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Human induced pluripotent stem cell-derived kidney organoids toward clinical implementations

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Abstract

The generation of kidney organoids from human pluripotent stem cells (hPSCs) has represented a relevant scientific achievement in the organoid field. Importantly, hPSC-derived kidney organoids contain multiple nephron-like structures that exhibit some renal functional characteristics and have the capacity to respond to nephrotoxic agents. In this review, we first discuss how bioengineering approaches can help overcome current kidney organoid challenges. Next, we focus on recent works exploiting kidney organoids for drug screening and disease modeling applications. Finally, we provide a state of the art on current research toward the potential application of kidney organoids and renal cells derived from hPSCs for future renal replacement therapies.

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Introduction

In the last decade, human pluripotent stem cell (hPSCs) differentiation methodologies have amazingly progressed toward the generation of three-dimensional (3D) tissue-like structures, so-called organoids. These partially resemble in morphology, cellular composition, and function to the *in vivo* developing organ. Nowadays, hPSC-derived organoids have been produced for many tissues and organs, including the brain, retina, intestine, lung, stomach, and kidney, among others. Recently, several works have demonstrated the potential of hPSC-derived organoids for disease modeling, drug toxicity testing, and drug discovery applications. Although the hPSC organoid field is rapidly growing, yet several challenges remain to be solved. These limitations include, among others, high variability in terms of differentiation extent and functional properties between organoids (even when derived from the same initial source), the lack of vascularization, and the presence of off-target cell types. Considering these drawbacks, the alliance between (bio)engineers and stem cell biologists is leading to the development of powerful approaches to control and externally direct hPSC organoid differentiation and function [1].

In this review, we will focus on the latest progress in hPSC-derived kidney organoid (hPSC kidney organoids) generation, making emphasis on how bioengineering approaches can enhance kidney organoid differentiation, vascularization, and complexity (in terms of cellular composition and functionality). In addition, we will discuss the immediate potential of hPSC kidney organoids for studying human kidney development and as valuable *in vitro* models for drug screening and disease modeling. Finally, we will pay special attention to current efforts examining the therapeutic potential of hPSC-derived nephron progenitor cells (hPSC-NPCs) and kidney organoids while highlighting the existing challenges and limitations. We will close the review by giving an outlook on the possible strategies toward the application of kidney organoids for renal replacement therapies.

Kidney organoid generation: challenges and bioengineering solutions

Current protocols for generating hPSC kidney organoids take advantage of the self-organization ability of hPSCs

in front of specific 3D culture conditions and renal inductive signals (Box 1. Protocols for hPSC kidney organoid generation). Self-organization of hPSCs depends in a significant way not only on the morphological and physical properties dictated from autologous mechanisms but also on the exogenous signals presented by the extracellular matrix (ECM).

The ECM provides both physical support and biochemical cues which are determinants during kidney development and homeostasis [2–4]. For decades, intense research has revealed the importance of the ECM remodeling and its role in mediating the transduction of developmental signals during kidney development [5]. Indeed, genetic alterations in the ECM components and the integrin receptors are responsible for diverse syndromes [6]. In the same manner, abnormal ECM deposition contributes to the progression of chronic kidney disease [7,8] and polycystic kidney disease [9]. This knowledge may inform the design of novel biomimetic materials for instructing nephrogenesis during hPSC kidney organoid generation *in vitro* (Box 2. Understanding the role of ECM in kidney development).

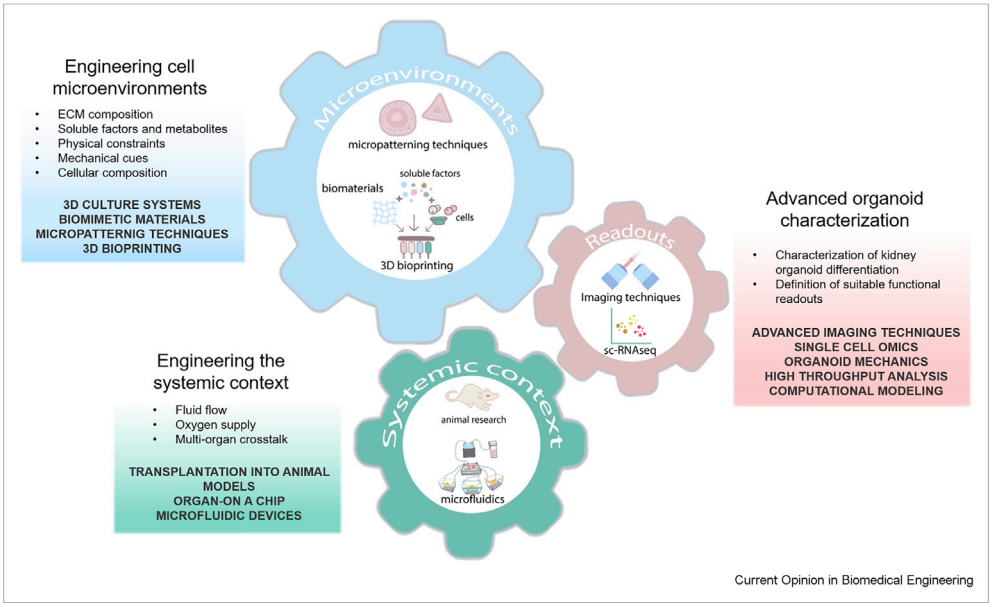
At the present time, the level of control that can be exerted externally over cellular self–organization processes occurring during kidney organoid formation and differentiation is still very imprecise. This may cause in part the high variability of kidney organoid phenotypes observed in current methodologies (even when generated from the same initial cell source) [10–12]. One possible approach to externally provide tissue-specific instructive cues that can robustly guide kidney organoid formation and differentiation might be the use of biomimetic materials as cell culture substrates that can be designed to contain growth factors and cell adhesion ligands at specific concentrations, controlled porosity, and viscoelastic properties [13]. The impact of natural- and synthetic-derived matrices in organoid generation has been recently studied in intestinal [14,15], endodermal [16], liver [17,18], and neural [19] organoids, among others. Alternatively, engineered hydrogel materials can be also used to fabricate microwell systems [20] or tissue-relevant morphologies [21]. These biomaterial-based engineering approaches offer great promise for improving kidney organoid differentiation, reproducibility, and scalability, characteristics that are specially required for disease modeling and drug screening studies (Figure 1).

Recently, enormous efforts have been directed to provide an accurate molecular, cellular, and functional signature of hPSC kidney organoids. In this regard, recent advances in single-cell omics are

Box 1. Protocols for hPSC kidney organoid generation.
Current hPSC kidney organoid methodologies take advantage of hPSC self-organization ability when applying specific 3D culture conditions and renal inductive signals guiding hPSC differentiation into intermediate mesoderm-committed cells and NPCs. At this stage of differentiation, 2D cultures are manipulated via enzymatic digestion for further aggregation by spin centrifugation, and the resulting spheroids are then maintained in 3D culture for several days. Making use of chemical compounds activating Wnt signaling, spheroids can mimic the metanephric mesenchyme condensation and mesenchymal to epithelial transition as occurs during kidney development, showing the formation of polarized renal epithelial structures, the so-called renal vesicles. Then renal vesicles further evolve into segmented nephron-like structures containing glomerulus-like structures composed of podocyte-like cells that are connected to tubules exhibiting proximal and distal tubule phenotypes. Taguchi et al. [127] were the first to develop a stepwise differentiation procedure to induce NPCs through posterior intermediate mesoderm commitment of mouse ESCs and hiPSCs that developed into nephron organoids when induced by coculture with mouse spinal cord tissue. Later, several independent laboratories published different methodologies for hPSC kidney organoid generation [128]. Although all these differentiation approaches resulted in the generation of kidney organoids containing segmented nephron-like structures, they mostly differ in the 3D culture system used (i.e. air-liquid interface culture [28,29,127] and in suspension cultures [129,130]). Other studies also reported the use of 2D culture settings using Matrigel coating [27,30]. More recently, by enhancing cell–cell and cell-ECM interactions, our laboratory developed a methodology that allowed the formation of kidney organoids that transcriptionally resemble the second trimester human fetal kidney [29].

Box 2. Understanding the biological significance of the extracellular matrix for applications in kidney organoid engineering
Cell–cell and cell-ECM interactions are known to provide signals to the cell niche. Through structural, physical, electrical, and biochemical signals present in their environment, stem cells acquire specific cell fate pathways during development. Importantly, ECM remodeling has been shown to play an essential role during branching morphogenesis in the lung, kidney (UB), mammary gland, and salivary gland (submandibular gland) [131,132]. In the kidney, spatiotemporally regulated reciprocal interactions between metanephric mesenchyme and UB progenitor cell populations dictate the repetitive formation of epithelial clefts and buds that invade the surrounding ECM. Of note, the role of ECM proteins in ureteric branching morphogenesis *in vivo* was revealed using mutant mice lacking Laminin-α5 (Lama5–/–) and Laminin-γ1 (Lamc1–/–) chains [133]. Decades of research have culminated in efficient protocols for organ decellularization enabling the community to understand the composition and organization of the native ECM and highlighting its role as a bioactive reservoir of growth factors, such as epidermal growth factor (EGF), fibroblast growth factor (FGF), and vascular endothelial growth factor (VEGF) [113]. In the future, the use of organ-specific ECM proteins may favor important processes such as self-organization and vascularization, which are key factors for hPSC organoid derivation and function. Alternatively, synthetic hydrogels hold great potential in the creation of defined organoid niches owing to their versatility producing highly tuneable environments in which stiffness, degradability, and tethered biochemical molecules (ECM-related and soluble factors) can be systematically assessed [13].

Figure 1



Bioengineering solutions for hPSC kidney organoids. In the next years major hPSC kidney organoid challenges would benefit from bioengineering technologies including biomimetic materials design, 3D bioprinting and microfluidics, among others, for engineering cell microenvironments and systemic conditions that would better replicate the in vivo context. These novel approaches together with the application of advanced characterization techniques would facilitate the generation of cutting-edge hPSC kidney organoid models for disease modeling and regenerative medicine applications.

providing quantitative means to relate the cellular composition of organoids to those of the native organs (including embryonic development, adulthood, or disease) [22]. The pioneer work by Freedman's laboratory performed single-cell RNA sequencing (sc-RNAseq) in hPSC kidney organoids revealing the presence of cell clusters with transcriptional signatures of proximal tubules, podocytes, 'early tubules,' 'early podocytes,' endothelial cells, and stromal cells, together with the presence of off-target cell populations expressing marker genes of neural, muscle, reproductive/endocrine, epithelial, undifferentiated, and proliferating cells [23]. In another study, sc-RNAseq of hPSC kidney organoids allowed the identification of cell lineage gene expression signatures shared with developing human kidneys. Interestingly, authors could identify a glomerular disease-relevant gene expression signature corresponding to the early developmental podocyte stages [24]. Wu et al. [25] performed a comparative sc-RNAseq in hPSC kidney organoids generated using two well-accepted procedures (from Little's and Bonventre's laboratories). The analysis was performed using fetal and adult kidney samples showing that while both approaches led to the generation of complex kidney organoids in terms of cellular composition, these also generated off-target cells (with neuronal and muscle identity) that accounted for around 20% of the total cells. In addition, this work further confirmed the immaturity of the renal cell types present in the organoids [25]. In another work by Phipson et al. [11], kidney organoid to organoid variability was assessed by means of sc-RNAseq reporting batch-to-batch variations between organoids generated in independent experiments. More recently, Subramanian et al. [26] compared kidney organoids generated from four different human-induced pluripotent stem cell (hiPSC) lines using two different protocols. Using sc-RNAseq analysis, the authors observed that the major cause of variability accounts from the original cell source which in turn is reflected in the presence of off-target cells.

To date, transcriptional profiling of hPSC kidney organoids together with immunofluorescence of kidney markers is frequently used to validate the differentiation of kidney organoids with nephron-like structures. In addition, hPSC kidney organoids have proved valuable as *in vitro* models for podocyte and tubule drug-induced toxicity screens, showing specific damage responses in both tubular [27–30] and glomerular structures [31–35]. In this regard, the implementation of automated high-throughput measurements and readouts for evaluating renal functionality in kidney organoids would facilitate drug screening and disease modeling applications. For example, reporter cell lines for specific renal solute transporters or injury biomarkers could be used for real-time monitoring of organoid responses by live-imaging analysis. Similarly, miniaturized biosensors for monitoring of secreted soluble biomarkers such as

metabolites or secreted proteins could be integrated into microengineered platforms for *in situ* monitoring of live organoids [36–38] (Figure 1).

Another major limitation of kidney organoids is the lack of vascularization that precludes tissue growth and renal blood filtration function. Recently, different laboratories have benefit of *in vivo* transplantation approaches to promote organoid vascularization [29,31,39,40]. When transplanted subcutaneously into immunodeficient mice, kidney organoids showed glomeruli-like structures with host vascular cell components and enhanced expression of vascular endothelial growth factor A and collagen IV [40] compared with *in vitro* cultured organoids. In another study in which kidney organoids were transplanted into the renal subcapsule of immunodeficient mice, the authors could demonstrate the ingrowth of perfusable host capillary-like structures within the glomeruli of implanted organoids [31,39]. Interestingly, our laboratory adapted the use of the chick chorioallantoic membrane (CAM) as a new approach to vascularize hPSC kidney organoids [29]. In contrast to organoids cultured *in vitro*, CAM-implanted kidney organoids exhibited enhanced glomerular differentiation features (i.e. presence of slit diaphragm-like structures between podocyte cell processes) and the detection of human endothelial-like cells in close contact with podocyte-like cells [29], indicating that this *in vivo* approach facilitated the organization of endogenous human endothelial-like cells within kidney organoids. Altogether these observations suggest that *in vivo* physiological conditions such as fluid flow may influence the organization of under-represented cell types within kidney organoids, as endothelial cells, thus contributing to improve kidney organoid vascularization. In this regard, microfluidic devices have been proved to emulate relevant fluid flow conditions and promote the formation of self-organizing perfusable vascular networks [41–43]. A recent example of the application of fluid flow to kidney organoids was reported by Homan et al. [44]. By using a printed fluidic chamber adapted to kidney organoid culture, the authors exposed hPSC kidney organoids to different fluid flow regimes, showing that shear stress enhanced the organization of endothelial cell networks within the kidney organoids [44]. In the next years, the application of microfluidic approaches to organoid culture will be used to provide physiologically relevant cues mimicking the native tissue microenvironment (i.e. biochemical gradients, fluid flow, mechanical stimuli, among others) (Figure 1).

Finally, another major challenge when trying to derive physiologically relevant hPSC kidney organoids involves the generation of a proper collecting duct system connected to the nephrons and a functional ureter. Early efforts toward this direction showed the derivation of ureteric bud (UB)-committed progenitors by exposing hPSC two-dimensional (2D) cultures to anterior intermediate mesoderm inductive signals [45–47]. In another work, Takasato et al. [28] claimed the generation of kidney organoids with a UB component based on the coexpression of GATA binding protein 3 (GATA3) and E-cadherin markers. Later, the same group reported that distal nephron segments in hPSC kidney organoids were prone to acquire a UB-like phenotype when isolated and cultured with a UB-inductive medium [48]. However, the generation of hPSC-derived UB organoids with tubular lumens or proper branching capability was not achieved. Following a different approach, Taguchi and Nishinakamura [49] independently induced NPCs and UB cells from mouse and human PSCs which were then reassembled to generate kidney organoids. Again, hPSC-derived UB epithelium failed to branch suggesting a critical role of the renal stroma to efficiently recapitulate branching morphogenesis. Recently, three independent laboratories have reported different methodologies to generate hPSC-derived UB organoids showing improved UB outcomes [50–52]. Mae et al. [46] adapted their previous reported UB protocol to achieve the generation of UB organoids within a period of two weeks. These modified culture conditions included the use of a retinoic acid agonist for nephric duct induction and the addition of low concentrations of Matrigel during the nephric duct aggregate culture to promote epithelial apicobasal polarity and lumen formation [51]. The induced UB structures contained tip and trunk domains. UB tips could be isolated, showing their ability to reconstitute new branching UB organoids on culture [51]. Notably, authors generated a heterozygous HNF1β-knockout (KO) (HNF1β+/−) hiPSC line by the Clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein-9 nuclease (Cas9) system and generated mutant UB organoids that partially recapitulated some phenotypes of the multicystic dysplastic kidney [51]. By separate induction of NPC- and UB-committed cells from hPSC, Uchimura et al. [50] proposed a coculture methodology to generate kidney organoids with nephron and collecting duct-like structures and established culture conditions for enhanced collecting duct differentiation in comparison with previous protocols [28]. In contrast to previous works, the newly formed organoids could faithfully model relevant kidney-specific molecular and cellular processes including tubular injury, collecting duct water channel translocation, and principal cell-intercalated cell interconversion [50]. Recently, Zheng et al. [52] reported 3D culture methodologies for the expansion and differentiation of primary mouse and human UB progenitor cells. These conditions were also adapted to generate expandable UB organoids from hPSC which differentiated into principal and intercalated cells [52]. Moreover, the authors used the CRISPR/Cas9 system to KO Ret/RET in mouse and human UB organoids which demonstrated the critical role of RET KO in arresting

branching morphogenesis, a phenotypical characteristic found in some forms of the congenital anomalies of the kidney and urinary tract [52].

In the future, bioengineering approaches combining ECM signals, biophysical/geometric cues, and biochemical gradients would help provide directional cues for driving correct organization of cells into kidney organoids with a proper UB tree and organized nephrons mimicking the *in vivo* tissue.

Modeling kidney disease with kidney organoids

To date, kidney organoids have started to show their utility as model systems to interrogate renal disease-related phenotypes by two different approaches: i) taking advantage of patient-specific iPSCs carrying mutations leading to renal disease; ii) making use of efficient genome editing tools, such as CRISPR/Cas9 system, in hPSCs to manipulate and/or introduce mutations related to renal diseases (Figure 2a).

A pioneering study exploiting CRISPR/Cas9 genome editing to model renal disease in kidney organoids was reported by the Bonventre laboratory. The authors developed a kidney organoid model for autosomal dominant polycystic kidney disease (ADPKD), that recapitulated tubule cysts formation, a hallmark of this kidney disease [53,54]. In another study, the same laboratory discovered a possible role for myosin in the early stages of PKD progression using a newly developed high-throughput screening platform [23].

Interestingly, a recent study has explored the different renal cyst phenotypes encountered when deriving kidney organoids from ADPKD patient-derived and gene-edited heterozygous and homozygous PKD1-mutant hiPSCs, showing how renal cysts in mutant kidney organoids responded to well-known chemical compounds that inhibit cyst formation in ADPKD [55]. Importantly, other kidney diseases that have been recently modeled with hPSC kidney organoids include congenital disorders as nephronophthisis [56], congenital nephrotic syndrome [57,58], autosomal recessive polycystic kidney disease [59], Mucin 1 kidney disease [60], cystinosis [61], and glomerulopathies [62,63].

Collectively, all these findings highlight the suitability of kidney organoids for testing safety and efficacy of potential therapeutic compounds and for drug discovery applications. Nonetheless, soon, further improvements in the composition and maturation capacity of the hPSC kidney organoid models would increase their potential for faithfully recapitulating pathophysiological mechanisms of the human kidney disease. In this regard, significant progress on the generation of UB/collecting duct structures from hPSCs has recently allowed to model human ADPKD in the collecting duct lineage [64].

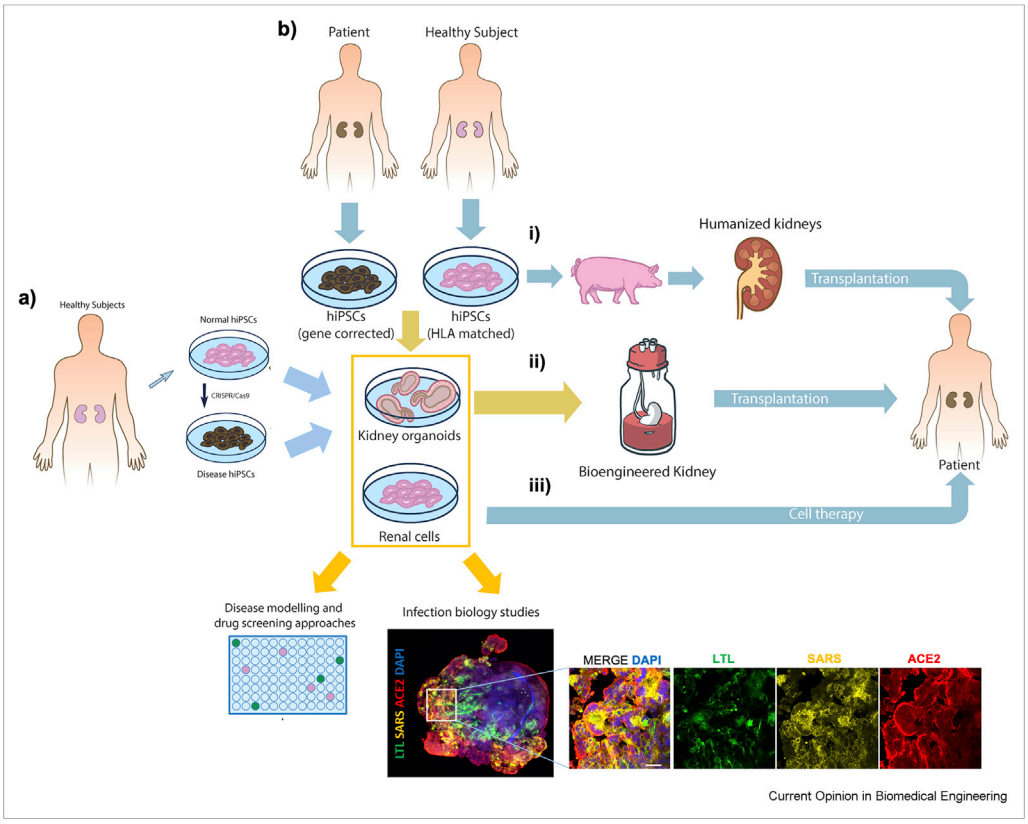
All in all, the mentioned studies have focused on recapitulating primary renal disease phenotypes. Now is expected that kidney organoids may be soon exploited to model kidney disorders accounting for prevalent diseases (i.e. diabetic nephropathy). In this regard, other works have already proved the amenability of hPSC-derived organoids to explore systemic conditions as diabetes under static culture conditions, including hPSC-derived vascular [65] and heart [66] organoids, and thus expand the utility of organoids to explore early hallmarks for diabetic systemic conditions in these tissues. Interestingly, by applying a bioprinting approach, a 3D vascularized proximal renal tubule model including fluid flow has faithfully recreated the renal reabsorption function and was used to model hyperglycemia-induced renal injury [67]. Based on these preliminary approaches, it is expected that the use of microfluidic devices will better recapitulate the critical disease-associated pathological responses of the *in vivo* kidney. Importantly, different studies have reported the establishment of glomerulus-on-a-chip mimicking the glomerular filtration barrier [68–71] and renal proximal tubule-on-a-chip [72,73] models for drug testing and disease modeling applications. In addition to present microenvironmental cues and dynamic culture conditions in a controllable fashion [42,43], these systems show the possibility to interconnect multiorganoid models and incorporate a perfusable vascular system, thus recapitulating crosstalk across different tissues [74]. Therefore, it is expected that the use of these systems will facilitate the creation of physiologically relevant models from hPSCs for studying complex kidney disorders (i.e. cardiorenal crosstalk malfunction, among others).

Studying severe acute respiratory syndrome coronavirus 2 infection using kidney organoids

Early in December 2019, a novel coronavirus — severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) — emerged in humans in Wuhan, China, and has since disseminated globally [75,76]. As of 21st April 2021, the confirmed case count of coronavirus disease 2019 (COVID-19) had surpassed 68 million with 1.5 million confirmed deaths.

From December 2019, several studies demonstrated that SARS-CoV-2 shares multiple similarities with SARS-CoV, including a high conservation in the receptor-binding domain, thereby suggesting a common host cell receptor [76–78]. At the present time, accumulated

Figure 2



Applications of Kidney organoids derived from hPSCs. (a) Kidney disease modeling. Recently several works have shown the possibility to model congenital kidney disorders via generation of patient-derived hiPSC carrying specific disease mutations or by CRISPR/Cas 9 genome editing of well-known disease mutations into hPSCs. Following hPSC differentiation into kidney organoids, these showed to partially replicate relevant disease phenotypes. Moreover, hPSC kidney organoids have demonstrated their utility as robust in vitro models for understanding SARS-CoV-2 infection. **(b) Kidney regeneration strategies.** The derivation of renal cells and kidney organoids from hiPSC (either gene corrected hiPSC from patients or human leukocyte antigen (HLA) matched hiPSC from healthy donors) holds enormous promise towards the development of therapeutic approaches for kidney regeneration. These include xenogeneic kidney organ generation approaches (i), the fabrication of kidneys de novo through bioengineering technologies including decellularization and 3D bioprinting (ii), or the transplantation of hiPSC-renal cells and kidney organoids into the damaged kidneys of patients (iii).

evidence shows that the surface unit of the spike protein (S) of SARS engages the angiotensin-converting enzyme 2 (ACE2) as the entry receptor.

To date, different works have shown ACE2 expression in multiple extrapulmonary tissues including heart, kidneys, blood vessels, and intestine [79—81], overall showing the wide distribution of ACE2 across the human body. Indeed, multiorgan damage and dysfunction associated with SARS-CoV-2 include myocardial dysfunction [82], gastrointestinal and liver [83] disorders, and acute kidney injury (AKI) [84].

Since the beginning of the COVID-19 pandemic, the study of ACE2-SARS-CoV-2 interactions has relayed in the use of animal models, animal cells from humans or primates (including Caco-2 or VeroE6 cell lines, respectively), and human immortalized cells. As these cellular models presented limitations such as species barriers and the potential lack of the specific target cell, the organoid community quickly started translating the use of organoid models to understand the first steps of SARS-CoV-2 infection and to explore therapeutic compounds blocking or disrupting these processes. In this regard, two early works generated human bronchial organoids for SARS-CoV-2 research [85,86] and evaluated antiviral effects of COVID-19 candidate therapeutic compounds [86]. Because SARS-CoV-2 affects several organs, we investigated the possibility to exploit kidney organoids for studying the early steps of SARS-CoV-2 infection (Figure 2a). Our work demonstrated that, after our differentiation procedure, kidney organoids predominantly express ACE2 in proximal tubular-like cells by sc-RNAseq [87], as occurs in the native human and mouse kidney [88,89]. Based on these findings, we evaluated the capability of kidney organoids

to be effectively infected by SARS-CoV-2 also demonstrating that these produce infectious progeny virus and verified human recombinant soluble ACE2 as an inhibitor of infection of SARS-CoV-2 in both kidney and vascular organoids [87]. In the same line, the work from Zhao et al. [90] has demonstrated that human liver ductal organoids are permissive to SARS-CoV-2 infection and support replication.

All in all, organoid model systems are becoming rapid and effective toolsets to interrogate and target ACE2-SARS-CoV-2 interactions as well as to explore therapeutic compounds blocking or disrupting these processes. Still, organoids cannot reproduce the systemic symptoms associated with whole-body responses to the viral infection. In this regard, the combination of microfluidic platforms to precisely present systemic conditions known to worsen or exacerbate COVID-19 progression will largely benefit the field when interrogating for complex processes that may occur in human organs during infection (i.e. diabetic microenvironments, hypertension, among others). In this regard, our group has started to investigate the impact of diabetic-like microenvironments in SARS-CoV-2 infection in hPSC kidney organoids establishing a direct link between hyperglycemia and ACE2 expression. Furthermore, through the generation of KO hPSCs for ACE2 using CRISPR/Cas9 genetic engineering, our work also identifies the essential role of ACE2 for SARS-CoV-2 infection in kidney organoids under normoglycemic or hyperglycemic culture conditions [91]. In the near future, we believe that the realization of loss-of-function screens taking advantage of CRISPR/Cas9 genetic engineering will pave the way to the identification of new pathways determinant for COVID-19 treatment.

Potential of kidney organoids for cell therapy and tissue replacement

In the future, it is expected that the development of new hiPSC-based therapies may ameliorate or cure patients suffering from kidney disease. Major hiPSC-based approaches toward kidney regeneration include the use of gene-corrected patient-specific hiPSCs for addressing genetic kidney diseases, the use of hiPSCs from healthy donors to differentiate kidney organoids or renal cells for cell therapy, and fabrication of functional kidneys *de novo* for kidney transplantation (Figure 2b). However, up to date, none of these approaches has reached preclinical or clinical trials.

Few studies have been undertaken to test the therapeutic potential of hPSC-derived renal progenitors by transplantation of hPSC renal progenitors into mouse models of [92,93] that showed partial recovery of kidney function. Additional investigations will be necessary to assess the therapeutic mechanisms (direct or paracrine) of hPSC renal progenitors. Moreover, it will be important to define culture conditions for long-term expansion of hPSC renal progenitors while maintaining their capacity to reconstitute nephrons [94,95].

Furthermore, the identification of a well-defined NPC population during hPSC kidney organoid generation is missing. At the present time, the different protocols for hPSC kidney generation rely on a single epithelialization induction step that differs from the process of differentiation in the native developing kidney in which renal progenitors at numerous stages of differentiation coexist within the organ as recently described by MacMahon laboratory [96]. Following this logic, the laboratory of Oxburgh has recently shown that mixing newly differentiated cells with cells that had been aggregated in organotypic conditions for two days results in the formation of organoids with increased numbers of proximal and distal tubule cells, connecting segment or collecting duct, podocytes, and stromal cells. Interestingly, the network of endothelial cells in organoids from heterochronic mixing was more extensive and complex than that seen in organoids generated from a single-directed differentiation [97]. Further studies exploiting this approach may increase our understanding on how to develop new venues to define differentiation procedures sustaining for the derivation of expandable NPCs with nephrogenic potential. This basic knowledge will be of major benefit when envisioning novel prospects for renal replacement applications.

In vivo transplantation of hPSC-NPCs and hPSC kidney organoids under the renal capsule of immunodeficient mice [31,39,40,98] or onto the chick CAM [29] produced hPSC kidney organoids with improved maturation features. Interestingly, a recent study used decellularized ECM hydrogel from a porcine kidney for transplantation of hPSC kidney organoids into the renal subcapsule of mice, showing that, under these conditions, kidney organoids exhibited less mal differentiation than those implanted in the absence of the hydrogel [99]. In the future, it will be necessary to develop new approaches to improve kidney organoid maturation and function before transplantation. In this regard, a compelling work has recently reported the engraftment of human liver organoids into human livers that were maintained *ex vivo* in a normothermic perfusion system [100]. These findings suggest that *ex vivo* human perfused organs can represent useful models for assessing functional engraftment of human cells and organoids into human organs. In the future, the implementation of this methodology to hPSC organoids could serve for validating functional organoid

engraftment and safety, thus paving the way toward the clinical translation of hPSC organoids for cell therapy and tissue replacement therapies.

With respect to *de novo* generation of functional human kidneys using hPSCs, the field is exploring different approaches. One approach consists in the generation of xenogeneic kidneys *in vivo* through interspecies blastocyst complementation [101]. This involves the injection of hPSC into blastocysts obtained from genetically modified animals in which the formation of the organ has been partially prevented, thereby providing the organ developmental niche in where hPSC would develop into the missing organ. Alternatively, another strategy for xenogeneic kidney generation, called the organogenic niche method, involves the depletion of NPCs from developing kidneys of a recipient animal and subsequent transplantation of hiPSC-NPCs [102–104]. This methodology has been successfully performed to regenerate mouse nephrons by *in vivo* injection of rat NPCs under the embryonic renal capsule (nephrogenic zone) of a transgenic mouse model [102,103]. In a follow-up study, a novel transgenic mouse model to ablate NPCs using tamoxifen was used to transplant hiPSC-derived NPCs, which differentiated into renal vesicles with connection to the host UB. However, human mature nephrons did not form [104], suggesting that divergence in renal developmental signals between the human and mouse prevents nephron formation. In this regard, different laboratories have investigated chimeric competency of hPSCs for interspecies organogenesis via blastocyst complementation [101,105–107]. In a recent study, human extended PSCs having improved chimeric capabilities [108] were used for chimera studies in the monkey, a host species evolutionarily close to humans [109]. Injection of human extended PSCs into monkey blastocysts showed contribution to both embryonic and extraembryonic lineages (though kidney lineage was not referred to) in cultured monkey embryos. sc-RNAseq analysis of human-monkey chimeric embryos when compared with control human and monkey embryos showed transcriptomic differences and identified signaling pathways with a potential role in modulating interspecies chimerism [109]. Future research will be fundamental to develop strategies that can improve human chimerism in other species such as the pig, paving the way toward the use of this technique for regenerative medicine applications including organ transplantation. Nonetheless, these xenogeneic organ generation approaches face major challenges that include ethical concerns, the potential immune rejection owing to the presence of host animal-derived cells into the newly formed kidneys [107,110] and the risk of cross-species viral transmission [111].

Another approach relies on the application of bioengineering technologies such as whole-organ decellularization/recellularization technique and 3D bioprinting for *the novo* fabrication of bioartificial kidneys *ex vivo* [112,113]. Although the use of simple engineered tissues such as the skin or cornea is already a reality in the clinical practice, the reconstruction of complex organs such as the kidney remains an unmet challenge. Importantly, encouraging results have recently reported the creation of engineered intestinal tissue using patient-derived intestinal organoids in combination with decellularized human intestinal scaffolds [114]. For the kidney, in the past decade, different methodologies have been established for the generation of kidney-derived matrices through decellularization, which relies on the removal of cellular material by the use of detergents and enzymes while preserving the composition and structure of the ECM [113,115]. The pioneering study by Song et al. [116] showed that decellularization and recellularization of the whole rat kidney with rat neonatal kidney cells could produce rudimentary urine after *in vivo* orthotopic transplantation into rats. More recently, other studies have used mouse or human PSCs for repopulating acellular kidney scaffolds from different species including mouse [117], rat [118], and monkey [119]. Nonetheless, this technology still faces important challenges before bioengineered kidneys can reach into the clinical practice: first, the need to meet specific culture requirements and efficient differentiation approaches to sustain kidney cell maturation; second, the need for efficient recellularization owning the complexity of the kidney, containing more than 26 different cell types; third, the potential problems of immune rejection [120]. Another line of research in the field of tissue engineering involves the application of 3D bioprinting for creating human-scale tissues and morphologies [121], which cannot be reproduced in classical 3D culture systems. Moreover, hydrogels fabricated from decellularized tissues can be used as bioink materials for 3D bioprinting [122–124]. These tissue-specific hydrogels have recently shown to support the derivation of mouse and human primary tissue-derived endodermal organoids [16] and improve the maturation of hPSC-derived brain [19] and liver organoids [125]. Recently, a bioink-free bioprinting strategy has been used to produce hPSC-derived kidney organoids with different conformations [112]. In another study 3D bioprinting was applied to guide the formation of intestinal morphologies using intestinal cells from primary mouse and human tissues [126]. Although much research is still required to achieve larger and functional kidney tissue analogs by 3D bioprinting, this technology might be applied to the fabrication of high-throughput organoid platforms for

drug screening and disease modeling. Future investigations in the tissue engineering field will need to provide strategies for scaling up the production of hPSC-derived renal cell types together with the development of organ bioreactor systems that support hPSC-bioengineered kidney maturation and function.

Conclusions

In the future, the production of hiPSC-derived cell products for renal cell therapy and the generation of bioengineered kidneys may provide effective therapeutic approaches for restoring kidney function in patients. Nonetheless, the applicability of these approaches into the clinic is still far from reality. Several hurdles are yet to be solved, including the immune competence of hiPSC derivatives, the scalable production of hiPSC kidney cells and organoids together with the development of new methodologies to evaluate their safety and therapeutic effectiveness, and the definition of kidney culture conditions for bioengineered kidney's maturation before transplantation, among others. Meanwhile, recent progress in hPSC kidney organoid generation in combination with bioengineering technologies to design cell microenvironments and emulate physiological cues offer great promise for improving kidney disease modeling and drug toxicity evaluation.

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.

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