CHAPTER 9

Kidney-on-a-chip

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Principles of Human Organs-on-Chips
ISBN 978-0-12-823536-2
https://doi.org/10.1016/B978-0-12-823536-2.00013-4

9.1 Introduction

Human kidney performs the function of maintaining the constant concentration of different ions and other important substances while removing waste products and other unwanted substances. Structurally, the kidney is composed of functional units, called nephrons, each consisting of glomerulus and tubules. The function of the glomerulus is filtering the blood [1]. The function of renal proximal tubule epithelial cells (PTECs) is active excretion of urea and uremic toxins and reabsorption of essential substances, such as water, salts, glucose, amino acids, and proteins [2]. In addition, renal proximal tubular epithelial cells (RPTECs) are tasked with providing a balance between influx from the kidney interstitial space and efflux to the glomerular filtrate through expressing specialized transmembrane drug transporter proteins while the imbalance between the two events results in susceptibility of RPTEC to drug-induced toxicity [3].

Nephrotoxicity is an important cause of drug failure during preclinical, clinical, and postapproval stages of drug development [4]. Drug toxicity can affect different parts of the kidney; however, the proximal tubule (PT) is the primary site of drug accumulation [5], and it can, therefore, be affected most. Enormous efforts have been dedicated to the investigation of the effect of drug-induced kidney injury using two-dimensional (2D) cell culture and animal models. However, 2D cell culture models are unable to recapitulate the in vivo biological functions of the kidney, and the effects of drug toxicity on tight junction, epithelial barrier, and cell polarization [6,7]. Kidney in vitro models are important for preclinical studies of drug toxicity; however, these models commonly suffer from partial dedifferentiation [8]. There are also reduced tight junction expression and the function of the epithelial barrier, which limits the replication of cell polarization-dependent drug-induced toxicity [7,9]. Thus, simulating the normal microenvironment of the kidney could prompt kidney histodifferentiation.

Animal models have been used, but they are inappropriate because of the difference in drug pharmacokinetics and pharmacodynamics response between animals and human. Therefore, the development of organ-on-a-

chip (OoC) platforms can help to recapitulate the microenvironment of the kidney and help to determine the transepithelial transport of anionic and cationic organic compounds. In addition, kidney-on-chip (KoC) models with epithelial polarization and localized drug transporters to study drug-induced toxicity have been developed [10–12].

Several research groups have been working on OoC systems and observed that exposure of kidney tubular cells to apical shear stress results in the rearrangement of their actin cytoskeleton, and upregulated tight junction expression as compared to 2D cell culture [13–15]. In this chapter, we focus on reviewing the state-of-the-art of KoC and discuss challenges and future directions.

9.2 Kidney-on-a-chip

9.2.1 Design and fabrication

Establishing 3D models that can recapitulate the anatomical complexity of the kidney will allow for carrying out physiological studies and developing successful therapeutics [16]. The development of KoC provides the capability to reconstruct kidney architecture at multiple scales, for example, tubule–tubule, tubule–interstitial, and tubule–vasculature interactions to investigate biological functionality [17]. KoC platforms comprise mainly three-microfluidic systems, single-layer, multilayer, and tubular microfluidic systems. A single-layer microfluidic system consists commonly of a single monolayer of kidney epithelial cells within microfluidic systems [16]. Moreover, this system can be used for coculturing different cell types to replicate distal nephron structure, and proteinuric nephropathy to investigate the behavior of kidney epithelial cells in coculture [18], and shear stress environment [15]. Microfluidic devices are usually composed of two channels separated by a thin polyester or polycarbonate membrane [19]. This approach imposes fluidic shear stress on cells seeded in the upper channel, while the lower microchannel is exposed to static media [20,21]. Further research to model the local microcurvatures of kidney tubules evolved into the development of circular microchannels, wherein cells could be seeded uniformly on the cylindrical walls, similar to what is seen in vivo. The tubular microfluidic device was fabricated with a single, cylindrical microchannel and coating PDMS microchannels with glass using sol–gel method [22]. This device was further improved by the incorporation of hollow tubular membranes to simulate in vivo transport phenomena through the lumen, and tubule–vasculature interfaces for better modeling of renal physiology (Fig. 9.1) [16].

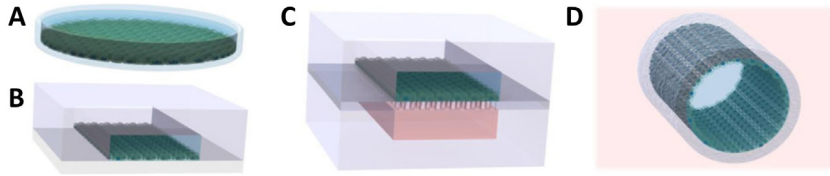

Figure 9.1 Schematic illustration of kidney-on-a-chip. (A) A 2D monolayer in Petri dish under static fluidic conditions. (B) Single layer of kidney epithelial cells in the microchannel that can be exposed to fluidic shear stress (C) Microfluidic system, where a cell monolayer is cultured in the lower chamber under static conditions while the upper chamber has seeded cells exposed to fluidic shear stress. (D) Cells seeded within a tubular membrane, where they are exposed to fluidic shear stress. Static fluid outside the tube represents the interstitial space. *(Reproduced from Sochol et al. [16], with permission from Springer.)*

9.2.2 Cells used for kidney-on-a-chip systems

The selection of an appropriate kidney cell source is an important step for the development of microphysiological systems (MPSs). Nephron consists of the functional units of the kidney, which have different epithelial cell types. The generation of these epithelial cells in vitro requires complicated efforts [23]. In general, cells used for KoC systems can be classified into three categories, cell lines, primary cells, and stem and progenitor cells.

9.2.2.1 Cell lines

Immortalized cell lines have been used to develop KoC systems as the most common cellular model [24]. They originate from different animals (such as pigs and dogs) or humans. The most common cell lines used for KoC applications are Madin–Darby canine kidney tubular epithelial cells (MDCK cell line) [25–27], Lilly Laboratories porcine kidney tubular epithelial cells (LLC-PK cell line) [28], and opossum kidney (OK) PT epithelial cells (OK cell line) [29,30]. These animal-derived cell lines show interspecies variation, especially in the expression of human-specific transporters [31,32]. Therefore, these cells cannot be recommended for human disease modeling and preclinical drug screening applications.

Human renal cortex cell lines such as human kidney two PTECs (HK-2 cell line) have also been reported for KoC systems [33]. These human cell lines can preserve the PT phenotypes with sensitivity to toxins [34]. Overall, cell lines have some advantages since they are relatively cheap and can easily be maintained. However, none of these immortalized cells completely mimics the primary cell phenotype or functional differentiation [4,34]. Also, continual growth and proliferation of these immortalized cells may obstruct hollow fibers or channels [28].

9.2.2.2 Primary cells

Primary cells can be isolated from kidney tissue, cultured in vitro, and used for the development of MPSs [35]. Primary human cells are better representative of native kidney tissue in comparison to cell lines because they retain their phenotype and functional properties [8,36]. For example, human PTECs are the most common primary cellular models that have been used in KoC systems [24]. Their application has been mainly for developing PT-on-a-chip systems [37], nephrotoxicity studies [4], therapeutic transport evaluation [38], and drug screening [33].

In contrast to their wide range of applications, primary cells lose their function, such as the expression of important genes through passaging, which restricts the number of duplication passages to a maximum of 12 doublings [35] (optimally 2–3 passages in terms of better purity, proliferation, and differentiation [39,40]). Therefore, obtaining an adequate number of primary kidney cells is challenging. The doublings of human PT cells can be enhanced by 3–5 folds via RNA interference or antisense nucleotides. In this regard, the siRNA-mediated lifespan extension has been used to increase the lifespan of the population doublings of human RPTEC by periodic transfection of the cells with small interfering RNA (siRNA) to tumor suppressor (p53) or the cyclin-dependent kinase inhibitor (p16INK4a) (to invalidate messenger RNA of cell cycle-related genes). Hence, a confluent layer of transfected cells can be created, which surrounds the internal surface of fibers and forms detectable microvilli on their apex [35].

In addition to hPTECs, human renal epithelial cells are another primary human cell type explored for kidney MPSs for developing tubular structures [41]. Furthermore, animal-derived cells such as primary rat inner medullary collecting duct cells have been investigated for developing the collecting duct part of the kidney [42].

The probability of contamination by other renal cells and donor-to-donor variability are other limitations of the use of primary kidney cells that should be considered before using them for KoC systems [43].

9.2.2.3 Stem and progenitor cells

Stem cells have been extensively used as cell sources in tissue engineering, microfluidics, and OoC because of two capabilities, including self-renewal and differentiation [44–46]. Their potential to differentiate into different functional cell types varies according to their type. For example, pluripotent stem cells, such as embryonic stem cells (ESCs) and induced pluripotent

stem cells (iPSCs) can develop into any cell type apart from extraembryonic cells [47,48]. ESCs are derived from the inner cell mass of the blastocysts and have differentiation ability to almost all of cell types. ESCs can be differentiated into renal cells by using several differentiation protocols [48–53]. However, these pluripotent stem cells have several limitations such as ethical controversies and legal issues due to involvement of destroying embryos to obtain them, rejection by the immune system due to their allogenic nature, and the possibility of developing neoplasms such as teratomas due to their high rate of proliferation [44]. Narayanan et al. showed that human ESCs could be differentiated from human PTECs, providing a favorable cell source for kidney tissue engineering [54]. However, characterization and application of these cells in the kidney MPSs should be carried out.

To overcome these limitations, obtaining pluripotent stem cells from patient's own cells is the best solution. To this end, iPSCs were generated by genetic reprogramming of adult somatic cells [55]. Reprogramming of cells led to pluripotency that can be achieved by inducing four transcription factors (OCT4, SOX2, KLF4, and c-MYC) in adult cells (such as fibroblasts). This method was a revolutionary step in physiology and medicine, resulting in winning the Nobel Prize by Shinya Yamanaka and John Gurdon in 2012 [55]. iPSCs possess many of the characteristics of ESCs in terms of morphology, proliferative capacity, surface markers, gene expression, epigenetic status, etc. Therefore, the use of iPSCs can be an efficient alternative to the use of ESCs as a cell source for OoC [56]. iPSCs maintain the genetic and epigenetic background of their original cells with a lower possibility of immunological rejection as compared to ESCs [57]. There are also established protocols to differentiate iPSCs to renal cells, which can be used for kidney tissue engineering and KoC systems [50,58,59]. For example, Musah et al. successfully differentiated human iPSCs to podocytes to fabricate glomerulus-on-a-chip (GoC) systems [60–62]. In addition, new gene-editing techniques such as CRISPR can be used in kidney organoids and KoC systems for drug discovery and disease modeling applications via pathogenic mutation of iPSCs [63,64].

Progenitor cells indicate the level of potency (self-renewal and differentiation capabilities) between stem cells and adult cells. These cells play a key role in the process of physiological cell turnover and substituting damaged or dead cells in vivo. Recent reports have demonstrated that adult kidney consists of renal stem/progenitor cells with high potential for use in autologous therapy [65–68]. In a study by Sciancalepore et al., adult renal

progenitor cells were used in a KoC system for drug testing applications [37]. However, the limited proliferative capacity of these cells is their main disadvantage for use in OoC research.

9.2.3 Structural materials used in organ-on-a-chip systems

The success of an OoC system is heavily dependent on the structural materials used for its fabrication [69–73]. Several criteria should be taken into consideration to select these materials. Biocompatibility, transparency, gas permeability, ease of the process, and cost are just some examples [74]. Since the introduction of OoC, several different materials were exploited to fabricate the chips. Broadly, these materials could be divided into organic and inorganic compounds. In the following paragraphs, we will describe these materials briefly.

The most common material used for OoC fabrication is the elastomer polydimethylsiloxane (PDMS) [59,75]. Elastomers are a family of materials that are defined as polymers with high flexibility, yield strain, and stretchability [76]. Several characteristics of PDMS are favorable for OoC development. PDMS is elastic, transparent, gas permeable, biocompatible, easily processable through several fabrication techniques, and cost-effective [77–79]. The elastic nature of PDMS helps scientists to fabricate OoC devices that are flexible and recapitulate some tissues with motion and mechanical strains [80]. One great example here is the system developed by Ingber and commercialized as Emulate [19,81,82]. The optical transparency of PDMS enables the fabrication of transparent chips, which are suitable for real-time monitoring of cultured organs and for cell imaging or imaging-dependent assessments. Gas permeability is another critical aspect of this elastomer, allowing for proper gas exchange for cell survival over long culturing times. Moreover, PDMS is capable of being shaped through several fabrication methods, including soft-lithography, use of sacrificial templates, hybrid stamp-based approach, razor-printing, and 3D stereo-lithography techniques [74].

Despite significant advantages, PDMS still has some pitfalls preventing it from being the "best" choice. Most importantly, PDMS absorbs small hydrophobic molecules [83]. PDMS can absorb several compounds available in the medium, including secreted biomolecules from cells or drugs/signaling molecules included in the medium [76,84,85]. Consequently, this can negatively affect the outcome and read-outs from OoC devices designed for drug toxicity evaluation and/or cell response to bioactive

molecules. For instance, in research by van Meer et al., PDMS-coated tissue culture plates (TCPs) were found to be more susceptible to the adsorption of heart-affecting drugs (namely verapamil, nifedipine, and Bepridil) as compared to nontreated TCPs [86]. They also showed that coatings could be a good option for the reduction of drug absorption. Although the gas permeability of PDMS is advantageous for oxygen (O_2) transfer (which is vital for cell survival), it could also play a negative role. Bubble formation due to PDMS high gas permeation and hydrophobicity is a prevalent problem and careful attention is needed to prevent its occurrence [76,87]. On top of these, its incompatibility and swelling within organic solvents, autofluorescence, and relatively time-consuming and expensive fabrication techniques (especially for the soft-lithography as the traditional fabrication technique for PDMS) could be considered as other major drawbacks of PDMS [88,89].

In addition to PDMS other elastomers such as methacrylated polyurethane, tetrafluoroethylene-propylene, poly(polyol sebacate), styrene-(ethylene/butylene)-styrene copolymer, poly(itaconate-co-citrate-co-octanediol), and poly(octamethylene maleate (anhydride) citrate) (POMaC) are used for development of microfluidic and OoC devices [90–92]. POMaC, a citric acid-based polymer, was first developed by Tran et al. through a polycondensation between citric acid, maleic anhydride, and 1, 8-octanediol [93]. They could show that this polymer is highly tunable in terms of mechanical properties, degradation, and other physical properties. In terms of OoC development, Zhang et al. used this elastomer to develop chips they called "AngioChip." These chips were used to simulate the vessels and were shown to be able to recapitulate several important characteristics of native vasculature and vascularized constructs and could be used as implantable devices [92]. The same group also used POMaC to develop a heart-on-a-chip device. Their new chip, called Biowire II, was capable of recording mechanical forces in developed heart tissue non-invasively and they could be used for disease modeling and investigating cell/drug interactions [94].

Plastics are another family of polymers that are used for the development of OoC systems. Like PDMS and other elastomers, thermoplastic polymers are biocompatible and thier use is cost-effective. Plastics are also easily processable and could be made into complex structures with various techniques [95]. In addition, they possess gas permeability properties to allow O_2 to be transferred to cells. Moreover, they are transparent, and their mechanical properties are suitable for developing different tissues and

OoC devices [96,97]. Typical examples of plastic polymers used for OoC fabrication are polymethylmethacrylate (PMMA), polycarbonate, polylactic acid, and thermoplastic polyurethanes [24,98]. In a study using PMMA, a microfluidic chip (UniChip) was developed to enable unidirectional tissue perfusion [99]. PMMA chips were fabricated by laser ablation and solvent-assisted bonding methods with great potential for resembling stress-sensitive tissues. Polycarbonate is also used in the development of OoC devices, mainly as a membrane in OoC systems [70,100]. For instance, in a work by Tian et al., a microporous polycarbonate membrane was sandwiched between two PDMS layers to form a kidney-liver-on-a-chip system [101].

Nevertheless, plastic polymers also show some pitfalls. Several plastic polymers are biodegradable, which could hinder their application for OoC development aimed for long-term screening. Moreover, they might be incompatible with organic solvents and show higher rigidity compared to elastomers, which may limit their application for specific investigations.

Hydrogels are 3D networks that are able to absorb significant amounts of water [102]. Due to their unique properties, and due to the fact that they can resemble the native ECM, hydrogels are widely used as cell culturing and encapsulation materials [103]. Hydrogels could be fabricated from synthetic polymers (e.g., PVA and PEG) or natural polymers (e.g., collagen, gelatin, and fibrin) [104,105]. It is possible to fine-tune hydrogels' physical, mechanical, and biological properties. Several works have shown that the swelling, degradation, stiffness, bioactivity, and cell adhesion properties of hydrogels are adjustable so they could meet required targets [106–109]. Considering OoC platforms, it is possible to use hydrogels and form microchannels inside them. For instance, in one study, Zhang et al. developed a thrombogenesis-on-a-chip platform to study the hemodynamics associated with thrombosis [110]. Using 3D bioprinting technique, they were able to fabricate biomimetic constructs from hydrogels. The constructs were developed from gelatin methacryloyl (GelMA), a well-known biomaterial for hydrogel development. They also implemented sacrificial 3D printing to embed microchannels within GelMA hydrogels [111]. The shortcomings of hydrogels, especially their fragility, relatively low mechanical properties, and possible biodegradation, limit their implementation as the main structural materials for OoC development, especially for long-term studies [112,113]. Therefore, they are primarily used in combination with other materials. In one approach, it is possible to use hydrogels to encapsulate cells within an ECM-relevant environment while

the system is made from another polymer/elastomer such as PDMS [114]. Nevertheless, hydrogels are greatly bioactive and tailorable. For example, they could be manipulated to develop smart structures that are responsive to stimuli such as changes in pH, temperature, and light [115,116]. Hence, they are still considered interesting choices for OoC and microfluidic chip fabrication.

In addition to these organic compounds, inorganic materials, especially silicon and glass, are exploited for the fabrication of OoC devices. Indeed, the application of glasses and silicones as structural materials in microfluidic devices, lab-on-a-chip, or OoC devices was investigated [117]. As a structural material, glass is a good option due to its transparency, low drug or molecule absorption, the possibility of surface modification, and biocompatibility [118]. However, glass is gas impermeable, and glass-based microfluidic devices are relatively expensive. On the other hand, silicon devices are cheap, and they are gas permeable [74]. Therefore, the combination of glass and silicone is a suitable approach for the development of OoC devices. Moreover, it is possible to combine organic and inorganic materials to develop OoC systems.

Paper is another interesting structural material for the development of OoC devices. Paper abundance, low cost, structural similarity to ECM, high porosity, ease of modification and sterilization, lightweight, and biocompatibility are considered as main advantages of paper-based MPSs [119]. However, this material is not commonly considered or investigated for the fabrication of OoC. Low mechanical properties in swollen state and low transparency are considered as the main reasons [76].

9.2.4 Components of kidney-on-a-chip

Nephron, the functional unit of the human kidney consists of different cells and functional parts. An appropriate KoC model should mimic cellular interactions (including interactions between glomerular vascular endothelial cells and podocytes), expression level of transporters, transcellular electrochemical gradients and osmotic pressure differences, architectural organization of renal tubules, cell metabolism and endocrine development, and fluid dynamics [120,121]. Different parts of the nephron have been modeled by researchers via kidney MPSs to recapitulate physiologically pertinent parameters of human KoC [24,122]. These systems include GoC, PT-on-a-chip, and distal tubule/collecting duct-on-a-chip.

9.2.4.1 Glomerulus-on-a-chip

Glomerulus is the functional part of the nephron, which is responsible for the filtration of blood and consists of podocytes, epithelial cells, and an array of capillaries [123]. Due to its importance, recapitulation of glomerular function in vitro by using MPSs has attracted the attention of many investigators. On this subject, Zhou et al. fabricated a GoC system having two different microfluidic channels, which mimics the architecture of the glomerulus and can be used for the modeling of hypertensive nephropathy. Immortalized human glomerular endothelial cells and murine podocyte precursor cells were utilized in organized layers lining two compartments. Interestingly, the effect of fluid flow on cytoskeletal reorganization, cell damage, and glomerular leakage was investigated was demonstrated [124]. Increased damage and leakage as a result of glomerular mechanical forces, which recapitulate the disease model, were also seen using this model. In another study, Wang et al. fabricated a GoC device having parallel channels by incorporating rat glomerular endothelial cells, basement membrane, and podocytes to replicate the glomerular filtration barrier and model diabetic nephropathy (a chronic renal disease). Hyperglycemia was indicated to be a cause of proteinuria due to higher barrier permeability to albumin and glomerular disorder. Also, pathological responses related to diabetic nephropathy were observed in this MPS, which can help to investigate disease mechanisms and therapeutic development [125].

To overcome the limitations to developing functional podocytes in vitro, Musah et al. cocultured human iPSCs-derived podocytes and human glomerular endothelial cells in a microfluidic device having distinct channels recapitulating blood and urinary fluids [60,62]. The differentiation protocol was highly efficient (more than 90% terminally differentiated cells) with high expression of maturation markers (such as nephrin, WT1, and podocin). This GoC successfully replicated the native glomerular tissue—tissue interface and filtration function for albumin and inulin clearance with the generation of basement membrane collagen. Also, albuminuria and podocyte damage were detected by using Adriamycin, mimicking in vivo drug nephrotoxicity. Furthermore, Petrosyan et al. cultured human podocytes and glomerular endothelial cells in a GoC device to mimic the structure and function of the glomerular filtration barrier. Retention of cellular morphology, creation of capillaries, upregulation of slit diaphragm proteins, and perm selectivity were observed in long-term cell culture. Interestingly, albuminuria proportional to patients' proteinuria was demonstrated only as a result of exposure to sera from patients with antipodocyte autoantibodies. In

addition, reproducibility was indicated by an assessment of 2000 devices, which confirms the authentication of this chip for high-throughput drug screening. This platform can also be used for modeling diabetic nephropathy and further pathophysiological studies [126].

9.2.4.2 Proximal tubule-on-a-chip

PT is the main part of the nephron responsible for drug elimination and therefore, a place showing renal drug toxicity. Therefore, several PT-on-a-chip systems have been developed for preclinical drug screening. Regulating reabsorption and secretion of compounds is an essential feature of PT-on-a-chip systems to be able to mimic native PT function. Researchers used various strategies for this development such as culturing PT cells in hollow fibers used as tubular scaffolds for cell culture, which can also immunoprotect them [127]. In this regard, a confluent monolayer of hPTECs was seeded on the inner surface of fibrin-coated hollow fibers showing great transport ability for creatinine and glucose [38]. Another model of biofunctionalized renal hollow fibers showed clearance of albumin-bound toxins and albumin reabsorption [128]. In addition, modeling of different renal diseases was demonstrated by the change in glomerular filtration rate, nephrolithiasis, hyperglycemia, and nephrotoxicity caused by different drugs such as cyclosporine and cisplatin [12,129].

Aiming to fabricate a cost-effective PT-on-a-chip model, Jang et al. seeded hPTECs on collagen-type IV-coated polyester membranes that separate microchannels into luminal and interstitial channels. Exposure of cells to fluid shear stress exhibited normal columnar shape, polarity, transporters, and primary cilia. Cellular uptake of albumin and recovery from cisplatin-induced damage were also enhanced [4]. Furthermore, Sciancalepore et al. developed a PDMS microfluidic device that had a fibronectin, laminin, or matrigel-coated porous polycarbonate membrane. Cultured human renal progenitor cells were polarized with a reduction in permeability of urea and creatinine under fluid shear stress, and fibronectin-coated surfaces exhibited the greatest metabolically active cells [37]. In addition, Weber et al. used a microfluidic device comprising an ECM-coated chamber with tubular architectures for hPTECs culture. Development of tubular structures, collagen matrix production, renal epithelial differentiation, cell polarization, and morphology with high cell viability (more than 95%) was confirmed for four weeks of cell culture. Interestingly, basolateral transport and apical uptake with intracellular enzymatic function were indicated as by ammonia genesis and activation of vitamin D. Therefore,

physiological conditions of PT cells can be recapitulated in vitro with higher differentiation and maturation [130].

In another study, Vriend et al. investigated renal drug-transporter interactions by using a predesigned microfluidic OrganoPlate cultured by immortalized PTECs upregulating organic anion transporter 1 (OAT1) [131]. As a result, proper cell polarization with gene expression of drug transporters, function of efflux transporters, and localization of F-actin were demonstrated. Recently, Vormann et al. fabricated a 3D high throughput microfluidic device for drug-induced kidney injury (DIKI) pharmaceutical applications [132]. Four drugs with reported nephrotoxicity effects were used including cisplatin, tenofovir, tobramycin, and cyclosporin A with readouts of cell viability, release of lactate dehydrogenase (LDH), expression of toxicity markers, and specific miRNAs. Accordingly, robust nephro-screening could be conducted for DIKI with high usability and reproducibility.

Emergence of 3D bioprinting allowed for the development of PT-on-a-chip structures with higher complexity [16]. Homan et al. printed a perfusive tubular structure using pluronic ink on a gelatin-fibrinogen ECM hydrogel [133]. The internal tubules were seeded with hPTECs and showed a more organized morphology with higher albumin uptake under perfusion in comparison to 2D culture. Also, dose-dependent exposure to cyclosporin A was observed with stability for more than two months. Moreover, King et al. developed a printed PT-on-a-chip device to mimic renal fibrosis by culturing renal fibroblasts, endothelial cells, and epithelial cells [134]. The stability of this system was more than 30 days.

9.2.4.3 Distal tubule/collecting duct on-a-chip

Distal tubule/collecting duct-on-a-chip has not been widely investigated as compared to other nephron componet-on-a-chip models, and there are only a few studies on this subject. As an early study in this field, Baudoin et al. fabricated a PDMS microchip, which consisted of a fibronectin-coated cell microchamber connected to microchannels for nutritient supply and waste product elimination [26]. Jang ad Suh developed a multilayer microfluidic PDMS chip with a fibronectin-coated polyester porous membrane for primary rat renal collecting duct cell culture [21]. A fluidic shear stress of 1 dyn/cm^2 was used by controlling flow rate, viscosity, and channel size to mimic in vivo renal tubules. This maintained stress resulted in higher cell polarity, improved cytoskeletal reorganization, increased cell-cell junctions, and enhanced molecular transportation. Furthermore,

collagen hollow fibers were developed as a model of renal tubular structures using sacrificial calcium-crosslinked alginate core and carbodiimide-crosslinked collagen shell. Renal tubular cells formed denser monolayers inside the hollow fibers with organized 3D morphology in comparison to less dense cells in 2D flat outer surfaces. Renal tubules also demonstrated higher activity, enhanced sodium and water transport, and overexpression of brush border enzymes of alkaline phosphatase and γ-glutamyl transferase [29,30]. Also, spatial and architectural cues, especially fiber diameter and surface curvature can significantly influence the functionality of distal tubular cells. It was reported that using a fiber diameter of 50 μm can recapitulate the native distal tubules and optimize cell function [135,136].

9.2.5 Multicomponent kidney-on-a-chip

KoC systems, with on-chip models ranging from simple to advanced, are continuously being developed to better elucidate renal physiology, the pathophysiology of kidney diseases, and drug-induced nephrotoxicity in vitro [24]. In particular, advanced on-chip models are at the stage of resembling structures and functions of individual nephron components including glomerulus, PT, and distal tubule/collecting duct [137]. However, considering that kidney is a highly structured organ, of which efficient function is dependent on a finely balanced interaction between different nephron components, research efforts aimed at developing multicomponent KoC systems comprising multiple parts of the nephron. Although the developments toward achieving this goal hold promise, multicomponent KoC systems reported in the literature are still in their infancy due to the difficulty in replicating the high structural complexity of the nephron [24,137].

One design of multicomponent nephron is the example suggested by Weinberg et al. to replicate the filtration by the glomerular unit, reabsorption by PT, and a large increase in urea concentration within the loop of Henle [122]. Accordingly, the device was composed of two layers and a dialysis membrane sandwiched between them and contained the three functional nephron components, namely the glomerulus, the PT, and the loop of Henle. In the device, a connector was used as an additional component to connect nephron parts with each other and with the blood and waste streams. Considering albumin, urea, Na^+, and Cl^- solutes, numerical calculations for the function of the glomerulus, the PT, and the loop of Henle within the device were reported, synchronized with the flow and transport properties of normal nephron. Another example is the multichannel microfluidic device (Fig. 9.2A) developed by Sakolish and Mahler to mimic the combined

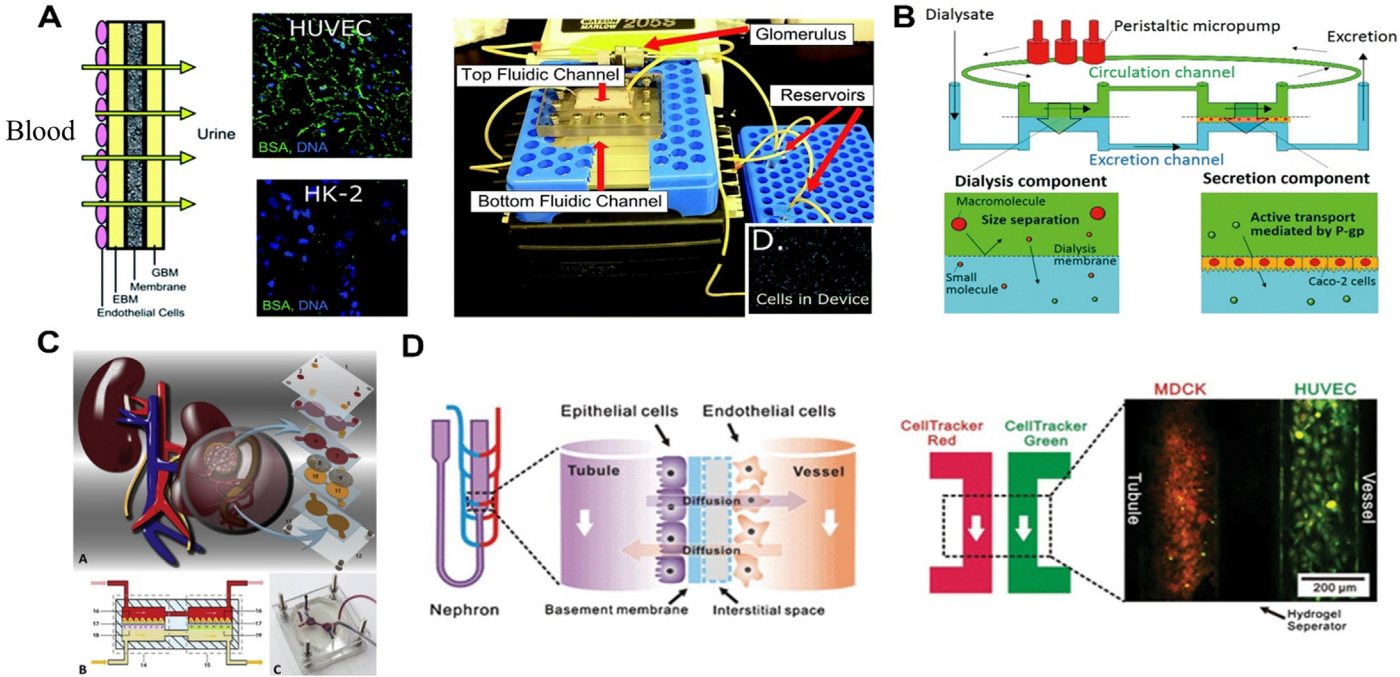

Figure 9.2 Example multicomponent kidney-on-a-chip systems developed to recapitulate the structure and functionalities of combined nephron components. **(A)** Left panel: Schematic showing the model used to recapitulate the structure of the glomerular barrier using endothelial cells along with epithelial basaement membrane (EBM) and glomerular basement membrane (GBM). Middle panel: Bovine serum albumin (BSA) protein was used to show the passive filtration of the glomerulus barrier and the active transport of the proximal tubule (PT) using endothelial cell-seeded glomerular filter cells (HUVEC) and PT epithelial cells (HK-2), respectively. Right panel: Micrograph shows the final assembly of the device, where subpanel D reveals the cell-seeded membrane. **(B)** Schematic of the microfluidic device

comprised of dialysis and secretion components to mimic combined functionalities of glomerulus and PT of the kidney, respectively. **(C)** Subpanel A: Schematics showing the on-chip nephron model (right part) mimicking the native nephron (left part). 1, top polycarboate (PC) plate; 2, inlet of "glomerulus"; 3, outlet of "peritubular capillary"; 4, inlet of "Bowman's capsule"; 5, outlet of "tubular lumen"; 6, "glomerulus"; 7, "peritubular capillary"; 8, interface in the "renal corpuscle"; 9, interface in the "PT"; 10, "Bowman's capsule"; 11, "tubular lumen"; 12, bottom PC plate; and 13, screw. Subpanel B: The longitudinal-section view of on-chip nephron model. the direction of "renal blood flow" and "glomerular filtrate drainage" is indicated by the pink and yellow arrows, respectively. 14, "renal corpuscle"; 15, "PT"; 16, primary renal endothelial cells; 17, porous membrane with basement membrane matrix extractant on; 18, primary podocytes; and 19, primary renal tubular epithelial cells. Subpanel C: Micrograph shows the final assembly of the device with red ("renal blood flow") and blue ("glomerular filtrate drainage") fluids. **(D)** Left panel: Schematic shows structure and passive diffusion of a physiological nephron. Right panel: The vascular network of a nephron was mimicked with independent hydrogel channels (analogous to tubules and vessels) using renal epithelial (MDCK) and endothelial (HUVEC) cells. *(A) Reproduced from Sakolish et al. [138], with permission from the Royal Society of Chemistry. (B) Reproduced from Sakuta et al. [139], which is freely available via the Creative Commons Attribution License (CC BY 4.0). (C) Reproduced from Qu et al. [140], with permission from Elsevier. (D) Reproduced from Naughton et al. [141], with permission from the Royal Society of Chemistry.)*

functions of glomerulus and renal tubule [138]. Through the application of physiologically relevant shear stress, a glomerulus-modeling filter attachment, and two independent fluidic channels, the device could provide an improved growth environment for renal endothelial cells, podocytes, and tubular cells compared to traditional methods. The device proved also effective both for the passive filtration barrier function of the human glomerulus, and, for the subsequent reuptake of serum proteins in the PT. More recently, another multicomponent KoC system was developed by Sakuta et al. to mimic the combined functionalities of glomerulus and PT of the kidney [139]. The system consisted of a peristaltic micropump to mimic the heart, a dialysis component (glomerulus and Bowman's capsule) to mimic glomerular filtration, and a secretion component (PT) to mimic renal clearance (Fig. 9.2B). In the device, a dialysis membrane was used to separate analytes by size in the dialysis component, where renal tubular cells, cultured in the excretion channel of the secretion component, demonstrated functions of filtration and secretion of the kidney.

Importantly, the study by Qu et al. demonstrated a multilayer microfluidic device (Fig. 9.2C) to generate the structure and function of the glomerulus, the Bowman's capsule, PT lumen, and peritubular capillary in a single chip [140]. Artificial renal blood flow and glomerular filtrate flow were used to mimic the function of the physiological nephron. As a result, developed nephron-on-a-chip recapitulated renal physiology, including the glomerular basement membrane charge-selective barrier, glomerular size-selective barrier, para-aminohippuric acid secretion, and glucose reabsorption. A developed device was also used to study drug-induced acute renal injury and nephrotoxicity using cisplatin and doxorubicin as model drugs. Another noteworthy study is the one conducted by Mu et al., where hydrogel microchannels were developed in a microfluidic device for mimicking the highly vascular network of the nephron (Fig. 9.2D) [42]. The study demonstrated that generated vascular network is mechanically stable for perfusing solutions and biocompatible for cell adhesion and coverage.

Clearly, combined functions of critically important nephron parts, such as glomerulus and PT, could successfully be mimicked in the reported multicomponent KoC systems. However, in view of the complex character of the nephron, constructing a nephron-on-a-chip that can completely recapitulate the filtration and reabsorption functions of the native kidney is challenging. This is due to fact that recapitulating all specific structures and functions of the nephron in a single KoC system would require developing on-chip models of different essential nephron parts along with

reconstituting tissue—tissue interfaces, recreating relevant chemical microenvironment, and recapitulating the physical microenvironment of the native kidney [13]. Although publications on KoC systems that involve building a multicomponent KoC is scarce, with advancements made in microfluidic technologies and cellular biology, the development of more structurally and physiologically relevant KoC systems is on the horizon.

9.2.6 Multi-organ-on-a-chip systems that include kidney-on-a-chip

Toxic effects of drugs can affect different sites in the kidney through various mechanisms [141]. They can be metabolized and absorbed by the small intestine and the liver and excreted by the kidney, suggesting key determined efficiency for therapeutic application. Thus, detection of these systemic effects of drugs requires the development of a reliable MPS that integrates different organ mimicking modules [4]. A four-organ-chip (4C) platform has been fabricated by Maschmeyer et al. to combine intestine, liver, skin biopsy, and PT. 4C comprised two PDMS layers that contained channels, micropumps, membranes, and openings for culture compartments (Fig. 9.3**)**. In this system, skin biopsy could be used as an absorption route for assessing the toxicity of drugs and their metabolites. It was used for in vitro analysis of absorption, distribution, metabolism, and excretion,

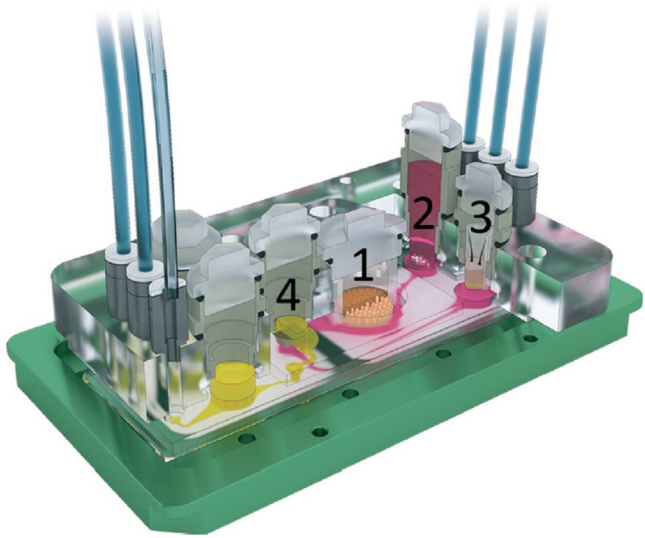

Figure 9.3 3D view of a four-organ-chip consisting of two polycarbonate cover plates, the PDMS-glass chip to accommodate a device with surrogate blood circulation (*pink color*), and an excretory flow circuit (*yellow*). It comprises intestine (1), liver (2), skin (3), and kidney (4) representing units. *(Reproduced from Maschmeyer et al. [142], with permission form the Royal Society of Chemistry.)*

along with repeated dose toxicity testing of drugs through predicting the interaction of metabolites with organs equivalents. A discrete medium reservoir at the apical side of the intestinal barrier was used to represent oral route for ingested drugs, which could be absorbed and get into circulation. Accordingly, drug will reach liver compartment of the system and undergo the first metabolism path, and the kidney for the second metabolism and excretion path [142].

9.3 Applications of kidney-on-a-chip systems

9.3.1 Physiological studies

KoC systems not only provide on-chip models to recapitulate the structure of a highly complex native kidney, but also resemble its physiology [143]. Indeed, assessing renal function in vivo using on-chip models could help in understanding mechanisms of kidney function at cellular and tissue levels, such as blood filtration in the glomerulus and secretion/reabsorption in renal tubules. For example, with a two-channel microfluidic chip, one representing the PT lumen and the other peritubular capillary lumen, Vedula et al. were able to replicate the reabsorptive barrier of PT in vitro by separating the channels of the chip with a porous membrane [144]. Within the device, renal epithelial and endothelial cells showed enhanced viability, metabolic activity, and compactness of the epithelial layer. The resulting tissue expressed kidney-specific transport proteins. Moreover, cells in the device showed inhibition and recovery of active reabsorption of 2-(N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino)-2-deoxyglucose (2-NBDG), which is a fluorescent glucose analog in response to the drug ouabain. In another study by Ferrell et al., single- and multichamber microfluidic bioreactors were developed to evaluate albumin handling by PT cells (Fig. 9.4A) [29]. In the single-chamber system, cells were perfused with physiologically relevant shear stress to evaluate the effects of mechanical stress on protein uptake. In the multichamber system, apical and basolateral compartments were separated by a porous membrane to evaluate the fate of protein following cellular metabolism. It was found that in cells exposed to flow, cellular uptake and/or degradation was significantly increased as compared to cells under static conditions.

With regard to the formation of tubular structures, Wei et al. developed a microfluidic chip where a monolayer of polarized epithelial cells was formed resembling the structure and function of native epithelia [146]. It

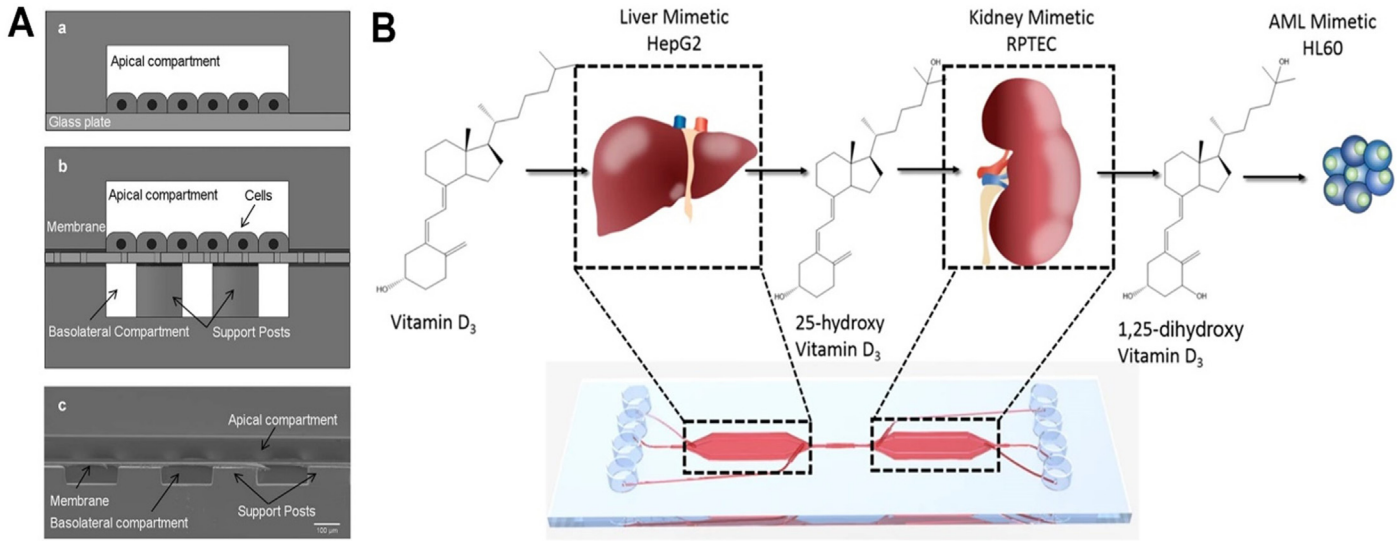

Figure 9.4 Example kidney-on-a-chip systems providing on-chip models to recapitulate the renal physiology. **(A)** Schematics showing the single- (subpanel a) and multichamber (subpanel b) microfluidic bioreactors developed to evaluate the albumin handling by proximal tubule cells. The electron micrograph in subpanel c shows the cross-section of the multi-chamber microfluidic bioreactor. In the bioreactors, a polycarbonate membrane was used to separate the apical and basolateral compartment, and cells are cultured on the membrane in the apical compartment. **(B)** Schematic showing an on-chip vitamin D3 metabolism and bioactivation using a microfluidic device, where the liver chamber is used to metabolize vitamin D3 to 25-hydroxy vitamin D3 and the kidney chamber is used to further metabolize 25-hydroxy vitamin D3 to 1,25-dihydroxy vitamin D3. The prodifferentiation effects of vitamin D3 were then investigated using myeloid leukemia cells (HL60) for vitamin D3 anticancer activity. *(A) Reproduced from Ferrell et al. [29], with permission from Wiley. (B) Reproduced from Jourde-Chiche et al. [145], with permission form, Nature Publishing Group.)*

was possible to assess the overexpression of specific proteins inside tubular structures, the functional kinetic information on Ca^{2+} in cells, in real time with Ca^{2+} transporting epithelia. Moreover, in a millifluidic chip developed by Homan et al., vascularized kidney organoids cultured under flow conditions had more mature podocyte and tubular compartments with enhanced cellular polarity and expression of adult genes as compared to those cultured under static conditions [147]. Further, a multicompartment microfluidic chip developed by Theobald et al. recapitulated hepatic vitamin D metabolism in humans (Fig. 9.4B) [148]. They showed that cultivation of HepG2 and RPTEC cells, which were used to mimic liver and kidney, respectively, in interconnected chambers of the chip led to enhanced expression of vitamin D metabolizing enzymes as compared to cultivation using conventional tissue culture.

Altogether, the aforementioned findings contribute to improving our understanding of the function of the kidney. However, more complex KoC systems need to be established to recapitulate complete renal physiology, which would require providing a physiologically relevant microenvironment to complex on-chip models.

9.3.2 Disease models and pathophysiological studies

Over a few decades, several advances have been made in developing in vitro on-chip models that can recapitulate normal physiology and pathophysiology, as well as cellular and molecular functions of the native kidney [24,142]. In this regard, KoC systems give a great opportunity for replicating key structures and integrated functions of the native kidney in lab settings, where more relevant disease models can be generated to assess pathophysiological mechanisms underlying kidney dysfunction mostly caused by acute kidney injury, chronic kidney failure, and viral infection.

9.3.2.1 Acute kidney injury and chronic kidney failure

Acute kidney injury occurs mainly when the blood flow to the kidneys is reduced or when the urinary tract is obstructed. In particular, in patients with renal hypoperfusion or urinary stone disease, acute injury leads to a reduced glomerular filtration rate [149]. Acute renal injury is also caused by toxic agents, such as drugs, metals, chemicals, and fungal toxins, which damage nephrons leading to nephrotoxicity [145]. With further worsening, acute injury leads to chronic kidney failure, which is associated with increased morbidity and mortality [150].

To study the effect of toxicities produced by drugs, a reusable microfluidic device containing on-chip models of PT and glomerulus was developed using human kidney epithelial cells (Fig. 9.5A) [4]. A chemotherapeutic drug, cisplatin, was administered to the PT cells under static and dynamic conditions, and cell injury was investigated. Cells cultured under flow conditions were less damaged than cells cultured under static conditions, as indicated by lactate dehydrogenase release. Moreover, cisplatin-damaged cells cultured under flow conditions recovered to a significantly greater extent than the cells cultured under static conditions as indicated either by suppression of apoptosis or the number of viable cells remaining attached to the extracellular matrix-coated substrates. These findings coincided with observations made in kidney failure patients, most of whom have recovered after cisplatin toxicity.

In another study, glomerulus was recapitulated, using human endothelial cells and rat podocytes, in a microfluidic device developed as a diabetic neuropathy on-chip model (Fig. 9.5B) [125]. The device, designed to reproduce high glucose-induced critical pathological responses in diabetic nephropathy, showed an increase in barrier permeability to albumin corresponding to high glucose concentrations on podocytes. The findings of this study were verified with a commercialized GoC, lined with human podocytes (primary, immortalized, or amniotic-fluid derived). Immortalized podocytes showed less filtration of albumin than primary and amniotic-fluid derived podocytes [126].

Chemotherapeutic or antiviral drugs might also injure the kidney leading to chronic kidney failure. To evaluate this, another microfluidic device was developed by culturing Madin–Darby canine kidney cells on a fibronectin-coated porous membrane to study the kinetics of gentamicin nephrotoxicity (Fig. 9.5C) [25]. Results of the study revealed that a higher concentration of gentamycin increases membrane permeability, reduces cell viability, and destroys tight junctions of the kidney, indicating that the drug toxicity may lead to chronic kidney failure.

9.3.2.2 Virus-induced disease models

OoC can be used to study viral diseases and their therapeutics [151,152,154,155] and can be applied to investigate kidney dysfunction caused by viral infections, for example, using distal tubule-on-a-chip [156]. In this study, an in vivo-like three-layered device (Fig. 9.5D) was fabricated using distal tubule cells, which were then infected with pseudorabies virus. Before infection, the on-chip model was tested for tight reabsorption

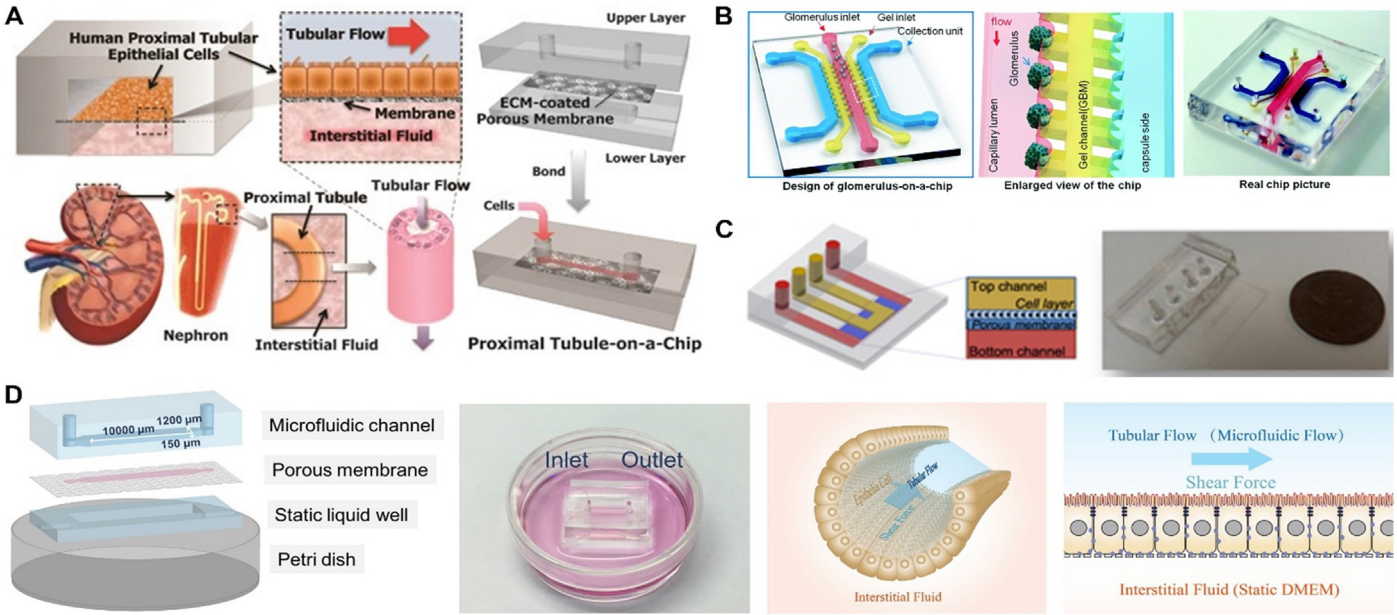

Figure 9.5 Example kidney-on-a-chip systems generating disease models for pathophysiological studies of the kidney. **(A)** Schematic showing the design of a microfluidic device consisting of an apical (intraluminal) channel and a bottom reservoir (interstitial space), where the compartments are separated by an extracellular matrix (ECM)-coated porous membrane. In the device, primary human proximal tubular epithelial cells are cultured in the presence of physiologically relevant apical fluid shear stress. The basolateral compartment is readily accessible for fluid sampling and addition of test compounds to study active and passive epithelial transport, and drug-induced cell injury. **(B)** Schematic design and micrograph showing a glomerulus-on-a-chip developed to study diabetic neuropathy. The device consists of capillary, gel, and collection channels, representing the capillary lumen, the glomerular basement membrane, and Bowman's capsule, respectively. **(C)** Schematic design and micrograph showing another kidney-on-a-chip developed to study the kinetics of cell injury due to gentamicin nephrotoxicity, caused by exposing kidney epithelial cells to various pharmacokinetic profiles of drug concentrations in the bloodstream in humans. **(D)** A 3-layered microfluidic chip developed to study virus-induced kidney dysfunctions. Illustrations depict the extracellular environment of epithelial cells in distal renal tubules and physiologically relevant fluidic flow within the tubule that generates a shear force to epithelial cells. (A) is reproduced from Ref. [4], with permission from the Royal Society of Chemistry. (B) is reproduced from Ref. [125], with permission from the Royal Society of Chemistry. (C) is reproduced from Ref. [25], with permission from IOP publishing. (D) is reproduced from Ref. [156], with permission from ELSEVIER.

barrier, apical microvilli, and sodium reabsorption. After 24 h postinfection, pathogenesis of the virus affected renal dysfunction in electrolytes regulation, decrease in sodium reabsorption, apical microvilli transformation, and disruption of polarized distribution of functional proteins. Although on-chip models other than KoCs have been applied for in vitro investigation of various viral infections, this study was the first one to investigate physiological and functional changes occurring in the kidney following viral infection.

9.3.3 Drug development and testing

While developing new drugs, there are various steps to perform for assessing drug toxicity. In general, the toxicity of drugs is tested using animal models, but these are expensive, and the results are species-specific. To overcome this limitation, on-chip cell culture models are necessary to recapitulate the structure of the kidney in vitro. Among them, the generation of PT-on-chip models attracts great attention for the creation of physiologic models of the PT, the primary site in the nephron for drug clearance and a primary site for drug-induced nephrotoxicity, because of at least in part due to accumulation of drugs in the PT [5,157]. When PT is exposed to drugs for prolonged periods, chronic kidney failure may ensue. Therefore, it is important that in preclinical trials newly developed drugs are assessed using appropriate and reliable models.

9.3.3.1 Drug screening using cellular models

For drug screening using cellular models, several studies aimed to generate KoC models. For example, in one study podocytes were cultured in a microfluidic device to form glomerulus for nephrotoxicity and drug assessment (Fig. 9.6A) [161]. In another study, the toxicity of two drugs, cisplatin and cyclosporine, were then assessed, showing more sensitivity with integrated glomerulus cells. In another study, PTECs were cultured in a single-channel Nortis microfluidic chip to assess nephrotoxicity caused by a model drug (polymyxin B). The results of the study showed a significant rise of kidney injury molecule-1 (KIM-1) and a panel of injury-associated miRNAs (Fig. 9.6B) [162].

Further studies reported microfluidic devices with more complex microenvironments. For example, in one study a microfluidic device was developed, and it comprised glomerulus, Bowman's capsule, PT lumen, and capillary tubules [140]. Adding drugs, such as cisplatin and doxorubicin, to the renal blood flow showed significantly increased biomarker levels such

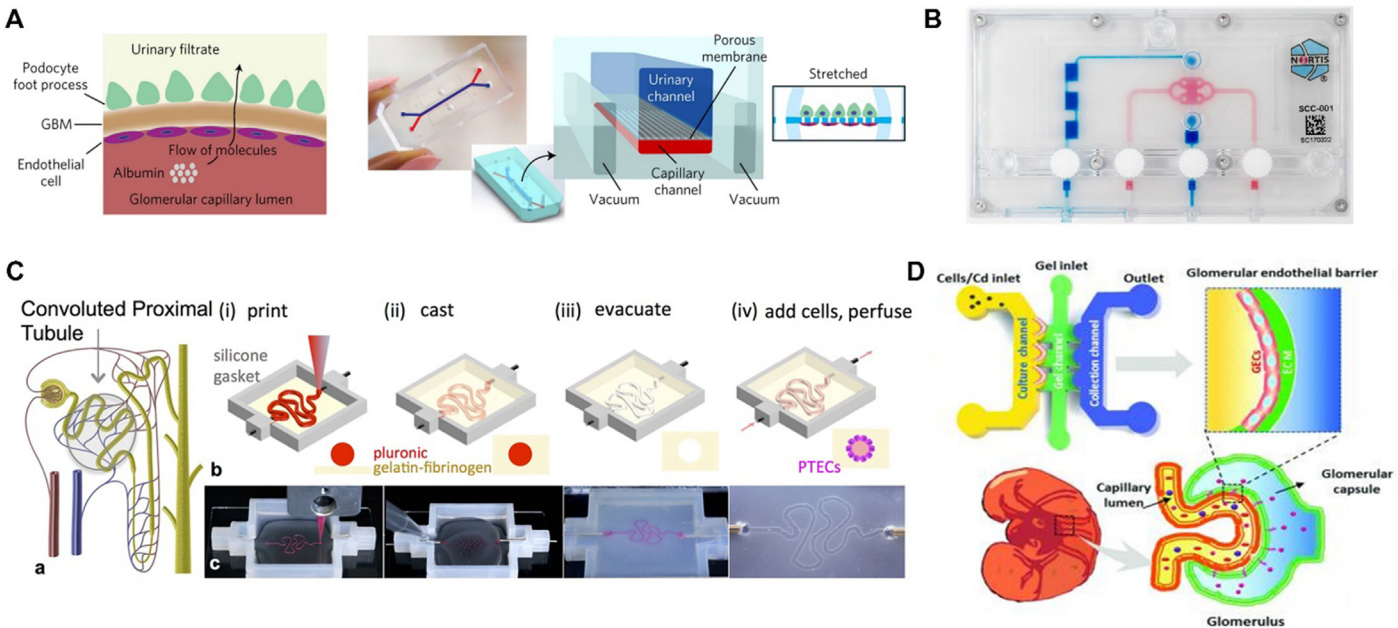

Figure 9.6 Example kidney-on-a-chip systems for nephrotoxicity and drug assessment. (A) From left to right: Schematic representation of the structure and function of a glomerular capillary lumen, where podocytes and endothelial cells are separated by a glomerular basement membrane (GBM). Micrograph and schematic design of the developed glomerulus-on-a-chip to mimic Adriamycin-induced albuminuria and podocyte injury, where cell layers were exposed to cyclic mechanical strain by stretching the flexible PDMS membrane using vacuum. (B) Micrograph showing a single-channel Nortis microfluidic chip developed for safety testing of polymyxin antibiotic nephrotoxicity on human kidney proximal tubule cells. (C) Schematic on the left panel showing a nephron where the convoluted proximal tubule is highlighted, whereas schematics and micrographs on the right panel show the bioprinting steps of 3D convoluted renal proximal tubules on perfusable chips to study the nephrotoxicity of cyclosporine A on the epithelial barrier. (D) Schematics showing a microfluidic device developed to test for cadmium toxicity on glomerular endothelial cells. *(A) Reproduced from Lentini et al. [158], with permission from Nature Publishing Group. (B) Reproduced from Lepist et al. [159], which is freely available via the Creative Commons Attribution License (CC BY 4.0). (C) Reproduced from Homan et al. [133], under a Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/). (D) Reproduced from Li et al. [160], with permission from The Royal Society of Chemistry.)*

VEGF and E-cadherin, resulting in injury of kidney cells. In another chip, renal tubular epithelial cells and endothelial cells were cocultured on a porous membrane integrated within a microfluidic chip, and model drugs were passed to generate concentration gradients. In two other studies, two-chamber microfluidic devices were developed to study the toxicity of drugs by cocultured kidney and liver cells. Developed systems metabolized the toxins and drugs that were administered and provided toxicity read-out of the metabolites that could be used for drug screening [163,164].

9.3.3.2 Metal-induced nephrotoxicity

Nephrotoxicity is not limited to drugs as exposure to heavy metals such as arsenic, cadmium (Cd), cobalt, copper, and mercury can also be toxic [158]. Although small amounts of these metals are beneficiary for human health as they represent important components of cellular pathways, large amounts contribute to organ damage [158].

To investigate nephrotoxicity induced by Cd [162], which severely damages internal layers of the kidney, KoC with three discrete champers was fabricated to determine Cd-induced nephrotoxicity. In this device, the capillary and the glomerular capsule sides of the glomerular filtration barrier were fabricated (Fig. 9.6D). Primary rat glomerular endothelial cells were cultured in the middle gel channel demonstrating selective permeability of the renal barrier for investigating the nephrotoxicity induced by exposure to Cd at different concentrations. The results of the study indicated that Cd-induced cytotoxicity led to the disrupted expression of tight junction protein ZO-1 in a dose-dependent manner, which enhanced the permeability of the endothelial layer to large molecules such as immunoglobulin G and albumin.

9.3.3.3 Drug transporter interaction

In drug development research, apart from drug toxicity, evaluation of drug transporter interaction is also important to evaluate, to avoid possible drug withdrawals at a later stage of clinical trials. Regulatory agencies such as the US Food and Drug Administration and European Medicines Agency endorsed the study of the drug–drug interaction using drug transporters [159]. Transporters such as P-glycoprotein are present in the PTECs and the transport of drugs from the blood takes place through organic solute carriers, namely organic cation transporter 2 and organic anion transporter 1 and 3 [165].

To study drug transporter interactions, conditioned PTECs and human primary PT cells have been used. For example, in one study a microfluidic device was developed using cultured human PT cells to study various functionalities of the kidney including drug efflux [4]. Cisplatin was applied, and the P-glycoprotein transporter efflux activity was assessed using calcein-acetoxymethyl-ester. The specificity of P-glycoprotein activity was confirmed by adding the inhibitor verapamil and measuring the fluorescence intensity. In another study, a 3D microfluidic PTEC model was developed for high-throughput drug-transporter interactions [166]. Using the chip, 3D drug efflux assays were analyzed using P-glycoprotein and multidrug resistance-associated protein transporters. Chip was further used in the detection of microRNAs released from kidney cells in response to toxic microenvironment.

Overall, KoC systems have been used to investigate biological acute kidney injury and chronic kidney failure occurring at cellular, molecular, and functional levels. Noteworthy, KoC models can also be applied to study molecular mechanisms of renal injuries caused by viruses, and potentially, renal pathology caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection [151–153]. This way, the developed disease models could help in assessing the chemotherapeutic drugs against virus-induced kidney dysfunctions in vitro.

9.4 Current challenges and future outlook

OoC is a technologically advanced medical device, which aims to investigate organ function, test drugs, and develop personalized therapeutics [72]. In addition, there is a rising demand for the drug toxicity assessment in the preclinical investigation, among which KoC represents a valuable model [12]. KoC has some benefits of exposing cells to physiological shear stress, which makes cells more sensitive to drugs in the kidney MPS. KoC could provide a platform for better testing of renal toxicity [167]. In this regard, PT epithelium is most prone to drug-induced toxicity, and it is a predominant focus of many studies to reconstitute physiologically relevant microenvironment to provide insights into drug discovery, efficacy, and toxicology.

Kidney MPS models with accurate functions offer the opportunity to investigate kidney physiology, pathophysiology, and drug toxicities. Having a reliable model of kidney biology, researchers have recommended the characterization of three different features of the mechanical environment, transport/metabolic function, and kidney-specific biochemical markers

[168]. In addition, it is critical to incorporate chemical, physical, and biological variables into the MPS models of the kidney. At the organ level, the kidney is composed of various, specialized cells, and different parts. Thus, specific kidney cell types are required for the development of disease models, and chemical screening [169]. Recent advances in iPSCs derived kidney organoids, and CRISPR gene-editing technology enable the development of functional experiments and establishing gene-edited organoids derived from pluripotent stem cells to gain insight into the cellular mechanisms of kidney disease [170].

To realize a more human-relevant in vitro model, it is critical to provide physiological flow to help the physiological function of cells and maintain cells in a highly differentiated state. With the advent of CRISPR/Cas9 technology, the functional consequence of genetic mutations can be investigated, thus new biomarkers for the detection of genes involved in drug-responsive phenotype.

9.5 Conclusions

Various KoC systems have been developed to recapitulate kidney function and model disease or study drug toxicity, and they represent potential tools for use in conducting preclinical studies in the future. To assess also secondary and systemic toxicity, multi-organ-on-a-chip systems that can have kidney as one component are required. With well-developed multi-organ-on-a-chip systems, the use of animal models can largely be reduced, and cost-effective drug development can be anticipated. To accomplish this, a multidisciplinary approach and sustained funding are required.

Acknowledgments

The authors acknowledge that they have no competing interests. The authors also acknowledge funding from National Institutes of Health (1UG3TR003148-01) and the American Heart Association (18TPA34230036, 442611-NU-80922).

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