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REVIEW

Design and strategy for manufacturing kidney organoids

Masaki Nishikawa[1] · Yasuyuki Sakai[1] · Norimoto Yanagawa[2,3]

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Abstract
Despite a continuing increase in the number of patients suffering from chronic kidney disease, currently available treatments for these patients, such as dialysis and kidney transplantation, are imperfect. The kidney is also a critical target organ vulnerable to the toxicity of various new drugs, and the lack of a reliable in vitro culture model imposes a severe limitation on drug discovery. Although the development of induced pluripotent stem cells (iPSCs) revolutionized strategies in biomedical fields, the complexity of the kidney imposed additional challenge to the application of this technology in kidney regeneration. Nonetheless, the recent advancement in our understanding on the developmental origin of kidney progenitor cells and the mechanisms of their reciprocal induction and self-organization has boosted research in kidney regeneration. Research since then has demonstrated that kidney organoids derived from iPSCs can serve as a useful model for drug discovery and toxicity screening, as well as for disease modeling, especially in combination with gene editing techniques. Moreover, attempts at kidney organoid implantation in animals have suggested their potential as an alternative source of kidney transplantation. In this review, we summarize recent progress on the generation of kidney organoids, as well as the obstacles that remain.

Keywords Kidney organoid · Kidney development · iPSC · Progenitor cell · Self-organization · Regenerative medicine

Introduction

The kidney is a vital organ for the maintenance of our body homeostasis, and the patients suffering from end stage kidney disease (ESRD) require renal replacement therapies. However, currently available renal replacement therapies, such as dialysis and kidney transplantation, are imperfect due to poor quality of life, high cost, and donor shortage. ESRD therefore remains a major health issue worldwide. Since the kidney is also a critical organ participating in drug metabolism and excretion, as well as a target organ vulnerable to various drug toxicities, the kidney has attracted attention from both healthcare and drug discovery fields.

✉ Masaki Nishikawa
masaki@chemsys.t.u-tokyo.ac.jp

[1] Department of Chemical System Engineering, School of Engineering, University of Tokyo, Rm. 5A01, Engineering Bldg. 3, Hongo 7-3-1, Bunkyo, Tokyo 113-8656, Japan

[2] Medical and Research Services, Greater Los Angeles Veterans Affairs Healthcare System at Sepulveda, North Hills, CA, USA

[3] David Geffen School of Medicine, University of California at Los Angeles, Los Angeles, CA, USA

The establishment of induced pluripotent stem cells (iPSCs) opened a new era of regenerative therapy and drug discovery [1, 2] and led to various novel strategies for organ and tissue regeneration, including the kidney [3–7]. However, because of the complexity of the kidney structure and function, kidney regeneration has been especially challenging. With the demonstration that random aggregates of dispersed kidney progenitor cells can undergo self-organization and develop into a more complex structure [8], kidney organoids have thus been considered to serve as a "seed structure" of human kidney that provides opportunity to study human kidney development, disease modeling, drug screening, and eventually regenerative therapy [9–13]. This review will focus on the recent progress of kidney organoids and the remaining obstacles that need to be overcome before they can be used for these applications.

Kidney progenitor cells and their developmental origins

Understanding of the developmental process is crucial for in vitro generation of organoids from iPSCs. The kidney is a complex organ that consists of more than 20 different types

of cells organized in an intricate three-dimensional structure, so de novo assembly of a fully developed adult kidney is beyond the capacity of our current technologies [14]. However, when we reflect on the development of all organs, they all develop from a rather simple structure, which may give us a more feasible starting point to assemble. The primordial kidney, called metanephros, consists of mainly three lineages of progenitor cells, i.e., nephron progenitor cells (NP), ureteric bud cells (UB) and stromal progenitor cells (SP) (Fig. 1). The development of the metanephros begins with the invasion of UB from the caudal end of the Wolffian/nephric duct into a neighboring region of mesenchymal cells, known as metanephric mesenchymal cells (MM). MM are a mixture of mainly NP and SP at embryonic day 10.5 (E10.5) in mouse. Upon UB invasion, NP start to aggregate surrounding the tip of the invading UB, called cap mesenchyme (CM), while SP form an outer layer covering this structure [15, 16] (Fig. 1). Thereafter, mutual interactions among these three lineages of progenitor cells control their self-renewal and differentiation, leading to the formation of glomeruli and nephron tubules from NP, the collecting system and ureter from UB, and the supportive interstitial tissues from SP [17–20] (Fig. 2). While the origin of SP is not yet fully elucidated, extensive investigations revealed the developmental processes of NP and UB in detail. Until recently, both NP and UB were thought to originate from the same cell population derived from intermediate mesoderm (IM). However, it was later shown that IM can be divided into at least two distinct populations: anterior and posterior IM, and that anterior IM gives rise to UB, whereas posterior IM gives rise to NP [21] (Fig. 3).

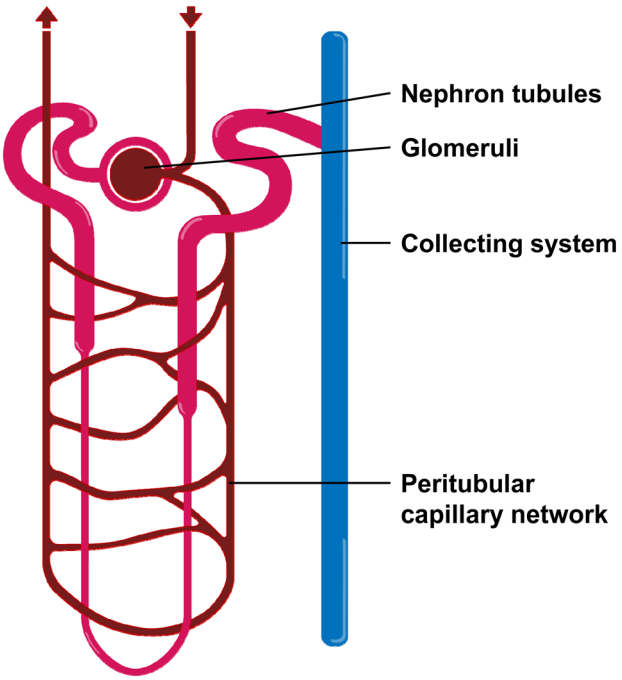

Fig. 2 The nephron structure. Glomeruli consist of capillary network and cells derived from NP and SP. Nephron tubules are also derived from NP. The collecting system and ureter are from UB

Current status of kidney organoids

The advancement in our knowledge on kidney development helped to advance the preparation methods of both kidney progenitor cells and kidney organoids [10, 21–26] (Fig. 4). Following the developmental process, Taguchi et al. have successfully induced NP from both mouse embryonic stem cells (mESCs) and human iPSCs (hiPSCs) [21]. When cocultured with spinal cord, the induced NP were shown to differentiate into series of epithelial cells, starting from podocytes down to the distal tubules. Of note is that both mouse and human share similar developmental cues and steps, except for the difference in the duration of each step. Takasato et al. [26] also generated a relatively simple method for NP induction, while Morizane et al. [25] reported a method to induce NP that requires a shorter induction period. These NP were shown to further differentiate into renal tubules in heterogeneous cell aggregates.

The induced proximal tubule like structures in these organoids can reproduce selective nephrotoxicity of certain drugs, indicating that they share some characteristics with in vivo kidney [10, 25, 27, 28]. These results suggest the potential of kidney organoids to serve as a patient-specific in vitro model to test drug-induced nephrotoxicity, which accounts for most cases of drug development failure in

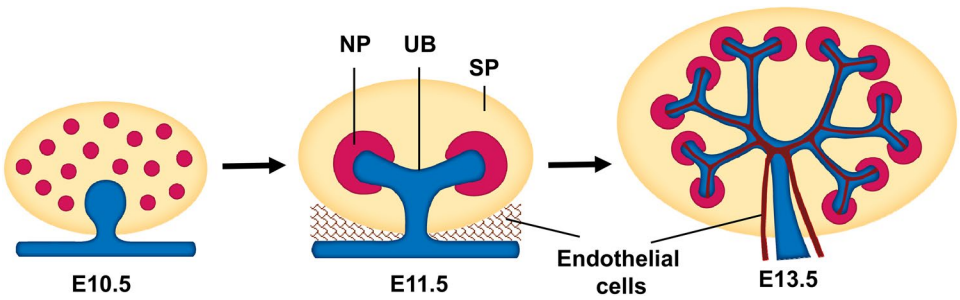

Fig. 1 Three progenitors of the kidney and the typical image of their topological positions in mouse metanephros. The reciprocal induction among these three progenitors renders the mechanism of the structural self-organization. Nephron progenitor cells (NP), ureteric bud cells (UB) and stromal progenitor cells (SP)

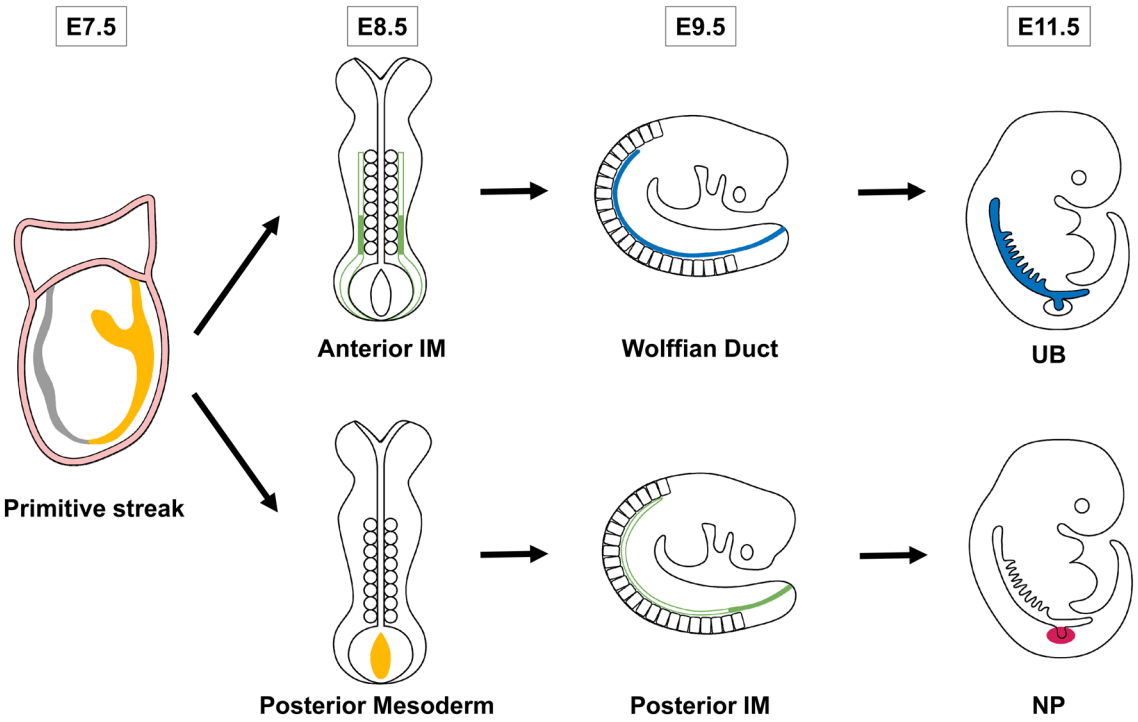

Fig. 3 Schematic summary of kidney development. Primitive streak gives rise to IM, which is the origin of the kidney. Recent progress demonstrated that IM can be divided into at least two distinct populations, anterior and posterior IM, and that anterior IM gives rise to UB, whereas posterior IM gives rise to NP. The developmental origin of SP remains not fully elucidated

clinical trials [29]. Kidney organoids derived from hiPSCs have also been shown to serve as disease models. Researchers have demonstrated that organoids consisting of cells with a mutation in a causative gene of a certain disease, such as polycystic kidney disease (PKD), congenital nephrotic syndrome, podocytopathy and nephronophthisis (NPH), could reproduce similar pathological condition in vitro [11–13, 30]. Thus, the combination of CRISPR-Cas9 gene editing and kidney organoids generated from patient-derived hiPSCs is becoming a powerful tool for kidney disease modeling and drug screening.

Induction of UB has also been reported [10, 24, 31]. Takasato et al. modified their NP induction protocol and generated GATA3 positive tubular structures in the induced kidney organoids. This protocol enables simultaneous induction of multiple lineages of kidney progenitor cells and may have an advantage in the applications for disease modeling and drug screening [10]. One potential obstacle, however, is the contamination of untargeted cells in the resulting organoids. Single-cell RNA sequencing revealed that these organoids contained non-kidney cell types, including neuronal and muscle cells [32, 33]. Wu et al. performed time course analyses of transcriptomes at the single-cell level during the differentiation of these organoids and revealed specific expressions of receptors and ligands in off-target cells. They also demonstrated that inhibition of these pathways could successfully deplete the related off-target cells in these organoids without affecting kidney differentiation [32]. These continuing efforts could significantly improve the quality of kidney organoids derived from such one-step induction protocols. On the other hand, Taguchi et al. reported a direct induction protocol specific for UB that enabled the generation of higher-order kidney organoids [24]. Despite the complexity of this induction protocol, it holds a clear advantage of minimizing contamination by non-kidney-related cells. Moreover, the induced UB underwent extensive branching and mimicked the well-organized structure of kidney epithelia when combined with both NP induced from hiPSCs and primary SP derived from mouse embryonic kidneys.

Setbacks and perspectives for in vitro assembly of transplantable kidneys

Functional and structural immaturity is being recognized as one of the limitations of kidney organoids. Transcriptomic analysis revealed that kidney organoids are equivalent to the first trimester of human [10] or E15.5 of mouse [24] embryonic kidney. The immaturity of kidney organoids could be related to the lack of blood supply and the concomitant lack of urinary flow.

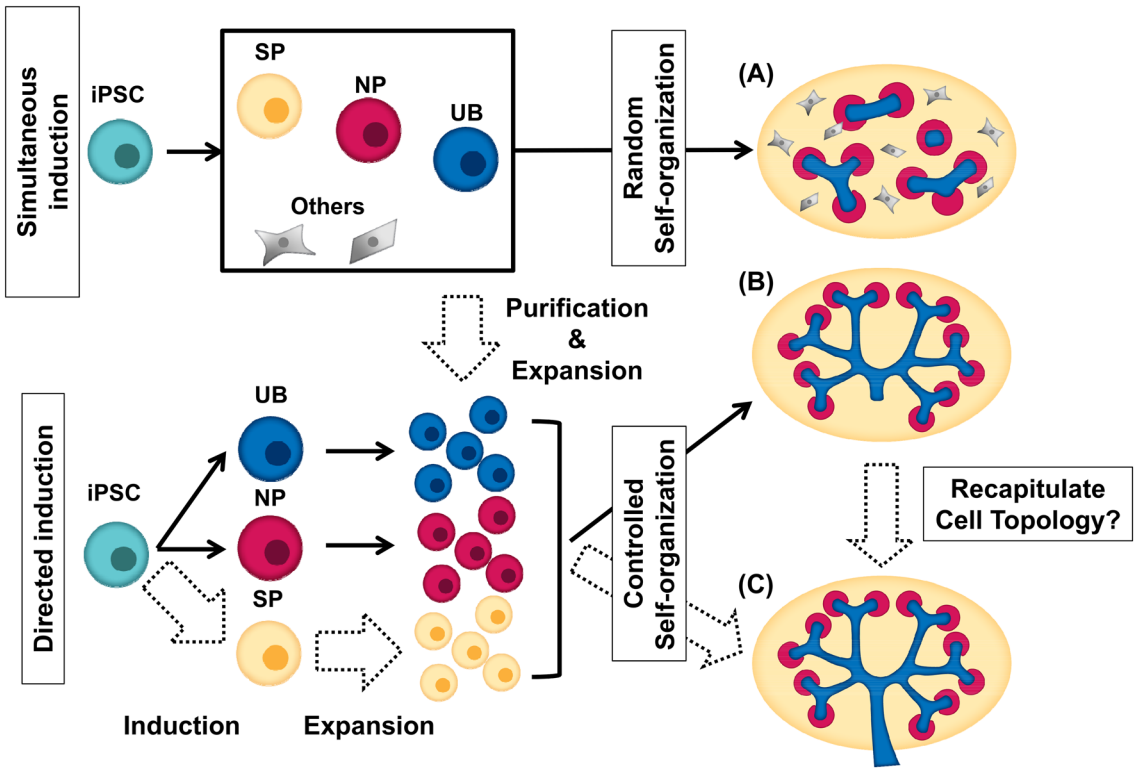

Fig. 4 Schematic summary of the current preparation methodologies of kidney organoids and future challenges. Induction and expansion protocols of SP are yet to be established. Random self-organization of dispersed cells results in partial organization with discrete UB (**a**). Controlled self-organization without (**b**) or with (**c**) preservation of cell topology may recapitulate in vivo kidney development and the structure

These defects may be solved by in vivo implantation. Many kidney organoids contain vascular endothelial cells, and taking the mechano-bioengineering approach, Homan et al. [34] reported improved vascularization and maturation of kidney organoids under flow using a microfluidic device. However, vascularized glomeruli are rarely observed in vitro [8, 10]. In previous reports, Rogers et al. [35] implanted E15.5 rat metanephroi, which are equivalent to E13.5 in mouse, onto the omentum of adult rat hosts, and observed vascularization into glomeruli with urine production from implanted metanephroi, while transplantation of developed kidneys failed to exhibit this functionality. Other reports have also demonstrated better integration and maturation of metanephroi when obtained from earlier developmental stages [36, 37]. This could be related to the degree of blood vessel development in the metanephroi. The embryonic mouse heart typically starts beating around E8.5 [38], and although E14 metanephros in mouse was previously considered avascular [39], Munro et al. [40] reported that kidney vascularization began as early as E11 from peri-Wolffian vessels, which are connected to the circulation of the embryo. The vascularization advances to the entire metanephros by E12.5 and forms vascular plexuses surrounding CM and UB by E13.5. Daniel et al. [41] also observed the onset of arteriovenous specification and renal arterial development at E13.5. Taken together, E13.5 metanephros, or kidney organoids at the equivalent stage of maturity, may be the latest developmental stage where implantation can be performed without vascular anastomosis.

For successful implantation of kidney organoids, it is also important to consider the relative contributions of intrinsic and extrinsic endothelial cells to vascularization. In the case of implantation of iPSCs-derived kidney organoids, the contribution of intrinsic endothelial cells was found to be limited [42, 43], while it was found to be dominant in implanted intact metanephroi [44]. Xinaris et al. [45] implanted kidney organoids from single-cell suspensions derived from E11.5 metanephroi and observed further maturation with vascularized glomeruli. Murakami et al. [9] reported implantation of reconstituted aggregates of sorted embryonic kidney progenitors combined together with an intact E11.5 UB structure, and observed the formation of an arteriolar network and glomerular capillaries. Interestingly, they found that the CD31-/Flk1-fraction in the embryonic kidney contains unidentified endothelial precursors that contribute to the majority of the renal vasculature upon implantation. These studies may suggest that the existing induction protocols for kidney organoids fail to produce these unknown endothelial

precursor cells. This unknown population seems to reside in the SP fraction [9] and may be identical or overlap with CD146 +/CD31-endothelial precursor cells that were found to contribute to the vasculature development in vitro [46]. However, the origin of the SP population remains largely unknown. Identification of the origin of this precursor cell and establishment of an induction protocol from iPSCs will facilitate the development of implantable kidney organoids.

The lack of a congruent urinary draining system is another major defect of currently available kidney organoids. Kidney organoids developed from dissociated metanephroi or induced iPSCs result in disconnected UB structures [8, 10]. Once vascularized upon implantation, these organoids may produce urine, but they soon suffer from hydronephrosis due to the accumulation of urine that cannot be drained. To circumvent this shortcoming, improved dissociation and re-aggregation systems have been reported, where UB branching was induced from a single UB structure that resulted in a higher-order structure capable of draining urine [47, 48]. A preliminary attempt has also been reported to induce a uroplakin-positive ureter-like structure from the part of UB exposed to BMP4 [49]. Nonetheless, reports thus far have yet to demonstrate successful induction of a functional ureter from the UB structure of kidney organoids with enough length to be connected to the host ureter when implanted [9, 24, 49]. Toward this end, Hauser et al. [50] proposed a method to generate a single tubule structure from dispersed UB cells on a micro-patterned mold that showed response to glial cell-derived neurotrophic growth factor (GDNF). However, the UB tube thus generated does not have peristaltic function, as is required for the ureter to expel urine into the urinary bladder. For this purpose, ureteric SP cells are required. Therefore, preservation of topology of progenitors in combination with proper tissue-engineering techniques is necessary for further improvement.

Besides these qualitative defects, kidney organoids created with currently available methods also hold quantitative shortage. It has been estimated that, with current technology, kidney organoids contain only up to ~ 100 nephrons, as compared to up to ~ 1,000,000 nephrons in human kidneys [51]. It might be enough for certain applications, such as drug screening and disease modeling, but intensive scale-up is clearly required before they can be used for organ replacement. Although optimization of organoid culture has also been attempted [49, 52, 53], the procedure of transplantation as a whole may need to be optimized to replace the function of kidney.

Considering the need for scaling-up, the development of efficient methods to induce and maintain the respective lineage of kidney progenitor cells is another important subject. The available protocols to induce kidney progenitor cells from iPSCs are often inefficient and costly. Improvement in such protocols to better maintain and expand the progenitor cells should make the technology of kidney organoids more affordable and accessible to those with different expertise. Several groups have reported the maintenance and expansion of NP using various signaling pathways, such as WNT-β-catenin, fibroblast growth factors (FGFs), and bone morphogenic proteins (BMPs) [54–57]. Li et al. [55] successfully maintained NP derived from human embryos and hiPSCs for more than 7 and 2 months, respectively, and demonstrated their ability to differentiate into glomerular and tubular cells. As for UB cells, Yuri et al. [58] reported the combination of factors, including GDNF, FGF, WNT-b-catenin, retinoic acid (RA), and Rho-associated kinase (ROCK) inhibitor, to expand dispersed single UB cells derived from mouse embryonic kidneys that retained the in vivo characteristics of the original UB and were capable of reconstructing UB-like branching structures from a single UB cell. Regarding SP, although the existence of differentiated SP is reported in kidney organoids [10, 59], currently neither induction nor expansion methods have been established yet. Due to their heterogeneity and obscure developmental origin, establishing protocols for directed induction and expansion of SP may not be straightforward; however, such protocols are certainly required for further refinement of kidney organoids, especially within clinical applications. Further improvement and/or establishment of efficient preparation methods of progenitors is required for more accessible and cost-effective kidney organoid generation.

To tackle these obstacles, animal models such as rodents are indispensable, but possible interspecies differences should be carefully considered. Anatomically, for example, a distinct lobed structure is observed in human kidneys but not in mouse. In genetic regulations, some distinct differences were reported in the expressions of marker genes of progenitor cells. Six1 continues to be expressed in human NP and function together with Six2, but in contrast only Six2 plays a critical role in mouse NP [60]. Some transcription factors that are expressed exclusively in mouse SP, such as Foxd1, are expressed in both SP and NP in human [61]. These interspecies differences may undermine the direct translation of the results between mouse and human, and thus careful examinations would be necessary.

Summary

The discovery of hiPSCs and the accumulation of basic developmental knowledge have brought enormous progress to the generation of kidney organoids. However, there remain obstacles that need to be overcome, such as the lack of vascularization and the lack of a draining system for urinary excretion. Also, methods for derivation and expansion of SP remain to be developed. As discussed above, reconstitution of proper topology of progenitor cells that mimics the

metanephros at early developmental stage is crucial to fill up these deficiencies, and under current technologies, further maturation can be achieved only by transplantation. These limitations and obstacles are common issues in the development of both implantable artificial kidneys and in vitro kidney models for disease and drug screening. It seems inevitable that close and synergetic collaboration between the fields of biology and engineering will be required for successful generation of kidney organoids useful in medical and pharmaceutical fields.

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Compliance with ethical standards

Conflict of interest The authors declare that there is no conflict of interest.

Ethical approval This study does not contain any studies with human or animal subjects performed by any of the authors.

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