Trends in Cell Biology

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Review

Advancements in kidney organoids and tubuloids to study (dys)function

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The rising prevalence of kidney diseases urges the need for novel therapies. Kidney organoids and tubuloids are advanced *in vitro* models and have recently been described as promising tools to study kidney (patho)physiology. Recent developments have shown their application in disease modeling, drug screening, and nephrotoxicity. These applications rely on their ability to mimic (dys)function *in vitro* including endocrine activity and drug, electrolyte, and water transport. This review provides an overview of these emerging kidney models and focuses on the most recent developments that utilize their functional capabilities. In addition, we cover current limitations and provide future perspectives for this rapidly evolving field, including what these functional properties mean for translational and personalized medicine now and in the future.

Highlights

Human kidney organoids and tubuloids are emerging models with the promise to overcome the limitations of conventional in vitro research models and to better recapitulate human kidney (dys) function.

Recent developments have shown that organoids and tubuloids can be applied in various applications which depends on their ability to mimic kidney specific (dys)function. To date, segment specific drug transport, response to extracellular microenvironment, endocrine functions, and functional electrolyte and water transport have been described.

Novel technological tools including multiomics, machine learning, automated high-throughput culture systems, and organ-on-a-chip can further advance kidney organoids and tubuloids to better understand these models and improve maturation for future applications of personalized disease modeling and reliable preclinical research models.

The urgency for novel kidney research models

The kidney is one of the most complex organs in the body and consists of >20 specialized cell types that are collectively responsible for filtration of the blood while regulating body fluid, electrolyte, and acid–base homeostasis, and performing endocrine activity. These functions are essential for health throughout the body and control processes including blood pressure regulation, bone density, and erythrocyte production (Box 1).

Chronic kidney disease (CKD) affects 7–12% of the global population and is responsible for an immense health and economic burden [1]. CKD develops when the functional units of the kidney called nephrons are damaged due to injury or aging. Due to the progressive nature of CKD, kidney function further declines and may lead to end-stage kidney disease (ESKD), where the kidney is unable to perform its functions. The only available therapy for ESKD is renal replacement, which includes dialysis or transplantation. Dialysis drastically reduces quality of life and although kidney transplant is the preferred treatment, there is a shortage of donor kidneys [2].

The limitations of current research models hamper further advancement towards novel (patho)physiological insights and the development of new therapies. Modeling the kidney *in vitro* is challenging due to its complex structure and the intricate interplay of many cell types that allow the kidney to perform multiple functions. Immortalized or primary cell and animal models for decades often lack translational value during preclinical trials as these models do not accurately mimic kidney (patho)physiology [3,4]. Most renal cell models are immortalized, leading to dedifferentiation, which greatly reduces resemblance to their *in vivo* counterparts due to the lack of heterogeneous kidney segment-specific cell populations and lack of cell-to-cell interactions [5]. Primary cell cultures that do have the expression profile of their *in vivo* counterparts can dedifferentiate and become senescent within days, making them unsuitable for long-term culture or expansion [6,7]. Animal models such as zebrafish and mice provide tissue complexity; however, interspecies variability can lead to poor clinical translation [8,9]. Therefore, novel research models are required to allow further advances in our understanding of kidney patho(physiology) and development of novel therapeutics.

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Box 1. Journey along the segments: kidney (patho)physiology *in vivo*

Kidneys receive around 25% of the cardiac output and filter ~180 l of plasma. Yet, the urine excretion per day is ~1.5 l due to the nephrons ability to concentrate filtrate though reabsorption [61].

The filtration process starts in the glomerulus, where the blood is filtered through the glomerular filtration barrier, consisting of endothelial cells, a glomerular basement membrane, and podocytes with foot processes that facilitate size-selective filtration [62]. Dysfunction of this process can lead to proteinuria, hematuria, and reduced glomerular filtration rate, which are associated with nephrotic and nephritic syndrome [63].

The filtrate then moves towards the nephron, which consists of specialized epithelial cells that form the proximal tubule (PT), loop of Henle (LoH), thick ascending limb (TAL), distal convoluted tubule (DCT), and finally, the nephron connects to a collecting duct (CD) [64]. During this process, the filtrate undergoes reabsorption, facilitated by passive and active transport.

In the PT, almost all the glucose, vitamins, and amino acids and bicarbonate are reabsorbed. In addition, 90% of phosphates, 65% of sodium, 65% of calcium, and 25% of magnesium, along with water, are reabsorbed [65,66]. Disorders in the PT lead to Fanconi syndrome, which includes proximal renal tubular acidosis and Lowe syndrome [67,68].

In the descending limb of the LoH, water is reabsorbed via osmosis, and in the ascending part of the LoH, 30% of the sodium is reabsorbed together with calcium, magnesium, potassium, and chloride. Dysfunction here results in Bartter syndrome, which involves polyuria, hypokalemia, and hyperchloremic metabolic alkalosis [69].

In the DCT, sodium is actively reabsorbed and partially regulated by aldosterone and calcium reabsorption which is regulated by parathyroid hormone [70]. Diseases arising here include Gitelman syndrome characterized by renal salt loss, hypomagnesemia, hypokalemia, and metabolic alkalosis with hypocalciuria [71]. Gordon syndrome arises from too much sodium reabsorption leading to hypertension, hyperkalemia, hypercalciuria, and metabolic acidosis [72].

Fine tuning of sodium and water reabsorption (<5%) occurs in the principal cells, which is regulated by aldosterone and vasopressin, respectively, and acid–base regulation in the intercalated cells of the CD [73]. Although a small part of the reabsorption occurs here, dysfunction can lead to severe phenotypes as there are no following segments to compensate. Diseases in this segment are related to excess (Liddle syndrome) or insufficient sodium reabsorption (pseudohypoaldosteronism type 1). The inability to reabsorb water in CD leads to nephrogenic diabetes insipidus, which leads to polyuria, polydipsia, and unresponsiveness to vasopressin.

In recent years, stem-cell-derived kidney organoids and tubuloids have emerged as versatile research models to study kidney (patho)physiology. These models aim to mimic kidney (dys)function *in vitro* and to overcome the limitations of conventional research models and increase translational value of preclinical experiments. The wide array of applications for these models relies on their abilities to exhibit kidney specific functions. This review provides an overview of emerging kidney models and focuses on recent developments that utilize their functional capabilities. In addition, we discuss the limitations of these models, and how to overcome them for use in translational and personalized medicine.

Resemblance of advanced models to *in vivo* kidneys

Organoids form complex miniorgans that recapitulate embryonic kidney development

Several protocols to develop kidney organoids from induced pluripotent or embryonic stem cells have been described with the promise to provide a novel platform to study (personalized) kidney development and disease [10–12]. Organoids are 3D structures that are composed of multiple organ-specific cell lineages that are differentiated and self-organized from (induced) pluripotent stem cells. The resulting organoids can recapitulate the complex 3D environment of the kidney to a certain extent, including segmented nephron-like structures (Figure 1). This organization is achieved through timely coordination of biochemical and/or biomechanical cues that mimic the microenvironment during nephrogenesis.

Kidney organoid models consist of >15 different cell types, including cells from different nephron segments, nephron progenitor, parietal epithelial, stromal and endothelial progenitor cells, and

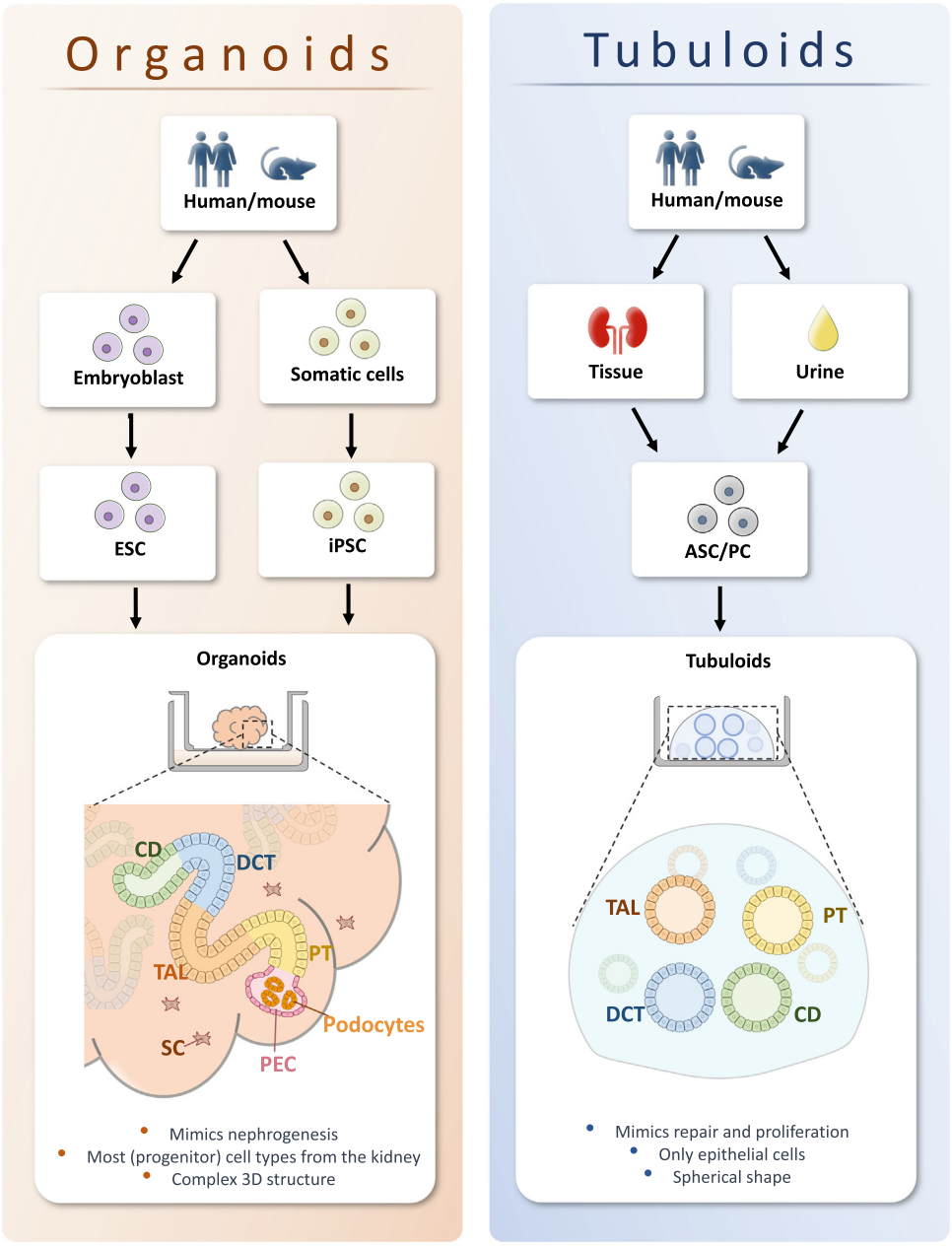

Figure 1. Overview of origin, development, and cellular composition of kidney organoids and tubuloids. Organoids can be obtained from either embryoblast-derived embryonic stem cells (ESCs) or somatic cell-derived induced pluripotent stem cells (iPSCs). These somatic cells can be either sourced from biopsy-derived fibroblasts or by noninvasive sources such as blood-derived endothelial cells or urine-derived epithelial cells. Tubuloids can be derived from adult stem cells (ASC) or progenitor cells (PC) from kidney tissue or urine from human or mouse. A closer look in the cellular composition shows that organoids and tubuloids consist of proximal tubule (PT), thick ascending limb (TAL), distal convoluted tubule (DCT), and collecting duct (CD). In addition, organoids also have podocytes, parietal endothelial cells (PEC), and stromal cells (SC). Organoids develop through nephrogenesis and develop into complex 3D structures. Tubuloids mimic repair and proliferation mechanisms and grow in spherical shapes in 3D.

off-target cells such as neuron progenitors [10,13,14]. The cellular composition varies depending on the differentiation protocol [13,15,16]. Recently, improved protocols also include a distinct collecting duct (CD) segment in the kidney organoids [17–19].

The complex multicellular kidney organoids resemble first to second trimester fetal kidney development [13,14]. Despite the premature state, some organoid cell types such as podocytes show high resemblance to their *in vivo* counterparts [20]. To date, organoids have been used for drug testing and disease modeling such as, idiopathic and congenital nephrotic syndrome [20], fibrosis [21,22], cancer [23], proteinopathy [24], and ciliopathies [25] including polycystic kidney disease (PKD) [26,27]. In addition, a PKD model of organoids has been used to screen compounds that inhibit cyst growth, ultimately resulting in the identification of a novel drug candidate [28]. Also, organoids implanted in rats to obtain perfusable vasculature have been used to evaluate the pharmacodynamics of a drug candidate against PKD [29,30]. Another study was able to perform viral and drug exposure assays using organoids with improved proximal tubule (PT) maturation [31].

Tubuloids display mature epithelial expression profile and recapitulate *in vivo* mechanisms of repair and proliferation

A recent kidney organoid model was developed by growing and expanding primary kidney tubular epithelial cells in 3D, which were called tubuloids [32]. In contrast to stem-cell-derived organoids, repair and proliferation mechanisms are induced that enable tubuloids to be cultured for >15 passages without chromosomal aberrations. Tubuloids consist of epithelial cells that likely arise from adult stem/progenitor cells. These cells can be obtained from either kidney tissue or urine [32] (Figure 1). Whether there are progenitor cells present in the kidney that respond to injury or that differentiated cells are dedifferentiating to become adult stem cells is still up for debate. In either case, tubuloids can be obtained from human, mouse and induced pluripotent stem cell (iPSC)-derived organoids [33,34].

The epithelial cells that make up the tubuloids have been characterized to predominantly express markers from the distal part of the nephron, but also from the PT. Recent developments have shown their plasticity by using differentiation media to enrich for certain segments including the PT and the CD, which results in expression of specific nephron markers [33–35]. The ability to culture tubuloids from urine allows for the possibility to obtain patient-derived tubuloids in a noninvasive manner for personalized medicine, clinical trials in a dish, and biobank formation [36].

Their high resemblance to the kidney in terms of expression profile, polarity, and functional epithelial layer of tubuloids have been utilized to recapitulate Wilms' tumors and to propagate BK viral infection *in vitro* [32]. In addition, functional characterization has been performed on tubuloids derived from patients with cystic fibrosis, which were subsequently tested for therapy efficacy [32].

Key differences between organoids and tubuloids

As these novel kidney *in vitro* models are emerging, they become more applicable and accessible for researchers to answer more complex research questions. Therefore, choosing the fit-for-purpose model becomes imperative. Here, we describe the key differences between the models.

Tubuloids consist of only tubular epithelial cells, whereas iPSC-derived organoids also contain, for example, stromal cells, including endothelial progenitors as well as glomerular cells such as podocytes and parietal epithelial cells. Also, organoids have a more complex 3D microenvironment in which different cell types interact, which makes them an interesting platform for fibrosis modeling *in vitro*. However, organoids do not yet mimic the adult kidney anatomy and this aspect

should be considered when interpreting data. As tubuloids are obtained from adult stem cells, they seem to have a more mature expression profile after differentiation, compared with iPSC organoids that remain immature using current protocols [37]. Also, tubuloids can be obtained from urine or kidney tissue, which takes 1–2 weeks to establish a differentiated tubuloid culture, thereby allowing for an easy personalized research pipeline within in a short time frame. Organoids can also be made from patient-derived somatic cells; however, this requires the time-consuming process (3–4 months) of reprogramming the cells into iPSCs. By contrast, the clonal expansion of iPSCs allows for genetic editing using established CRISPR/Cas protocols, which is currently more challenging in adult stem cell derived organoids, including tubuloids [38]. Also, easier genetic modification allows for the creation of isogenic controls in iPSC organoids, which is also more challenging in tubuloids. The differentiation of kidney organoids from iPSCs is challenging and can require optimization for each iPSC line. In our experience, culturing of tubuloids has a more reliable success rate, but is limited in passage number. After around ten passages, their functional capabilities can be reduced and would require an additional donor or sample [32].

These differences show that both models have their (dis)advantages, which can be more suitable for certain research questions. It should also be noted that there is still much to be discovered and improved regarding these models.

Recent advances in kidney (dys)function *in vitro*

The advances in the kidney organoid and tubuloid field in recent years have enabled these models to be utilized in many applications. Here, we provide an overview of reported functionalities in kidney organoids and tubuloids in recent years (Table 1 and Figure 2).

Drug transport and nephrotoxicity

The kidneys are essential in excreting endogenous waste (e.g., uremic toxins) and exogenous factors (e.g., drugs) that occurs in the PT through transporters, such as organic anion transporters (OATs), organic cation transporters (OCTs), and multidrug resistance protein (MRP). Upon nephrotoxicity, these cells express kidney injury molecule (KIM)1, which is a marker for PT toxicity screening [39]. Exposure of organoids to nephrotoxic compounds aristolochic acid and cisplatin resulted in the expression of KIM1 [40]. As proof of concept, ten anticancer molecules were used to assess time-dependent severity of toxicity on the tubuloids using this platform. Similarly, bioprinted kidney organoids were used to study nephrotoxic effects induced by aminoglycoside antibiotics by measuring KIM1 expression and intracellular ATP concentrations for cell viability [41]. A similar approach for real-time nephrotoxicity assessment in organoids was demonstrated by incorporating an ATP/ADP biosensor [42]. In addition to toxicity to epithelial cells, this approach allowed the evaluation of toxicity to podocytes by measuring DNA damage, which gave more accurate results compared with a conventionally used podocyte cell line.

To measure the transport capacity of multidrug resistance (MDR)1, also known as P-glycoprotein, and OCT2 in organoids, rhodamine-123 was used as a fluorescent MDR1 substrate [43]. The accumulation of rhodamine-123 in the tubular lumen of organoids was reduced by inhibiting MDR1 and/or OCT2 indicating that this accumulation was mediated by the activity of MDR1 and OCT2. In another study using tubuloids, organic anion transporter (OAT)1 function was confirmed by fluorescein uptake that was regulated by the OAT1 inhibitor probenecid [35]. This uptake capacity was enhanced after exposing the tubuloids to extracellular vesicles derived from immortalized kidney tubular proximal epithelial cells, which led to increased maturation of PT and OAT1 activity. Another recent approach generated tubuloids that are derived from the epithelial cells

Table 1. Overview of studies describing kidney specific (dys)function in kidney organoids and tubuloids[a]

	Cell model	Segment	Functional assay	Refs
Drug transport and nephrotoxicity	Human tubuloids	PT	Injury (KIM1), nephrotoxicity (aristolochic acid and cisplatin)	Wiraja *et al.*, 2021 [40]
	iPSC organoid	PT	Injury (KIM1, ATP), nephrotoxicity (aminoglycoside antibiotics)	Lawlor *et al.*, 2021 [41]
	iPSC organoid	PT, podocytes	Injury (ATP/ADP biosensor, DNA damage)	Susa *et al.*, 2023 [42]
	iPSC organoid	PT	Drug transport (MDR1, OCT2), rhodamine-123	Rizki-Safitri *et al.*, 2022 [43]
	iPSC organoid isolated PT cells on a chip	PT	Drug transport (OAT1), fluorescein	Lindoso *et al.*, 2022 [35]
	iPSC organoid-derived tubuloids	PT	Drug transport (MRP-2 and MRP-4), Inulin-FITC	Yousef Yengej *et al.*, 2023 [34]
Response to microenvironment	Human tubuloid	PT	Shear stress and drug transport (OCT1, OCT2, and MDR1), rhodamine-123	Schutgens *et al.*, 2019 [32]
	iPSC-organoid derived PT cells	PT	Shear stress and drug transport (OCT2, OAT1, and OAT3), FITC-inulin	Aceves *et al.*, 2022 [45]
	iPSC-organoid microdissected segments	PT, TAL, DCT	Flow induced calcium transport (mechanosensitive PIEZO-1 channel, Fura-2)	Carrisoza-Gaytan *et al.*, 2023 [46]
Physiological response to (bio) chemicals	Transplanted iPSC organoid	Glomeruli, podocytes, PT	Size selective glomerular filtration (fluorescein dextran), VEGF secretion	Low *et al.*, 2019 [47]
	Transplanted iPSC organoid	Glomeruli, PT	Size selective glomerular filtration (RITC/TRITC-dextran and FITC-albumin)	Van den Berg *et al.*, 2020 [48]
	iPSC organoid	Glomeruli, podocytes	Calcium signaling during development (Fluo-4)	Djenoune *et al.*, 2021 [49]
	Patient-derived tubuloids	PT, TAL, DCT, CD	Forskolin induced CFTR (dys)function	Schutgens *et al.*, 2019 [32]
	iPSC organoid	Juxtaglomerular cells/PT/DCT/CD	RAAS regulation and forskolin regulated renin production	Shankar *et al.*, 2021 [50]
	iPSC organoid	PT	PTH dependent Cyp27b1 regulation and 1,25-dihydroxyvitamin D production	Yoon *et al.*, 2023 [51]
Water and electrolyte transport	iPSC organoid derived DCT and CD cells	DCT, CD	Sodium and potassium transport (ENaC, $BK_{Ca,}$ and ROMK) using patch clamp	Montalbetti *et al.*, 2022 [52]
	iPSC organoid	CD	Aldosterone and amiloride dependent sodium transport (ENaC) using transepithelial voltage and current	Shi *et al.*, 2023 [17]
	Mouse tubuloids	CD	AQP2 stimulation (desmopressin and forskolin induced) and sodium transport (amiloride dependent ENaC)	Olde Hanhof *et al.*, 2023 [33]
	iPSC organoid-derived tubuloids	CD	Sodium transport (amiloride dependent ENaC)	Yousef Yengej *et al.*, 2023 [34]
Protein transport	Bioprinted iPSC organoid	PT	Protein uptake (TRITC-albumin)	Lawlor *et al.*, 2021 [41]
	Patient-derived iPSC-organoid	PT, DCT, CD	Protein misfolding and rescue	Dvela-Levitt *et al.*, 2019 [24]
	Patient derived iPSC-organoid	Glomeruli, podocytes	Enzymatic activity (lack of GLA induced Gb-3 accumulation)	Cui *et al.*, 2023 [53]

[a]Abbreviations: CD, collecting duct; DCT, distal convoluted tubule; FITC, fluorescein isothiocyanate; PT, proximal tubule; TAL, thick ascending limb.

from iPSC kidney organoids [34]. These organoid-derived tubuloids were capable of further maturation and differentiation towards the distal tubules and their functionality was shown by multidrug resistance protein (MRP) activity.

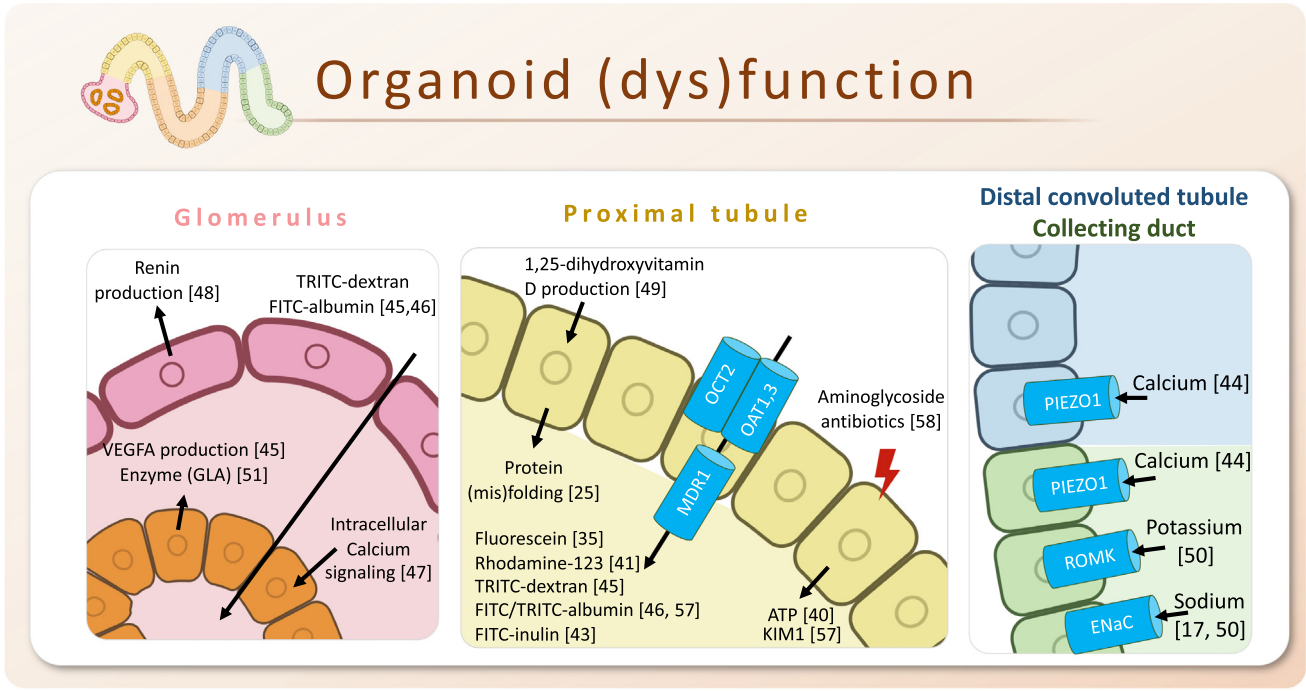

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Figure 2. Segment specific kidney (dys)function recapitulated in organoid and tubuloid models. Kidney organoids have podocytes that are capable of vascular endothelial growth factor A (VEGF) secretion, galactosidase α (GLA) enzyme activity, and calcium signaling. In addition, transplantation into mice has led to size-selective glomerular filtration and renin production. In the proximal tubule, the presence of solute carriers and drug transporters including organic cation transporters (OCTs), organic anion transporters (OATs), multidrug resistance transporters (MDRs), and multidrug resistance-associated proteins (MRPs) allow the transport of fluorescently tagged

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Physiological response to microenvironment: flow

Shear stress induced by flow is an essential biomechanical cue that is present in kidney cells *in vivo*. Tubuloids that express OCT1 and OCT2 in an organ-on-a-chip models form a polarized and leaktight tubule in a perfusable Mimetas 3-lane OrganoPlate, where the cells are exposed to bidirectional flow [32,44]. Tubuloids-on-a-chip demonstrated transepithelial transport of fluorescent rhodamine-123, by showing fluorescence presence on the apical compartment after addition to the basolateral compartment. The authors concluded that this was mediated by the basolaterally localized OCT1 and OCT2 and the apically localized MDR1 transporters. Furthermore, the apical presence of rhodamine-123 could be reduced upon pharmacological inhibition of OCT1, OCT2, and MDR1. In another study, PT cells from an organoid were isolated and cultured on an organ-on-a-chip model [45]. This resulted in improved polarity, expression, and function of OCT2, OAT1, and OAT3.

Another approach to allow flow-mediated transport was demonstrated by microdissection of a nephron tubule obtained from maturing organoids obtained with long-term culturing. Long term organoid culture led to the increased level and polarized expression of the mechanosensitive PIEZO-1 channel along the organoid tubules [44]. Isolated tubules were perfused and showed calcium signaling through the mechanosensitive PIEZO-1 channel via the Fura-2 fluorescent probe measurements [46]. Notably, organoids cultured for less than 30 days expressed lower levels of nonpolarized PIEZO-1 and these tubules were not capable of PIEZO-1-mediated calcium signaling, emphasizing the importance of maturation for functionality.

Physiological response to (bio)chemicals and endocrine function

Although current organoid models do not have a mature vasculature, a recent study showed the capability of organoids to interconnect with host vasculature upon transplantation into mice and establish a glomerular filtration barrier [47]. After injection into mice, a 70-kDa dextran molecule was found in the glomerular capillary tufts and PTs of transplanted organoids, indicating their filtration and reabsorption capacity. Smaller 10-kDa dextran was able to pass into the Bowmans capsule, whereas larger 200-kDa dextran and albumin was retained in the blood vessels, indicating size-selective filtration [48]. Blocking calcium release in organoids resulted in malformation of the glomerulus and foot processes, emphasizing the importance of calcium regulation during kidney development, and providing a tool for developmental studies [49].

Tubuloids obtained from cystic fibrosis patients demonstrated the functionality of cystic fibrosis transmembrane conductance regulator (CFTR) [32]. In healthy conditions, tubuloids were responsive to forskolin, a compound that increases cyclic AMP and opens the CFTR channel, leading to water influx-induced swelling of tubuloids. The swelling was not observed with tubuloids derived from cystic fibrosis patients, which have impaired CFTR function, and this phenotype was rescued using a CFTR-potentiator drug.

Organoids are also capable of endocrine activity and production. The presence of multiple components of the renin–angiotensin–aldosterone system (RAAS) were measured in kidney organoids including angiotensin-converting enzymes and angiotensin II receptors that are

compounds. Also, injury responses including kidney injury marker 1 (KIM1), nephrotoxicity, protein (mis)folding, and hormonal activity has been observed. In the collecting duct, organoids are also capable of electrolyte regulation including renal outer medullary potassium channel (ROMK) mediated potassium, epithelial sodium channel (ENaC) mediated sodium, and PIEZO1 mediated calcium regulation. The tubuloids also have solute carriers that allow the transepithelial transport of compounds. Also, injury response and nephrotoxicity has been observed in proximal tubule tubuloids. Cystic fibrosis transmembrane conductance regulator (CFTR), that is present in multiple segments, can be regulated by forskolin which can lead to chloride (dys)regulation and consequent swelling. Also, AQP2 regulation upon stimulation with desmopressin and ENaC mediated sodium uptake was shown in the collecting duct segments of the tubuloids. See [17,25,32–35,38,40,41,43–48,50,51,57]. Abbreviations: FITC, fluorescein isothiocyanate; TRITC, tetramethylrhodamine isothiocyanate.

important in regulating blood pressure [50]. Stimulation with forskolin, that is known to stimulate juxtaglomerular cells to release renin, led to increased synthesis and secretion of renin. In addition, subcutaneous implantation of these organoids into mice led to the maintenance of renin production for 2 months.

The use of kidney organoids also revealed the regulation of Cyp27b1 via parathyroid hormone (PTH) [51]. This process is essential for the enzyme that converts vitamin D into its active from. Organoids expressed the PTH receptor and showed accumulation of phosphorylated protein kinase (PK)A substrates in PT segments and Cyp27b1 mRNA upregulation. In addition, 1,25-dihydroxyvitamin D was produced, which was regulated by adding inhibitors to trigger Cyp27b1 expression, thereby increasing 1,25-dihydroxyvitamin D.

Electrolyte and water transport

Maintaining electrolyte and water balance in the body is an essential function of the kidney. Kidney organoids are capable of sodium and potassium uptake [52]. After dissociating organoids into single cells, a patch–clamp technique was used to measure sodium and potassium currents on single cell level. This study revealed that distal tubule cells are capable of potassium uptake through large-conductance calcium-activated potassium (BK_{Ca}) channel and the renal outer medullary potassium (ROMK, Kir1.1) channel, and sodium uptake through the epithelial sodium channel (ENaC). ENaC activity was also shown in CD organoids on Transwell filter systems, allowing access to both compartments and measurement of transepithelial resistance (TEER), and epithelial voltage and current [17]. Upon addition of the diuretic amiloride, an ENaC blocker, the TEER levels increased and abolished transepithelial voltage and current, indicating ENaC-mediated transport. In addition, ENaC activity was regulated by the mineralocorticoid steroid hormone aldosterone.

Electrolyte and water regulation was also observed in mouse-derived tubuloids that were differentiated and enriched towards the CD [33]. A physiological response of the water channel AQP2 was shown with apical translocation of the channel upon stimulation with desmopressin, which is the analog of antidiuretic hormone vasopressin. Using radioactive tracer sodium in mouse and organoid-derived tubuloids revealed amiloride sensitive ENaC-mediated sodium uptake [34].

Protein transport, folding, and enzymatic activity

Kidney organoids can also be used to mimic protein transport and folding. Indeed, bioprinted organoids had increased maturity, reproducibility, and nephron numbers [41]. These organoids were capable of albumin uptake into PT cells as shown by the accumulation of fluorescent-tagged protein substrate tetramethylrhodamine isothiocyanate (TRITC)–albumin. In another study, patient-derived organoids could recapitulate mucin 1 kidney disease (MKD) [24]. MKD is a tubulointerstitial disease that is characterized by misfolding and aggregation of proteins. The organoids revealed that MUC1 mutation leads to MUC1 entrapment in vesicles of the early secretory pathway, which was also validated in a mouse model. In addition, a candidate drug was identified to rescue this phenotype.

Kidney organoids are also capable of enzymatic activity in patient iPSC-derived organoids with two different *GLA* gene mutations, leading to different severities of the Fabry's disease phenotype. Patient-derived kidney organoids could recapitulate the accumulation of globotriaosylceramide (Gb-3), in podocytes, due to lack of GLA, which impairs their function in Fabry's disease, [53].

Overcoming limitations with innovations: towards a functional kidney *in vitro*

Organoid differentiation protocols are subject to intra-and interbatch variability, which reduces reproducibility [14,54]. Main sources of current reproducibility limitations of kidney organoids

include multistep differentiation protocols resulting in off-target cell types, the residual undifferentiated cells in culture, clonal differences of pluripotent stem cells, and variation of experimental reagents. Tubuloids are also variable among lines that are obtained from different patients. Although this can be considered as an experimental hurdle, it also reflects interpatient variability *in vitro*. Nevertheless, these differences can be a confounder for differences between control and treatment conditions. Another limitation is that organoids resemble first or second trimester fetal kidney development and lack vascularization. Although they express many kidney markers, immaturity can be a limitation for certain applications and research questions. For example, it can be argued that solute carriers OAT and OCT levels and associated pathways in adult human kidneys are significantly different compared with the prenatal situation that kidney organoids represent [55]. A protocol with enhanced metanephric specification resulted in more mature PTs as shown by increased OAT1 and OCT2 expression levels with a functional capacity for drug response [31]. These results demonstrated the feasibility of enhancing PT maturation in kidney organoids *in vitro* and may support the use of kidney organoids for studying renal pharmacology *in vitro*.

As tubuloids are obtained from primary adult cells, they have an expression profile, polarity, and functional epithelium that more closely resembles an adult kidney. Nevertheless, they seem to require additional stimuli to reach near human kidney expression levels [33,35]. Further advancement of culture conditions may include the introduction of additional cell types (e.g., stromal cells, endothelial cells) to promote tubulointerstitial crosstalk in tubuloid cultures to support maturation of kidney tubules. In addition, scalability can be a limitation for large-scale screening or regenerative medicine applications, since a human kidney consists of ~1 million nephrons, whereas the number of nephrons in, for example, the Takasato protocol is ~100 nephrons per organoid [10]. Although the multicellular heterogeneity of organoids and tubuloids is one of their most important advantages, this can pose a challenge for functional studies that focus on cell type specific processes. The anatomical organization of these models is random as it is not controlled by external cues. The accessibility of the basal and apical compartment is challenging as both organoids and tubuloids have an apical polarity towards the inside of the structure. Innovative and advanced tools can be used to address these challenges to improve organoid and tubuloid culture, and thereby recapitulate (dys)function *in vitro*. These promising approaches are discussed later.

Computational tools for improved culture conditions

Tools such as single cell transcriptomics and spatial profiling can be used to unveil new insights [13,14] (Figure 3). For example, single cell transcriptomics has been used to pinpoint and inhibit a pathway responsible for many off-target neural cells, which reduced these off-target cells by 90% [54]. In addition, using multiomics multimodal integration approaches to gain insights in organoid and kidney development could teach us how to better define improved organoid differentiation protocols [13]. Similarly, omics approaches can be employed to better understand the repair and proliferation mechanisms to improve tubuloid culture. The large datasets that emerge could be utilized in artificial-intelligence-based machine learning and developing knowledge graphs to interpret and predict optimal culture conditions.

Better recapitulation of *in vivo* microenvironment through bioengineering

Biomechanical cues that define the microenvironment can influence many cellular and tissue behavior such as proliferation and differentiation [56]. Providing an *in vivo* environment by transplanting organoids have resulted in increased maturity and function. A promising alternative to animal models to mimic the organoid microenvironment and stimulate maturation might be the use of synthetic hydrogels [16,48,57]. Hydrogels can be modulated for stiffness and elasticity

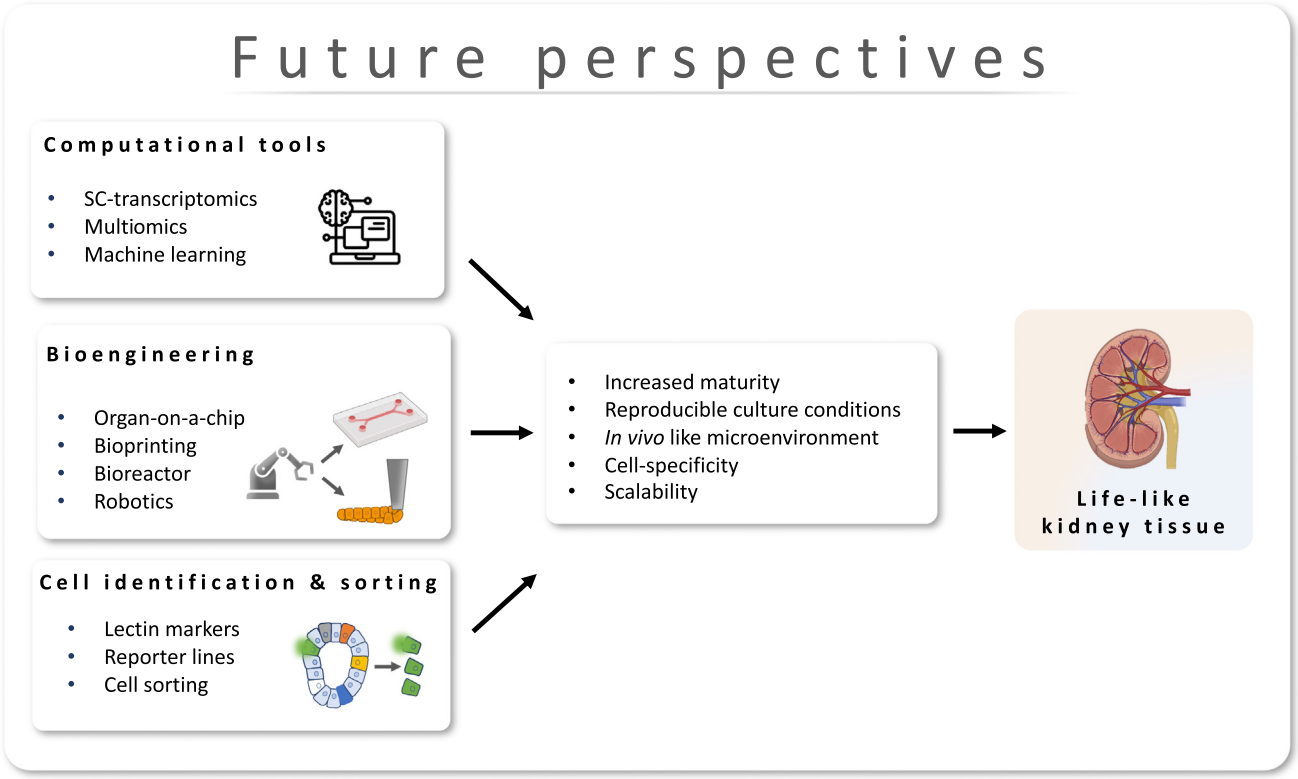

Figure 3. Using advanced tools to overcome current limitations in organoid and tubuloid cultures to enable development towards a life-like kidney tissue *in vitro*. Novel computational tools allow for careful study of [single cell (SC)] transcriptomics and combinations thereof (multiomics). These large datasets in combination with machine learning can provide novel insights. Novel technologies in bioengineering can enable combination of and/or automated organ-on-a-chip applications, bioprinter, and bioreactor applications. Finally, approaches to identify and possibly isolate specific cells can enable more specific studies. Possible combinations of these emerging technologies can aid in overcoming the limitations of kidney organoids and tubuloids and increase maturity, reproducibility, specificity, and scalability. In the long term, these efforts will enable advancements towards life-like kidney tissue cultures *in vitro*.

and allow incorporation of, for example, growth factors and predefined shapes. A more detailed recapitulation of the *in vivo* environment using advanced hydrogels in combination with organ-on-a-chip technologies could be the next step for organoid and tubuloid cultures [58]. In addition, approaches including bioprinting, bioreactors, and automated culture systems can allow for standardization and large-scale culturing [41,59,60]. These techniques can utilize precise robots in combination with machine learning for fully automated high-throughput cultures that can maintain cells, assess viability, and guide differentiation while improving reproducibility and scalability.

Live cell identification and isolation of specific cell types

Individual cell type identification is often required for organoids and tubuloids to understand the complexity of their heterogenous cell populations. Earlier examples demonstrated strategies including directed differentiation and segment identification using reporter lines. Reporter lines and fluorescently labeled lectins can be excellent tools to identify or isolate cells of interest [41,45]. The disruption of cell–cell interactions is minimized and therefore approaches including manual microdissection or automated large particle sorters can be considered. Subsequent culturing of these isolated microsections or (groups of) cells can be applied in current culture methods.

Concluding remarks

After relying for decades on immortalized cell cultures and animal models to study kidney (dys) function, the rising prevalence of kidney diseases, urgency for novel therapies, and increasing complexity of research questions require novel research models. Kidney organoids and tubuloids have been described as promising research models to answer this need (see Outstanding questions). This review covers the ability of organoids and tubuloids to recapitulate the essential (dys)functions of the kidneys. These include size-selective glomerular filtration upon transplantation of organoids and endocrine activity by RAAS regulation, response to PTH and renin, and vitamin D production. Injury response and functional protein, drug, electrolyte, and water transport are demonstrated. Further advances in these approaches have been shown with bioprinting, organ-on-a-chip applications, and single cell transcriptomics. The aforementioned examples demonstrate that kidney organoids and tubuloids in their current state can mimic kidney (dys) function *in vitro* to a certain extent, which provides opportunities for future studies. Nevertheless, further advances are required to overcome limitations, including maturity and reproducibility [15]. Computational tools including multiomics and machine learning in combination with hydrogels and organ-on-a-chip models can provide optimal culture conditions and enhance the (dys)functional capabilities of kidney organoids and tubuloids. These advanced kidney models hold great promise for kidney research in areas of disease modeling, therapy development, and personalized and regenerative medicine today and in the future.

Outstanding questions

To what extent do current organoids and tubuloids recapitulate the kidney-specific functionalities of the kidney *in vivo* and what are the specific advantages of one model over the other?

Can we use organoids and tubuloids as models to better understand development and (dys)function of the kidneys *in vivo*?

Are the functional properties observed in kidney organoids and tubuloids sufficient to be used in applications such as disease modeling, nephrotoxicity, and drug screening?

How do the limitations of maturity, reproducibility, and scalability effect advancements in the organoid and tubuloid field? How can we overcome these limitations with the use of emerging technologies, such as multiomics and organ-on-a-chip applications?

Can organoid and tubuloid models be used for personalized medicine and kidney replacement therapy in the future?

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Declaration of interests

No interests are declared.

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