Unlocking the full potential of human pluripotent stem cell–derived kidney organoids through bioengineering

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Human pluripotent stem cells hold inherent properties, allowing researchers to recapitulate key morphogenetic processes. These characteristics, coupled with bioengineering techniques, have led to the definition of early procedures to derive organ-like cell cultures, the so-called organoids. With regard to kidney organoids, challenges stand ahead, such as the need to enhance cellular composition, maturation, and function to that found in the native organ. To this end, the kidney organoid field has begun to nourish from innovative engineering approaches aiming to gain control on the externally imposed biochemical and biophysical cues. In this review, we first introduce how previous research in kidney development and human pluripotent stem cells has informed the establishment of current kidney organoid procedures. We then discuss recent engineering approaches to guide kidney organoid self-organization, differentiation, and maturation. In addition, we present current strategies to engineer vascularization and promote *in vivo*–like physiological microenvironments as potential solutions to increase kidney organoid lifespan and functionality. We finally emphasize how working at the cusp of cell mechanics and computational biology will set the ground for successful translational applications of kidney organoids.

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Editor's Note

Most methods for generating kidney organoids from human pluripotent stem cells (PSCs) aim to recapitulate the developmental trajectory of the mammalian kidney. However, current differentiation protocols often rely on the self-organization capability of human PSCs or PSC-derived nephron progenitors. Although this strategy enables the generation of diverse kidney cell types, its dependence on spontaneous self-organization, without well-defined external physical and chemical cues, can lead to inconsistent and less robust differentiation outcomes. In this insightful mini review, the third installment in our series on regenerative medicine and nephrology, Goux Corredera *et al.* first summarize the current strategies for generating kidney organoids from human PSCs. They then walk through a range of engineering approaches designed to more precisely guide organoid self-organization, enhance vascularization, and promote functional maturation. Finally, the authors highlight emerging technologies for measuring and monitoring physical parameters in cells and tissues, and consider how these tools, together with computational biology, can offer new insights into human kidney development and disease. See more topics in the Regenerative Medicine and Nephrology series at https://www.kidney-international.org/content/regenerative-medicine-nephrology.

Over the past 10 years, stem cell research has advanced our understanding of key aspects of kidney organogenesis through the exploitation of the self-organizing capacity of human pluripotent stem cells (hPSCs). Such advances have led to the development of cell culture procedures

to generate hPSC-derived kidney organoids (hPSC-kidney organoids), which capture key features of the *in vivo* organ, including cell composition and morphologic organization and the recapitulation of cell type–specific functional characteristics.[1] Current hPSC-kidney organoid methods commonly rely on 3-dimensional (3D) culture techniques that exploit cell-autonomous self-organization responses of hPSCs along with the timely addition of soluble renal inductive signals to recapitulate early stages of kidney development. In general, hPSCs are first induced into intermediate mesoderm (IM), followed by nephron progenitor cell (NPC) commitment, and then cultured in 3D as cell spheroids, leading to the formation of hPSC-kidney organoids containing cellular structures resembling the nephrons. In parallel, the convergence of such developments with the bioengineering field is leading to technological advances, allowing researchers to provide controlled instructive environments (physical and chemical) to better guide the formation of multicellular structures. Besides these advances, hPSC-kidney organoids still face several challenges that include lack of reproducibility, limited organoid lifespan, incomplete matureness and functionality, and lack of relevant tissue components, such as vascularization and a proper connection of nephrons to a collecting duct system. In this review, we first introduce key information to understand early developmental cues driving kidney development in mammals while looking back on the historical origin of kidney organoid technology exploiting hPSCs. Then, we discuss the application of bioengineering approaches to overcome current hPSC-kidney organoid challenges. We foresee that this knowledge will guide the scientific community to generate higher-grade hPSC-kidney organoid models for developmental biology studies, drug screening, disease modeling, and personalized medicine applications.

Understanding kidney development for the generation of hPSC-kidney organoids

Mammalian kidneys arise after 3 sequential overlapping stages (namely, the pronephros, mesonephros, and metanephros), with the latter being the permanent or metanephric kidney. The kidney is derived from the IM.[2,3] The anterior-posterior patterning of the IM generates the anterior IM that elongates to form the nephric duct and ureteric bud (UB), which will develop into the collecting duct system. Conversely, the posterior IM differentiates to form the metanephric mesenchyme (MM) that will form all the epithelial cells of the nephron and the renal stroma, as well as vascular progenitors.[4,5]

Reciprocal interactions between the UB and the MM tightly regulate UB branching and nephron formation.[4,5] A subset of cells within the MM condensates to form the cap mesenchyme, representing the NPCs that will produce all epithelial cells of the nephrons. The cap mesenchyme is surrounded by the cortical stroma, which will contribute to the cortical and medullary interstitium, mesangial cells, and pericytes. Developmental studies performed in the mouse have revealed that fibroblast growth factor 9, fibroblast growth factor 20, bone morphogenetic protein, and low canonical Wnt signaling maintain the pool of self-renewing NPCs, whereas Wnt signaling via *WNT9b* expression in the UB tips triggers mesenchymal-epithelial transition of NPCs initiating the process of nephron patterning.[2] During this process, epithelial cells subsequently self-organize into polarized spheres (renal vesicle), transitioning into a comma-shaped body, and then an S-shaped body (Figure 1). Successively, the 3 segments of the S-shaped body emerge to produce the segmented nephron with the proximal, mid, and distal compartments, with the latter being oriented adjacent to the UB tips. The proximal segment differentiates into glomerular epithelial cell (podocytes), the mid section forms the proximal tubule and loop of Henle, and the distal segment evolves into the distal tubule, readily connected to the UB branches.

Differentiation strategies to generate hPSC-kidney organoids

The knowledge gained from kidney developmental studies has been key in the definition of stepwise differentiation methods toward kidney cell types and organoids from

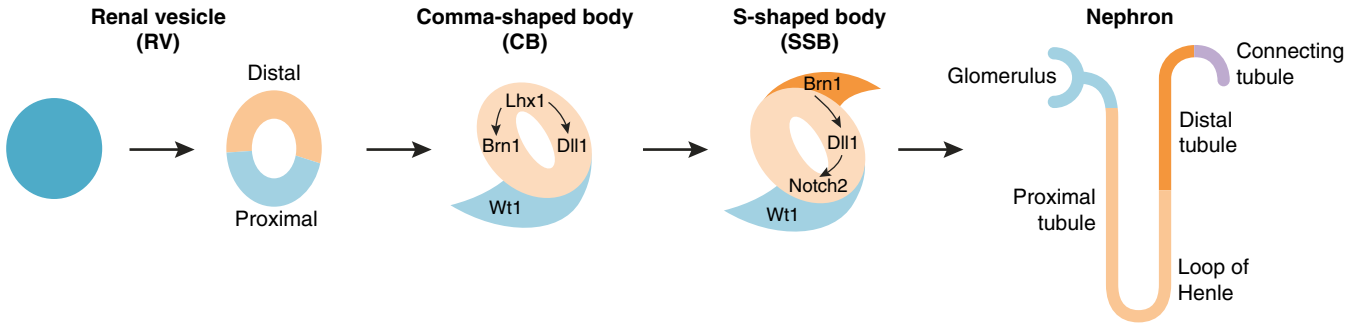

Figure 1 | Schematics showing main nephron patterning events. Reciprocal signaling between the cap mesenchyme and the ureteric epithelium induces the formation of the renal vesicles. These epithelial structures acquire proximodistal polarity and elongate to morphologically transition into the comma-shaped body (CB) and the S-shaped body (SSB) that will further pattern into the segmented nephron. Wnt–ß-catenin signaling induces LIM homeobox 1 (LHX1) in the distal region of the CB, later specifying into the tubule segment that will connect the nephron to the collecting duct system, and Notch signaling is required for proximal tubule specification. The distal and medial regions of the SSB will specify into the distal convoluted tubule, the loop of Henle, and the proximal tubule, whereas the proximal region of the SSB will generate the glomerulus and Bowman's capsule. Relevant marker genes for distal, medial, and proximal nephron fates are indicated. Brn1, brain-specific homeobox/POU domain protein 1; Dill1, delta like canonical notch ligand 1; Wt1, wilms tumor 1.

hPSCs[6–16] (Figure 2 and Table 1). The establishment of hPSC-kidney organoids may be traced back to a pioneering study conducted by Taguchi *et al.*, hailed as a milestone in the field.[6] In this study, the authors started with the formation of embryoid body aggregates, then induced the posterior nascent mesoderm with activin A and CHIR99201 (a Wnt agonist), which further differentiated into MM-committed cells. hPSC-derived MM cells required co-culture with mouse embryonic spinal cords acting as mesenchymal-epithelial transition inducers, to yield kidney organoids with nephron-like structures.[6] The following year, Morizane *et al.*[7] and Takasato *et al.*[9] developed similar chemically defined methods that recreated kidney organogenesis through the timely administration of a cocktail of renal inductive factors (including activin A, CHIR99201, and fibroblast growth factor 9) to hPSCs, generating kidney organoids with nephron-like structures using U-bottom 96-multiwell or transwell culture systems, respectively. Notably, Takasato *et al.* further revealed the impact of CHIR99201 duration in anterior-posterior specification of the IM, producing kidney organoids with segmented nephron-like structures as well as endothelial and stromal cell lineages, and cells expressing some markers of ureteric epithelium.[9] Alternatively, Freedman *et al.* described a sandwich Matrigel culture method to directly induce mesoderm differentiation from hPSCs using CHIR99201 and further formation of nephron-like structures.[8] Interestingly, the generated organoids contained some off-target neuronal cells.[8] Using 3D floating culture conditions, Przepiorski *et al.* generated kidney organoids by differentiating embryoid bodies under shaking conditions using a spinner bioreactor, showing the formation of nephron-like structures in 14 days.[11] Remarkably, Garreta *et al.* demonstrated a differentiation method to generate segmentally patterned nephron-like structures transcriptionally reminiscent of second-trimester human fetal kidney (Figure 3).[10] Recently, Vanslambrouck *et al.* have underscored the importance of enhancing metanephric specification for producing NPCs with improved metanephric identity, thus impacting on more functional kidney organoids in terms of proximal tubule maturation and segmentation as well as improved spatial organization of the nephron structures.[12] On the other hand, in the past 3 years, different efforts have been fruitful in the generation of UB organoids from hPSCs.[13–16] Nevertheless, future research is required to delineate novel approaches to recreate higher organized kidney organoids containing relative proportions of nephron, UB/collecting duct, stroma, and vascular components that better reproduce the native kidney regarding composition, morphology, and functionality.

Engineering approaches to enhance hPSC-kidney organoid self-organization

Engineering approaches have the potential to offer better control over initial cellular density, geometry/shape, and size of cell aggregates and cell–to–extracellular matrix (ECM) interactions during organoid formation, thereby providing controlled instructions to enhance hPSC-kidney organoid self-organization.

For example, microfabricated microwell systems have been used to force cell-to-cell contact when aggregating hPSC-derived NPCs or hPSC-derived UB progenitors to generate hPSC-kidney[17] and UB organoids,[16] respectively. This approach represents a highly reproducible method to produce 3D cell aggregates, opening the door into scalable and robust applications in personalized medicine or regenerative medicine.

In vivo, ECM dynamics can define tissue boundaries, guiding tissue pattern formation.[18] Moreover, ECM mechanical properties can modulate cell behaviors, such as proliferation and differentiation.[19,20] Cell-ECM interactions can be emulated *in vitro* through the development of biomimetic materials for hPSC differentiation. For example, the use of polyacrylamide hydrogels that mimic soft environments improved the differentiation of hPSC-derived renal cells into kidney organoids.[10] Moreover, encapsulating these cells in soft, fast-relaxing hydrogels results in more mature organoids compared with using stiffer or slow-relaxing hydrogels,[21] highlighting the importance of selecting appropriate mechanical properties of matrices. Current kidney organoid engineering focuses on simultaneously modulating biochemical and mechanical cues to enhance organoid self-organization.[22,23]

Notably, the Engelbreth-Holm-Swarm matrix, which is a reconstituted basement membrane harvested from mouse sarcoma,[24] also known as Matrigel, Geltrex, and Cultrex basement membrane extract, has been pivotal in the development of hPSC-organoids (i.e., cerebral, optic cup, or gastric organoids) and in 2-dimensional organoid differentiation cultures (i.e., kidney organoids).[7,8] Conversely, hPSC-kidney organoids have been obtained in both air-liquid interface[9,10] and suspension cultures.[11,25] Up to date, Matrigel batch-to-batch variability and undefined biochemical composition represent important drawbacks to the development of organoid reproducibly, limiting their potential for translational and personalized medicine applications. In this regard, synthetic hydrogels can help in the creation of defined controllable organoid niches because of their versatility in producing highly tunable environments.[22,23,26] For example, using photosensitive hydrogels, key biomolecules can be locally positioned in specific areas in the hydrogel network in a reversible manner, producing 3D patterns and gradients of biochemical cues that can be dynamically tailored on light exposure.[27] Light-induced patterning can be also applied to locally modulate hydrogel physical properties,[28] and even to create stiffness gradients.[29] Recently, a 2-photon–mediated bioprinting approach has been used to impose specific 3D constraints guiding organoid growth in a spatiotemporal dynamic manner.[30] Alternatively, hydrogels derived by decellularization of tissues can retain the complex composition of the native organ-specific ECM.[31–33] A major consideration in material selection should be to facilitate ECM

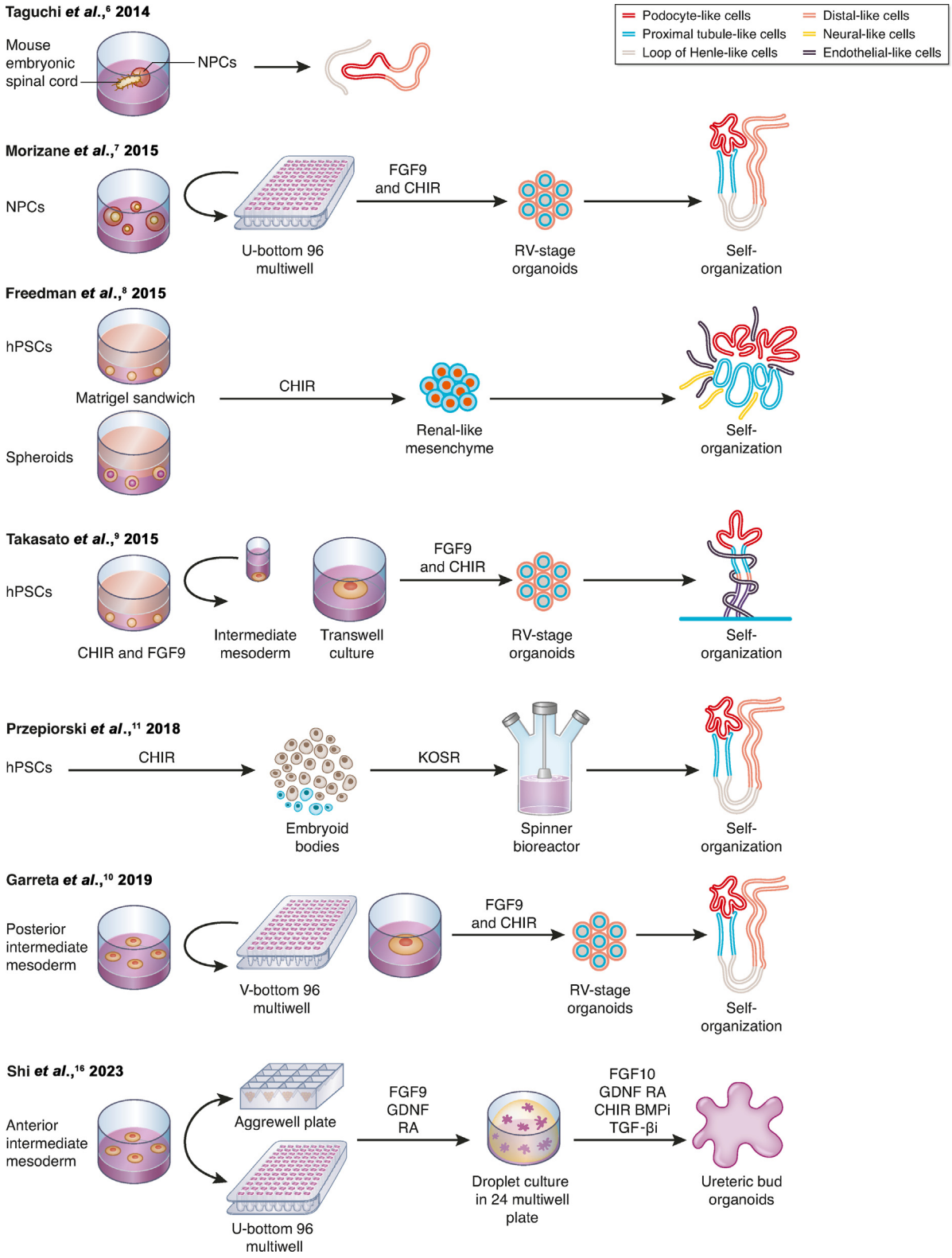

Figure 2| Overview of main methods and cell culture systems to generate kidney organoids from human pluripotent stem cells (hPSCs).[6–11,16] Comparison of the main current methods to generate kidney organoids from hPSCs. Generally, methods to produce hPSC-kidney organoids rely on a first stage of 2-dimentional monolayer differentiation in the presence of CHIR99201 (CHIR) and fibroblast growth factor 9 (FGF9) to allow intermediate mesoderm (IM) commitment followed by nephron progenitor cell (NPC) induction. Subsequent self-aggregation of IM-committed cells or NPC-committed cells is accomplished using v-bottom multiwell plates or microwell culture plates in the presence of renal inductive signals. Three-dimensional spheroids can be then differentiated under organotypic culture conditions using the transwell system or as floating spheroids, resulting in the generation of kidney organoids with segmented nephron-like (continued)

remodeling during organoid differentiation and growth, guiding this process toward a stable end point that best mimics the ECM properties appropriate for the given organ(oid).

Engineering approaches to promote hPSC-kidney organoid lifespan and vascularization

Envisioning strategies extending the utility of hPSC-kidney organoids for disease modeling and regenerative medicine applications require the development of engineering approaches to increase lifespan, maturation, and vascularization. Although main kidney organoid methods show a relatively short lifespan, often ranging from 20 to 30 days of *in vitro* culture (Table 1),[7–10,25,34] recent studies have demonstrated the culture of kidney organoids for extended time periods of 50 to 64 days, that produce elongated tubular structures with improved differentiation characteristics.[35–37] Future efforts to enhance organoid's lifespan and maturation should develop culture regimens addressing kidney organoid metabolic demands through the application of bioengineering approaches involving biomaterials and microfluidics that recreate physiological conditions. Moreover, early exhaustion of NPC identity during hPSC-kidney organoid differentiation may limit nephron patterning and differentiation as well as result in short organoid lifespan. Thus, the definition of improved differentiation procedures that support the maintenance and expansion of NPCs will be key to enhance and control nephrogenesis within kidney organoids.[12]

On the other hand, the lack of functional vascularization in current kidney organoids might be in part responsible for their limited lifespan *in vitro*. Although scattered endothelial networks are present in many protocols,[7–11,38–40] these tend to decrease with time in culture. To date, strategies, including transplantation into animal models,[10,41,42] treatment with exogenous growth factors or ECM cues,[31,33] or application of controlled shear stress through microfluidics,[43] have enhanced organoid's characteristics by enabling glomerular vascularization, podocyte differentiation, and prominent tubular polarization with escalated expression of ciliary proteins.[43] Currently, bioengineering technologies, including microfluidics and 3D bioprinting, are regarded as promising tools to promote kidney organoid vascularization. Microfluidic devices could be used to induce the self-assembly of an organ-tailored microvasculature within kidney organoids from the initial steps of organoid formation.[44–46] Interestingly, a recent study has shown that incorporation of hPSC-derived macrophages within a vascularized heart-on-chip model promoted vessel stabilization, unveiling a key role of macrophages in supporting long-term vascularization of cardiomyocyte tissue-like structures.[47] Overall, the synergism between microfluidics and multilineage self-organization may represent a potential strategy for promoting organoid vascularization and maturation.

Conversely, 3D bioprinting can design perfusable channels within cell clusters, recreating a vascular network,[48] eventually enabling the generation of macroscopic "organ-building blocks" with a vascular network architecture replicating those of specific organs. Although vascularization can promote lifespan and maturation, further investigations are needed for the full realization of kidney organoid lifespan and generation of mature renal cell types. On the other hand, the development of new vascularization approaches will be of extreme importance when aiming to increase as well as to provide a stable and functional vascular network to connect different organ-specific compartments, and eventually for transplantation purposes.

Engineering approaches to boost hPSC-kidney organoid functionality

Evaluating current hPSC-kidney organoid functionality. Current hPSC-kidney organoids are still unable to faithfully reproduce the physiological functions of the native kidney.[49] Evaluating kidney organoid's capacity to perform glomerular filtration, for instance, is achievable on their successful *in vivo* implantation and vascularization. After injection of fluorescein isothiocyanate–labeled dextran into the host's circulation, this molecule could be traced into the engrafted organoid's tubular structures, indicating glomerular filtration of this substance.[50,51] Conversely, evaluating kidney organoid's tubular function is feasible in *in vitro* conditions. A standard approach for assessing proximal tubular reabsorption consists of pulse-chase experiments with fluorescent molecules[8,9] or interrogating the organic anion transporter family.[34,52] Moreover, it is of interest to interrogate kidney organoid's competence in renin synthesis,[53] which is accountable to salt reabsorption, as well as for blood pressure regulation.

Engineering higher-order tissue architecture. Current efforts in the field are directed to provide kidney organoids with the missing cellular compartments, majorly the UB, the stroma, and the vasculature. Recent efforts showed the generation of kidney organoids with improved renal architecture.[54,55] Nevertheless, organoids with a fully branched collecting duct system connected to the nephrons and proper vascularization have not been realized yet. In the next years, 3D bioprinting approaches[56] could be used to deposit different cellular components together with biomaterials to recreate the complex multicellular and cell-ECM interactions occurring in the native kidney. For example, ECM remodeling has been shown to play an essential role during branching morphogenesis in the kidney.[18,57–60] On the basis of these

Figure 2 | (continued) structures. Ureteric bud (UB) organoids have been derived by differentiating hPSCs into anterior intermediate mesoderm. Aggregates of UB progenitors are then embedded in Matrigel drops in the presence of UB branching signals to generate UB organoids. BMPi, bone morphogenetic protein inhibitor; GDNF, glial cell derived neurotrophic factor; KOSR, knockout serum replacement; RA, retinoic acid; RV, renal vesicle; TGF-βi, transforming growth factor-beta inhibitor.

Table 1 | Methods for the generation of hPSC-kidney organoids

Protocol	Duration of IM induction, d	Gene expression at the IM stage	Duration of NPC induction, d	Gene expression at the NPC stage	Total protocol duration, d	Cell types in the organoids
Taguchi *et al.*,[6] 2014	11	*OSR1*$^+$, *WT1*$^+$, *HOX11*$^+$	3	*SIX2*$^+$, *SALL1*$^+$, *PAX2*$^+$, *WT1*$^+$	20–22	WT1$^+$/NPHS1$^+$ early podocytes; CDH6$^+$ proximal tubule structures; CDH1$^+$ distal tubule structures
Morizane *et al.*,[7] 2015	7	*OSR1*$^+$, *WT1*$^+$, *HOX11*$^+$, *PAX2*$^-$, *LHX1*$^-$	2	*SIX2*$^+$, *SALL1*$^+$, *PAX2*$^+$, *WT1*$^+$	21–28	WT1$^+$/ PODXL$^+$/NPHS1$^+$ podocytes; LTL$^+$/CDH2$^+$/AQP1$^+$ proximal tubules; CDH1$^+$/UMOD$^+$ loop of Henle; CDH1$^+$ distal tubules; FOXD1$^+$ stromal cells; ENDOMUCIN$^+$ endothelial cells; α-SMA$^+$ fibroblasts
Freedman *et al.*,[8] 2015	4.5 (mesendoderm induction) + 2.5	*PAX2*$^+$	ND	*SIX2*$^+$, *WT1*$^+$	23	WT1$^+$/PODXL$^+$/ SYNPO$^+$ podocytes; CDH1$^+$/LTL$^+$ proximal tubules; CDH1$^+$ distal tubules; CD31$^+$ endothelial cells; TUJ1$^+$ neuronal cells
Takasato *et al.*,[9] 2015	7	*HOXD11*$^+$ (PIM), *GATA3*$^+$ (AIM)	5	*PAX2*$^+$/*ECAD*$^-$ (MM induction)	18–25	WT1$^+$/NPHS1$^+$ podocytes; CDH1$^+$/LTL$^+$ proximal tubules; CDH1$^+$ distal tubules; CD31$^+$/KDR$^+$/SOX17$^+$ endothelial cells; FOXD1$^+$/MEIS1$^+$ stromal cells; PAX2$^+$/GATA3$^+$/CDH1$^+$ ureteric bud-like cells
Przepiorski *et al.*,[11] 2018	ND	Early mesoderm markers on day 4	ND	ND	14	WT1$^+$ early podocytes; LTL$^+$ proximal tubules; CDH1$^+$ distal tubules; MEIS1/2/3$^+$ stromal cells; PECAM1$^+$ endothelial cells
Garreta *et al.*,[10] 2019	4	*PAX2*$^+$/*OSR1*$^+$/ *HOXD11*$^+$	5	*OSR1*$^+$/ *WT1*$^+$/ *PAX2*$^+$/ *SIX2*$^+$	20	PODXL$^+$/ PODOCIN$^+$/ NEPHRIN$^+$/ NEPH1$^+$/ WT1$^+$ podocytes; LTL$^+$/AQP1$^+$/SLC3A1$^+$ proximal tubules; ECAD$^+$ UMOD$^+$ loop of Henle; UMOD$^-$ ECAD$^+$ distal tubules; CD31$^+$ endothelial cells

AIM, anterior intermediate mesoderm; AQP1, aquaporin 1 ; CDH1, cadherin 1; CDH2, cadherin 2; ECAD, E-cadherin; FOXD1, forkhead box D1; GATA3, GATA binding protein 3; hPSC, human pluripotent stem cells; IM, intermediate mesoderm; KDR, kinase insert domain receptor; LTL, lotus tetragonolobus lectin; MEIS1/2/3, meis homeobox 1/2/3; ND, not defined; NEPH1, kirre like nephrin family adhesion molecule 1; NPC, nephron progenitor cell; NPHS1, nephrin; PAX2, paired box 2; PECAM1, platelet endothelial cell adhesion molecule 1; PIM, posterior intermediate mesoderm; SLC3A1, solute carrier family 3 member 1; SOX17, SRY-box transcription factor 17; α-SMA, α-smooth muscle actin; SYNPO, synaptopodin; TUJ1, class III beta-tubulin; UMOD, uromodulin; WT1, wilms tumor 1.
Duration of the IM and NPC induction steps and the expression of relevant markers are shown for each protocol. The total duration of each protocol and cell populations within organoids are also indicated.

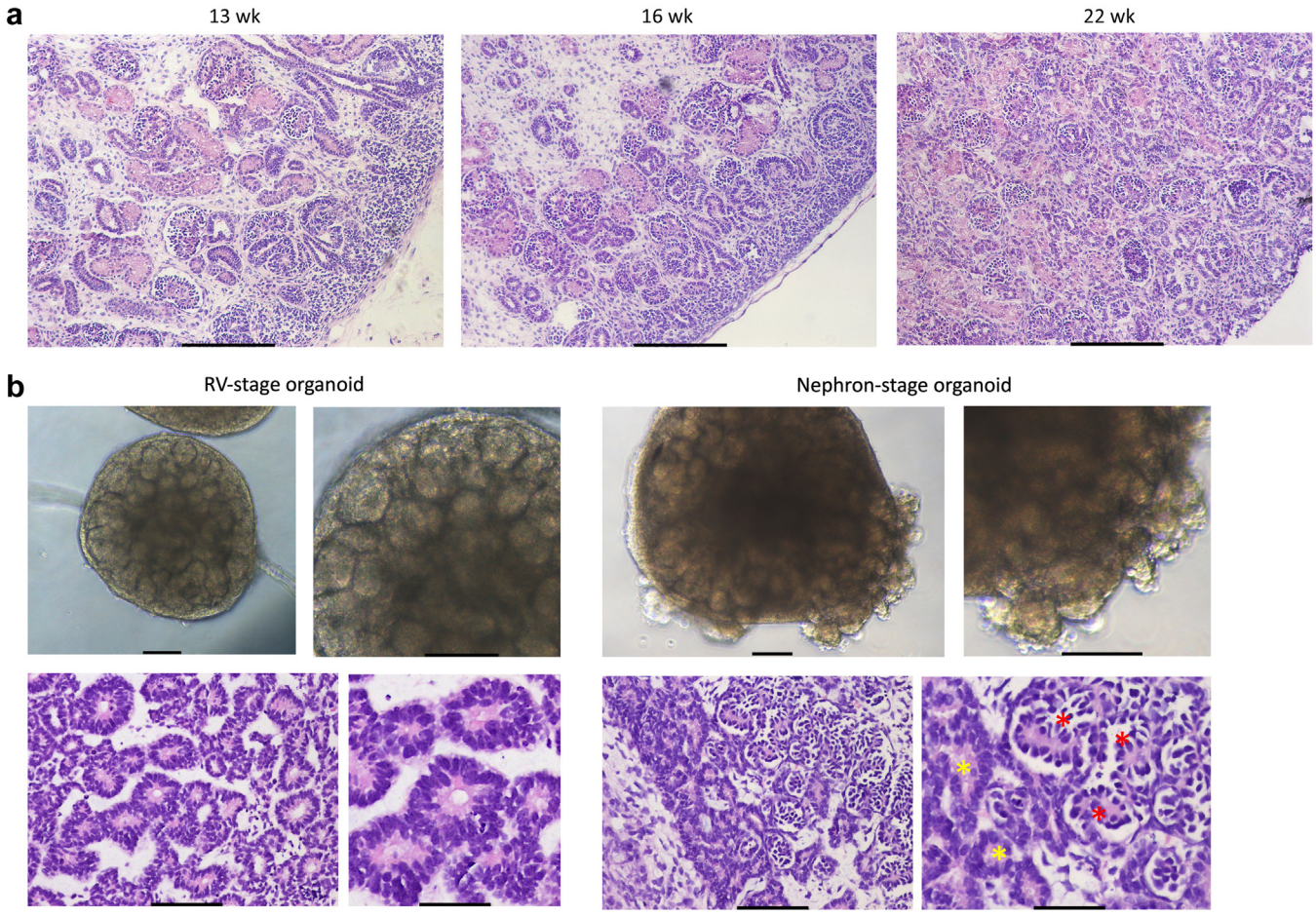

Figure 3 | Human pluripotent stem cell (hPSC)–kidney organoids can recapitulate early events of nephron induction and patterning. (**a**) Representative hematoxylin and eosin staining of human fetal kidney paraffin sections at 13, 16, and 22 weeks of gestation. Bars = 200 μm. (**b**) Representative bright-field images of hPSC-derived kidney organoids at different moments during the differentiation process. Bars = 100 μm. Histologic analysis of renal vesicle (RV)- and nephron-stage organoids. Magnified views show details of RV structures (RV-stage organoid) followed by the formation of nephron-like structures (nephron-stage organoid) that contained tubule-like (yellow asterisks) and glomeruli-like structures (red asterisks). Bars = 100 μm and 50 μm (magnified views). To optimize viewing of this image, please see the online version of this article at www.kidney-international.org.

findings, a compelling approach would be to deposit specific cell compartments (i.e., UB, NPC, and stroma) together with ECM proteins (i.e., laminins) essential for branching morphogenesis[18,57] to promote cell-cell and cell-ECM cross-talk, leading to proper branching of UB epithelial structures in hPSC-kidney organoids (i.e., collecting duct system connected to nephron-derived structures).

Engineering physiological-like environments through organ-on-a-chip technology. Microfabrication technologies have allowed the creation of miniaturized cellular models, namely organ-on-a-chip, which can partially reproduce the complex physiology of an organ.[61–63] These microfluidic systems can generate chemical gradients as well as control culture conditions in an automated manner,[64,65] which could help robustly establishing culture conditions to enhance organoid maturation and function. For example, glomerulus-on-a-chip mimicking the glomerular filtration barrier has been developed using hPSC-derived podocyte-like cells and endothelial cells.[66,67] Similarly, hPSC-derived kidney proximal tubule-on-a-chip approaches have been designed, showing promise for drug testing and disease modeling applications.[68–72] In the future, multiorgan devices[73] readily connected through a vascular network could capture key aspects of human physiology, ultimately providing unique opportunities to directly study multiorgan diseases and for personalized treatment discovery. We refer the readers to the recent mini review by Tabibzadeh and Morizane for more detailed discussion of organ-on-a-chip systems to advance organoid disease models.[62]

Future directions: toward the convergence of cell mechanics and organoid technology to understand tissue morphogenesis

Morphogenesis is controlled by chemical and physical pathways, such as deformation, remodeling, and flow, that ultimately shape tissues and organs. During organogenesis, multicellular morphogenesis-related programs (such as patterning and segmentation) are also driven by intrinsic

tissue mechanics. These processes involve a tight control over the generation of local internal forces and dynamics of ECM stiffness and viscosity, allowing the development of complex tissue shapes through deformation, local growth, and remodeling.[74]

Our current understanding on how cell biological and physical mechanisms interact to drive cell shape changes, control cell behaviors, and create tissue properties (i.e., stiffness, viscoelasticity) has largely relied on top-down approaches, whereby the mechanisms that lead to shape changes in tissues or whole organisms are deciphered combining imaging, genetic manipulations, and force inference methods.[75,76] In recent years, the convergence of hPSC-organoids and tissue mechanics techniques offers new opportunities to study human tissue development and morphogenesis. However, long-term challenges remain, such as the lack of experimental and theoretical insights to organoid mechanics.

In the past years, several techniques to probe cell and tissue mechanical forces have been developed.[74] Despite the existing challenges, some of these techniques are starting to be applied to organoid systems. Traction force microscopy maps traction stresses at the interface between a cell and its surrounding ECM in 2-dimensional and 3D settings by computing the cell-induced deformation of the ECM.[77] Current limitations toward its application in 3D organoids include the capacity of the algorithms used to calculate the forces as well as the need to know the mechanical properties of the ECM. Alternatively, genetically encoded fluorescence resonance energy transfer sensors are used to determine molecular forces both inside cells and at the plasma membrane. For instance, a fluorescence resonance energy transfer–based sensor that responds to mechanical forces exerted by the ECM on the cell can be harnessed to measure the tensions between cells and their ECM.[78] However, the low signal-to-noise ratio of the sensor may pose a challenge for precisely accessing the interior region of 3D organoids. On the other hand, the stiffness of cells or tissues can be assessed by atomic force microscopy[79] or micropipette aspiration technique.[80] Another approach to probing cellular forces involves measuring the deformations of inert materials with known mechanical properties, such as cell-sized soft droplets or gels, that are introduced into organoids or tissues.[81] The use of these droplets in organoids is often limited by the challenge of precisely microinjecting them into specific regions within the organoid. Another tool used to quantify mechanical forces is laser ablation, whereby a high-power laser pulse is used to sever cellular structures.[82] Force is then calculated from the resulting recoil of the tissue surrounding the ablation area. Because of its invasive nature, laser ablation is only suitable for measuring mechanical forces at a specific time point. Finally, optical force inference technologies[83] represent a highly innovative means to infer forces that cause a given change in organoid or tissue shape. These technologies often rely on advanced imaging techniques, such as traction force microscopy and optical tweezers, to measure forces at a microscopic scale. Machine learning algorithms are often used to process large and complex imaging data sets generated by these techniques, enhance image analysis, and infer mechanical forces more accurately.

In the next years, the establishment of new approaches to measure and monitor 3D organoid mechanics coupled to robust computational models integrating biochemical and mechanical information will represent a powerful strategy to understand and predict the emergence of self-organizing multicellular structures (i.e., nephron structures) and shapes during hPSC-organoid differentiation. In this regard, we expect that controlled *in vitro* studies using hPSC-kidney organoids could prove useful to model the interactions established between diverse cell populations within the developing kidney and study how the interplay between cell-generated forces, biochemical and biophysical signaling, and genetic programs defines hPSC-kidney organoid self-organization and differentiation.[1] This information may represent a powerful approach to provide new insights in kidney development and disease in the human context.

Outlook

The lessons learned from these past years show that externally guiding the inherent properties of hPSCs through bioengineering becomes an essential need to drive kidney organoid generation. Similarly, it is expected that the application of transcriptomics and computational tools may help in generating workflows and identity scores to infer in a quantitative, systematic, and rapid way how effective and efficient the used differentiation protocols are. We foresee that within the next years bioengineering techniques together with the convergence of sophisticated machine learning and organoid modeling will soon allow researchers to track and monitor responsive characteristics to explore healthy and disease states. For instance, automated 3D imaging of kidney organoids coupled to machine learning algorithms and deep learning techniques represent powerful tools to effectively enhance the high content phenotyping of kidney organoids.[84,85] We expect that these developments and their interaction will soon open new facets of implementation of precision medicine and health care engineering for kidney.

DISCLOSURE

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