curable, and biocompatible polymer used for the generation of microfluidic devices, including organ-on-a-chip.

Organ-on-a-chip
Microfluidic-based cell culture system recreating a specific organ's physiological features. By simulating shear stresses associated to fluid flow, these models ultimately recreate more physiologically accurate responses than conventional cell culture methods.

Multiorgan-on-a-chip
Systemically connected organ-on-a-chip compartments, which recapitulates the interaction mechanisms between different organs. These systems ultimately mimic the complex physiological responses of the human body.

Declaration of competing interest

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

Data availability

No data was used for the research described in the article.

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Using a microfluidic device constructed with a 2-photon nanoscribe printer and a customized hydrogel, the model demonstrated the generation of synthetic capillary-like vessels and showed successful long-term perfusion of neural, liver, and cerebral organoids with neural tissues showing enhanced differentiation. The design involves a perfusable grid having capillary like tubes made of hydrogel that allows diffusion inside a chip which is linked to a pump circulating cell culture medium. This is the first study to show the construction of large de-novo tissues which were viable over long periods of time. Overall, this platform addresses the major issue of lack of large viable engineered tissue constructs and can serve as a promising tool for drug development and disease modelling.

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The study uses kidney organoids derived gene-edited hiPSCs to show that cysts grown in organoids under flow are more segment specific as compared to organoids grown in static culture thus establishing mechanosensitive signals as key drivers of cystogenesis in autosomal recessive polycystic kidney disease (ARPKD). The study also tested two U.S. Food and Drug Administration-approved and one investigational new drug that target RAC1 and FOS in organoid-on-a-chip model, which suppressed cyst formation.

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