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Kidney bioengineering in regenerative medicine: An emerging therapy for kidney disease

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Abstract

The prevalence of end-stage renal disease is emerging as a serious worldwide public health problem because of the shortage of donor organs and the need to take lifelong immunosuppressive medication in patients who receive a transplanted kidney. Recently, tissue bioengineering of decellularization and recellularization scaffolds has emerged as a novel strategy for organ regeneration, and we review the critical technologies supporting these methods. We present a summary of factors associated with experimental protocols that may shed light on the future development of kidney bioengineering and we discuss the cell sources and bioreactor techniques applied to the recellularization process. Finally, we review some artificial renal engineering technologies and their future prospects, such as kidney on a chip and the application of three-dimensional and four-dimensional printing in kidney tissue engineering.

Key Words: *extracellular matrix, kidney decellularization, kidney recellularization, regenerative medicine, tissue engineering*

Introduction

The kidney has a function in the filtration and excretion of waste products and excess material from the blood in addition to performing metabolic, hemodynamic, immunologic and endocrinologic roles [1]. Patients who undergo dialysis have impaired renal function when compared with people in the general population [2]. Although renal replacement therapy (RRT) may be more effective than dialysis, a shortage of organ donors is the main obstacle to restoration of patient quality of life. In European countries such as Austria, Norway, the Netherlands and the United Kingdom, the number of patients older than 65 years requiring RRT has increased significantly over time, whereas the number of actual renal transplants did not match the demand [3]. In North America, approximately 100,000 patients are awaiting RRT; the mortality rate is 5% to 10% for patients on the waiting list, and approximately 40% of transplant recipients will die or lose graft function within 10 years of transplantation [4].

In recent years, decellularization technology has emerged as a promising field in the field of regenerative medicine [5–7]. Decellularized scaffold derived from a whole organ has the advantage of providing structural integrity of tissue, as synthetic and natural polymers cannot replicate accurate spatial organization of complex cellular architecture as is found in the native kidney tissue [8]. The use of a decellularized kidney scaffold is based on the mechanical and biological property of the extracellular matrix (ECM), which can maintain natural cellular architecture and some residual molecules that may enhance recellularization, differentiation and proliferation of the decellularized cells [9–11].

Perfusion methodology in different species

The scaffold derived through perfusion should contain a vascular tree that facilitates *in vitro* perfusion and reconnection to the blood stream, which will provide nutrient and oxygen delivery in addition to removal

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of waste in the tissue-engineered construct [12]. The decellularization process of xenogeneic or allogeneic donor kidneys, using cell-lysing solutions perfused through the renal vasculature, has been demonstrated in recent years by a number of techniques.

In the rodent, Bonandrini et al. [13] were able to overcome the time-consuming perfusion method by using sodium dodecyl sulfate (SDS) alone as a lysis buffer. Transmission and scanning electron microscopy showed preservation of the three-dimensional (3D) architecture of blood vessels, glomeruli and tubuli and used a novel evaluation method of micro-computerized tomography (micro-CT) scanning to demonstrate the integrity of the vascular network. For a systematic evaluation of scaffold preparation, Caralt et al. [14] developed a histologic scoring system based on two semi-quantitative grading scales, which were used to evaluate biochemical characteristics of decellularized rat kidneys, with the use of two protocols using 1% Triton X-100, 1% Triton X-100/0.1% SDS. The scoring system evaluated preserved renal microarchitecture and matrix-bound basic fibroblast growth factor and vascular endothelial growth factor. A combined and sequential detergent approach was used to procure intact rat kidney ECM by Yu et al. [15]. The team developed a new protocol to generate rat kidney scaffolds by perfusion using continuous detergent through the infrarenal abdominal aorta, with heparin, Triton X-100, deionized water and SDS. Evaluation of this combination perfusion approach showed good retention of both a range of cellular cytokines and an intact architecture of the vascular tree. Hematoxylin and eosin staining and electron microscopy were used to confirm the clearance of nuclei in the kidney structure; Periodic acid-Schiff and Masson's staining define the number of positively stained structures present as without architectural damage.

In the larger porcine kidney, Orlando et al. [16] applied hypotonic distilled water that induced cell swelling and consequent blast of cell membrane, followed by perfusion with SDS. Methenamine silver staining was performed to estimate the kidney structure of glomerular basement membrane, and the degree of cell clearance was also evaluated by staining of glomerular transmembrane proteins, which showed negative results. To better evaluate the influence of perfusion pressure, a Millar MPC-500 Mikro-Tip pressure transducer catheter and MPVS-400 signal conditioning hardware were used, which showed a good relationship between pressure an increased flow of the decellularization lysis solution. In a protocol that also used SDS alone as the lysis buffer, Sullivan et al. [17] compared the effectiveness of the concentration of single perfusion regimen by using a high-throughput system designed and constructed to provide decellularized scaffold from porcine kidney. Results showed that the SDS-treated decellularized scaffolds were non-cytotoxic to primary human renal cells and that 0.5% SDS was the most effective detergent, with <50 ng remnant DNA/mg dry tissue. In addition, glomerular and tubular structures in the cortex-medulla regions of the ECM were well preserved, and an intact boundary structure between the vascular and filtrate collection systems was determined by means of CT imaging. In attempting to mimic a procedure that could be applied to human kidney, Nakayama et al. [18] perfused rhesus monkey kidney with 1% SDS at 4°C and found this to be the most effective regimen for preservation of native cellular architecture and to cause minimal changes in morphology.

Discarded human kidneys are a desirable source of renal ECM, and their application for tissue engineering may be more clinically compatible than kidneys derived from other species. The perfusion of a discarded human kidney is performed by connecting the renal artery and the ureter and perfusion with a 0.5% SDS solution [16,19]. After perfusion, the matrix is rinsed with phosphate-buffered saline (PBS) to reduce the toxic effect of detergent. Under these conditions, the ECM framework retained its architecture and biochemical properties, and this process has the advantage of cell clearance despite radical glomerulosclerosis and interstitial fibrosis. Another method achieved clearance of human leukocyte antigens, confirmed by immunostaining, which is needed to prevent organ rejection and essential for positive clinical outcomes in the future [20]. A further study that used discarded human kidneys confirmed the preservation of glomerular microarchitecture, vascular mechanical properties and retention of cytokines and growth factors essential for recellularization [21].

Multiple techniques combined in the perfusion process

The addition of new solutes to kidney perfusion solutions and a systematic comparison of the pros and cons of different solutions may allow an improvement in decellularization methodology. Biological and physical techniques are both used in some protocols to decellularize the kidney. Caralt et al. [14] used 0.02% trypsin, 0.05% EGTA, 1% Triton X-100 to decellularize the kidney; however, it should be noted that a trypsin enzyme solution may cause structural damage and loss of growth factors. DNase has also been used in many protocols to help clear residual nucleic acid [17,21]. Wang et al. [22] described a protocol using different detergents, 1% SDS, 1% Triton X-100, 1% peracetic acid (PAA) and 1% sodium deoxycholate (NaDOC) perfused through the renal artery of the kidney and followed by a rinsing step with PBS. On comparison, the SDS-treated group showed the most efficient clearance of nucleic acid. However, Triton

X-100, PAA and NaDOC were unable to completely remove cellular components and xenoantigens completely, and Triton X-100 and NaDOC have been reported to disrupt the 3D micro-structure of the ECM scaffold [23]. A recent finding is that Triton X-100 may effectively clear residual SDS, which may ensure that the scaffold shows no or reduced cell cytotoxicity. Kawasaki et al. [24] used a novel detergent, sodium lauryl ether sulfate (SLES), and on examination of the cell microarchitecture and glycosaminoglycans (GAG) content showed better preservation of ECM with SLES than with SDS. In addition, the use of SLES was associated with less platelet adhesion and a relatively low inflammation, which over time may promote recellularization. Freeze-thaw cycles were also used to help decellularization protocols [22,25,26]. A single freeze-thaw cycle can decrease immune responses such as leukocyte infiltration in the vascular structure of ECM scaffolds, which may present a hazard for decellularization and subsequent use of the scaffold [27]. Osmotic shock has the advantage of disrupting DNA-protein interactions, effectively lysing cells, although it is ineffective in removing cellular residues [16,26,28,29]. A physical pressure method may also be applied by a constant pressure of 40 mm Hg during the perfusion step. With the use of 1% SDS during perfusion, the study reported an increase in vascular resistance during decellularization and a decrease after re-endothelialization. This may be explained by the observation that microemboli and immature vascular beds were found in heart and lung. Immunohistochemical staining confirmed the presence of key ECM components such as laminin and collagen. The use of higher perfusion pressure, for example, 120 mm Hg, did not cause increased albumin or glucose loss in bioengineered kidneys [5,29,30].

The main goal of organ decellularization is the removal of all cellular material without adversely affecting matrix component, biologic activity or mechanical integrity [31]. However, it is difficult to achieve complete organ decellularization, and most ECM scaffolds contain residual DNA and other cytoplasmic components [32]. To date, there is no accepted consensus view on a standardized procedure. However, a broad view has been proposed, based on the findings of other studies using decellularized tissue, and can be summarized. The ECM should contain less than 50 ng of DNA per milligram of extracellular matrix dry tissue, any residual DNA fragments should be less than 200 bp in length and the ECM should lack any visible nuclear material as confirmed by standard hematoxylin and eosin or 4′-6-diamidino-2-phenylindole (DAPI) staining [33–35]. An overview of the paradigm that summarizes recent strategies for producing decellularized ECM from kidney is shown in Table 1; the typical procedure for decellularization of human kidney is presented in Figure 1.

Factors associated with kidney decellularization and recellularization methods

Any perfusion technique that uses exposure of tissue to chemicals or pressure to remove cellular components may disrupt ECM structure [5–7,30,36,37]. Non-ionic detergents, for example, Triton X-100, is less effective than SDS [38–42] and is commonly used. Differences in effective lysis may occur, depending on the tissue, and may be more effective in removing cellular components, depending on tissue thickness, and may cause some disruption of microstructure and loss of GAG. Ionic detergents, for example, SDS, whose function is to cause cell lysis and remove nucleic membranes, will denature scaffold proteins unless the lysis conditions are carefully controlled. Although SDS can effectively remove residual nuclear matter and cytoplasmic proteins from dense tissues, it may adversely disrupt ultrastructure, reduce GAG content and growth factor levels, and the degree of unwanted ECM damage may depend on tissue thickness [43–47]. Interestingly, the age of the tissue donor appears an important factor in kidney recellularization efficiency in which cellular repopulation was greatest with scaffolds from the youngest kidney donors compared with juvenile and adult rhesus monkey kidney [48]. Nakayama et al. [18] showed that the temperature of the perfusion solution is another factor that may affect decellularization, with the use of SDS at 4°C to be most effective in preserving native kidney architecture [18]. Tsuchiya et al. [49] developed a protocol to test several pH conditions (pH values tested: 8, 10, 12) and showed that lung tissues decellularized at pH 8 retained the greatest preservation of tissue architecture and the least ability to induce a host immune response. As the kidney and lung have a similar organogenesis [50], further research should be performed to explore and derive the optimum pH value for decellularization of the kidney.

Other factors that may influence the quality of derived ECM include the effect of freezing and thawing and sterility. The effect of freeze/thaw cycles on native and decellularized whole porcine kidneys was studied in the absence of cryoprotectants, and results indicated that the elastic modulus of native kidneys was reduced [51]. Although various physical and chemical factors may damage the scaffold during the process of a whole-organ decellularized kidney, it is essential that a pathogen-free scaffold is provided for transplantation. The choice of sterilization method, however, has an impact on scaffold quality. Clinically, commonly used sterilization methods such as pressurized steam and dry heat have the potential to cause protein

Table 1. Critical decellularization strategies.

Author	Species	Decellularization strategy	Time consumed	Main decellularization result	Sterilization choice	Reference
Nakayama et al.	Rhesus monkey	PBS washed twice; either 1% (v/v) SDS at 4°C or Triton X-100; then washed with PBS.	7 to10 days	Removal of cellular material; preservation of native expression patterns of ECM proteins; a decrease in the compressive modulus of ECM.	In 10% (v/v) penicillin/streptomycin.	[18]
Orlando et al.	Human	Starting with distilled water at 12 mL/min for 12 h; 0.5% SDS for 48 h; finally using PBS rinsed for 5 days at 6 mL/min.	60 h plus 5 days	95% of DNA was removed; ECM retained its architecture and biochemical properties; vascular network is intact.	Not mentioned	[19]
Song et al.	Sprague-Dawley rats	Perfusion of 1% SDS, then 1% Triton X-100 at a constant pressure of 30 mm Hg.	108 h	Preserved the structure and composition of the renal ECM and arterial elastic fiber network.	10,000 U/mL penicillin G, 10 mg/mL streptomycin and 25 μg/mL amphotericin B.	[29]
Sullivan et al.	Porcine (Yorkshire pigs)	10 USP units/mL sodium heparin, then 0.5% SDS and 1% Triton X-100/0.1% ammonium hydroxide, then 0.0025 w/w% DNase with 10 mmol/L magnesium chloride, using a high-throughput system to perfuse.	36 h	<50 ng DNA/mg dry tissue; intact microarchitecture.	Exposed to 10.0 kGy gamma irradiation.	[17]
Yu et al.	Rat	8 mL/min in the following order:50 U/mL heparin in 0.01 mol/L PBS for 30 min; 0.1% Triton X-100 for 3 h; deionized water for 30 min; 0.8% (v/v) sodium lauryl sulfate (SDS) for 3 h; and deionized-water for 24 h.	28h	The kidney scaffolds lose renal cells but keep normal vascular tree and continuous extracellular matrix.	100 U/mL penicillin and 100 mg/mL streptomycin.	[15]

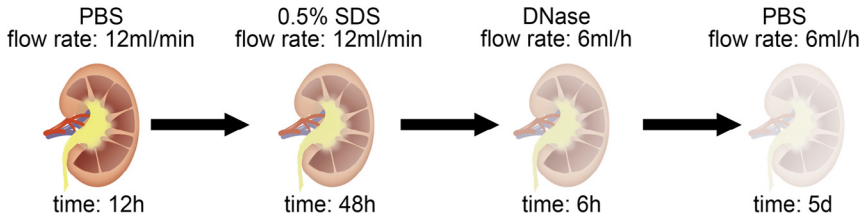

Figure 1. Flow chart illustrates the decellularization method of a typical discarded human kidney, using gradual color fade to note the extent of decellularization.

denaturation [52]. Other methods of sterilization in ECM such as gamma irradiation and electron beam can change the mechanical properties of ECM [53–56]. Although chemicals such as ethylene oxide gas have the advantage of not destroying common cytokines, it can also change mechanical properties of the ECM [42,57]. Fortunately, PAA and supercritical carbon dioxide are emerging as promising alternative choices of sterilization. PAA has the advantage of effective removal of bacteria, fungi and spores, without causing disruption of ECM proteins [58]. The use of supercritical carbon dioxide as a sterilization agent has received some attention because it may cause multi-log reductions in bacterial and viral products within the ECM, although it may cause minor changes of mechanical properties when compared with PAA [59].

Although we have many ways (hematoxylin and eosin, DAPI staining, terminal deoxynucleotidyl transferase staining, corrosion cast model) to evaluate the quality of the scaffold, it may be necessary to systematically define an algorithm of properties to optimize a protocol of decellularization that will ultimately form a gold standard for clinical application. A standard and strict evaluation method is clearly required. For example, Caralt et al. [14] used histologic scoring systems to quantify fundamental characteristics of decellularized rodent kidney, and Song et al. [29] applied a histology-based morphometry protocol to assess the microarchitecture of acellular kidney scaffolds [60]. For the scaffold component, Peloso et al. [21] applied a method for evaluation of 40 key growth factors (GFs) after decellularization with the use of a glass chip-based multiplex enzyme-linked immunosorbent assay array and *in vitro* immunofluorescence. There is no doubt that a systematic scoring evaluation strategy can facilitate and optimize the decellularization process; thus, further development of scaffold evaluation strategies is needed.

For methods supporting recellularization, it is obvious that different organ sizes of either xenogeneic or allogeneic origin require different numbers of cells [14,17,19,61,62]. In addition, each organ relies on different conditions such as recellularization perfusion rate, temperature, CO_2 concentration, GFs, and nutrients [63]. Different cell types also have different properties and cell seeding within the kidney tubules, and peritubular capillaries through the vascular tree or ureter result in different cell distribution. Seeding through the renal artery is a routine method, which may achieve a balanced distribution of cells, and more than 97% attachments have been reported by Bonandrini et al. In contrast, Caralt et al. [14] presented a protocol that showed that 50% coverage of the renal area is attained, and Ross et al. [28] compared seeding methodologies through the renal artery and ureter, which showed >95% retained versus ~50% retained, respectively [28]. Very recently, a new recellularization strategy through the renal artery, followed by perfusion of the neonatal kidney cells through the ureter, has been developed. The technique maintains a negative pressure (about 40 mm Hg), after which the scaffold culture can produce urine *in vitro* and *in vivo* [29]. Rosines et al. [64] found that hyaluronic acid (HA) has the ability to simultaneously modulate ureteric bud (UB) branching, facilitate mesenchymal-to-epithelial transformation and to promote the differentiation of the metanephric mesenchyme. The study also showed that UB branching was dependent on both the concentration and the molecular weight of HA [64]. Exploring factors affecting decellularization and recellularization is critical for potential clinical application; these factors are summarized in Figure 2.

Cell sources for renal scaffold seeding

The ECM scaffold structure is vital to facilitate cell seeding and construction of organ-like cellular compartments [31]. In a routine physiologic state, the kidney has the potential for relatively low regenerative capacity compared with other organs; the tubular epithelium has the highest potential for self-renewal [65,66]. After damage, such as acute tubular necrosis, tubular epithelial cells can restore damaged tubules [67]. Renal adult stem cells express cytokines, for example, CD24, CD133, CD146 and Pax-2 [68–72]. However, today, we lack a detailed understanding of the molecular mechanisms for regeneration.

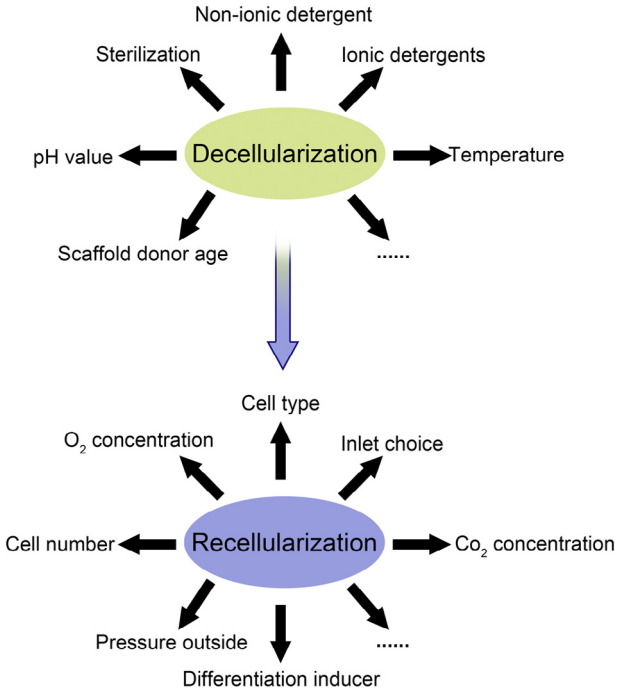

Figure 2. Summarized factors based on recent studies that associated kidney decellularization and recellularization.

For the final aim of recellularization, which is to generate full kidney function, which includes filtration, secretion/reabsorption and concentration of urine, constructs comprising nephrons and other supportive cells need to be populated [73]. Based on the current research on scaffold decellularization, the minimal function of a recellularized kidney is the secretion of urine by the repopulation of renal medulla cells [31].

Embryonic stem cells (ESCs) are pluripotent cell sources that can give rise to all three germ layers and they also have the ability to proliferate to large numbers without aging. Ng et al. [74] suggested that predifferentiation of ESC could increase the chance of organ-specific differentiation [74]. Furthermore, predifferentiated ESC lack the epigenetic modifications that may enable immediate response to *in vitro* stimuli when compared with more differentiated stem cells or progenitor cell populations [75]. However, there is an ethical and moral debate concerning the derivation of ESC in addition to the risk that ESC have the potential to give rise to teratomas if transplanted into an undifferentiated environment [76,77]. Bonandrini et al. [13] used murine embryonic stem (mES) cells to recellularize the kidney through the renal artery. mES were produced for recellularization in a bioreactor for 24 and 72 h with adjusted pressure and applied in a regimen of perfusion recycling. Differentiation toward a meso-endodermal lineage was observed. Ross et al. [28] perfused mES through the artery or retrograde through the ureter, achieving the first renal recellularization; the results showed differentiation and proliferation of seeded cells. The study by Nakayama et al. [50] suggested that decellularized kidney scaffolds have an intrinsic ability to induce human embryonic stem cell (hESC) differentiation and proliferation into appropriate structures and phenotypes.

Primary tissue or whole-organ–derived cell sources have a nonimmunogenic property and can rapidly give rise to mature functional tissue [31]. With the use of a perfusion-based medium, Caralt et al. [14] perfused human renal cortical tubular epithelial (RCTE) cells in an antegrade pulsatile perfusion regimen through the renal artery, and the results showed approximately 50% coverage of the renal area [14]. Human renal cells have also been utilized for seeding porcine kidney scaffold [17], although the main disadvantage of cells from primary tissue is that the number of cells is limited.

The main advantage of using fetal cells is that they preserve their proliferative ability while being committed to a proliferation end point and have less likelihood than ESC to result in teratomas. Human amniotic stem cells (HASC) express surface markers and transcription factors distinctive of ESC [78] and can proliferate to about 250 doublings and have no known tendency for tumorigenesis. Song et al. [29] perfused the scaffold with neonatal kidney cells and added human umbilical vein endothelial cells (HUVECs) through the ureter. Bioengineered kidneys produced urine when compared with cadaveric kidney [29].

Human inducible pluripotent stem cells (iPSC) have emerged as an ideal source for regenerative medicine because they can be derived from autologous sources, can be produced in large numbers *in vitro* and may give rise to either parenchymal or supportive cells needed for complicated tissue engineering [29,79–83]. However, Hong et al. [84] showed that undifferentiated autologous iPSC can differentiate to mature teratomas in a dose-dependent manner accompanied by an inflammatory reaction. This is in contrast to the situation with differentiated iPSC, in which no evidence of teratoma formation has been reported. For parenchymatous cells, Song et al. [85] first reported on the directed differentiation of iPSC to form kidney cells with podocyte features. Other protocols have reported on the differentiation of iPSC in the pattern of intermediate mesoderm tubules expressing kidney proximal tubular markers [86]. For inducers of iPSC, Araoka et al. [87] discovered two retinoids, AM580 and TTNPB, through high-throughput chemical screening, and showed them to be efficient and stable intermediate mesoderm inducers, which achieved rapid (5 days) and efficient (80% induction rate) intermediate mesoderm [87]. We have summarized the

Table 2. Summary of recellularization cell sources.

Author	Cell type	Cell numbers	Inlet choice	Basic recellularization result	Reference
Ross et al.	Mice ESC	Not mentioned	Antegrade through the artery or retrograde through the ureter.	Primitive precursor cells populated and proliferated within the glomerular, vascular and tubular structures.	[28]
Caralt et al.	RCTE	40×10^6	Renal artery.	Resided on the basement membrane and formed what appear to be tubular structures.	[14]
Song et al.	HUVEC	$50.67 \times 10^6 \pm 12.84 \times 10^6$	Arterial cannula.	Grafts produced rudimentary urine *in vitro* and *in vivo*.	[29]
Lam et al.	hPSC	Not mentioned.	Not mentioned.	hPSC form tubules that express proximal tubular markers.	[86]
Song et al.	iPSC	Not mentioned.	Not mentioned.	The first report of the directed differentiation of iPS to generate kidney cells with podocyte features.	[85]

principally used cell sources for recellularization strategies in Table 2.

Recellularized scaffold bioreactor strategies and potential applications

As the final aim, kidney bioengineering in clinical application, developing a robust and reproducible recellularization strategy is a mandatory clinical and regulatory requirement. Provision of the right conditions with bioreactor systems is essential to recellularize cells to exact compartments of the kidney ECM scaffold, such that a 3D architecture can develop by cell proliferation and differentiation to replicate renal function. The ideal bioreactor should properly mimic the *in vivo* environment, deliver nutrients within perfused medium and monitor physiological parameters of tissue development with appropriately defined levels of sensitivity. Caralt et al. [14] established a perfusion-based bioreactor from two glass flanges placed against each at their ends, with a valve and exclude pad for media sampling. Results for recellularization studies showed that cells located in the parenchyma or peritubular space and attached to the basement membrane. Twenty-four hours after infusion of RCTE cells, about 50% of the renal area was recellularized. Song et al. [29] produced a bioreactor with improved cell delivery and retention that was achieved when kidney scaffolds were embedded in a seeding medium and under a vacuum that generated a pressure gradient across the scaffold. For monitoring the bioreactor, Uzarski et al. [88] designed a bioreactor capable of maximizing cell seeding of small-animal whole-organ scaffolds and showed preservation of long-term cell survival. They further developed noninvasive monitoring capabilities for tracing dynamic statistical changes within scaffolds by evaluation of hydrodynamic pressure drop. These bioreactor studies illustrate some routes to achieving a stable environment around the scaffold and are a step forward toward application for clinical transplantation.

The desirable application of an ECM scaffold is as a source for organ regeneration; therefore, given the limited research studies reported, there appears a considerable distance to go and scope for improved methodologies. Despite the challenges, the ability of iPSC to recapitulate various diseases helps us understand and model disease at both a molecular and pathogenic levels [89–93]. The production of induced iPSC-podocytes and their potential differentiation into epithelial cells allows an culture strategy that may promote both quantitative and qualitative modeling of kidney cell function in specific renal disease phenotypes *in vitro* [94]. Furthermore, iPSC-kidney cells in a 3D scaffold may act as a whole-organ application for high-throughput toxicology screening, studies of drug distribution and population-based toxicology studies, in addition to supporting personalized medicine [94]. This may help in the situation in which studies in animal models do not always translate into successful human response [95]. Thus, further research into kidney scaffold fabrication may not only reduce the use of animal model experimentation but also improve the reliability of kidney research models and progress the field of kidney bioengineering [4,96–98].

Chip kidney

The basic architecture and functional unit of the kidney is the nephron. The chip kidney is a novel artificial construct aimed at mimicking kidney function through the use of microfluidic devices that can cannulate to fluid pumps and to detection probes embedded within specific cell types [1]. Jang and Suh [99] presented a study of chip kidney; in 2010, when they integrated a polydimethyl siloxane (PDMS) microfluidic channel on a porous membrane to culture and analyze the

function of renal tubular cells. Their results showed that it was possible to develop a renal tubule system suitable for *in vivo* testing, which may have potential applications in drug screening and in advanced bioengineering. Similarly, Gao et al. [100] reported culture conditions of human renal proximal tubule epithelial cells (RPTECs), which were able to establish a basic structure and environment to support cell proliferation. Jang et al. [101] explored factors such as fluid-shear-stress, which may affect the translocation of aquaporin-2 and the reorganization of the actin cytoskeleton inside the renal tubular epithelial cells. Recently Jang et al. [102] described a protocol that showed proximal tubule-on-a-chip may have an application to toxicology research, using primary human kidney proximal tubular epithelial cells to construct a "kidney-on-a-chip". The application in toxicology appears promising, and other systems have been developed previously in this field [103–105]. It addition, Choucha-Snouber et al. [105] presented a co-culture system of liver-kidney, a two-organ interaction chip using micro-fluidics, which studied the interaction between both organs and noted the development of systemic multi-organ interactions. Very recently Maschmeyer et al. [106] established a microphysiological system capable of preserving the specific functions of four organs after nearly 28 days in co-culture. In addition to toxicology research, the kidney chip has also been applied in a pathology study, and Wei et al. [107] first reported conditions for the establishment of a layer of polarized cells, embedded within the microfluidic device. This system was able to function as a model of epithelial cells and as an organ model to study the molecular and pharmacological theory of calcium phosphate stone formation inside the epithelium. As great advancements in techniques based on organ-on-a-chip are made, an extension is to consider the future aspect of body-on-a-chip technology, which should have a sophisticated and complicated set of tools for micro-patterning cell cultures in 3D structure to create interconnected and interactional multi-organ like structures [108]. Although many achievements have been made, bioengineering of a fully replicate kidney is not yet achievable. There is still a long way to go because a fully functional kidney requires tubular and glomerulus components together with a functional vascular network tree capable of supporting compartmentalized fluid flow.

Three-dimensional printed kidney

Three dimensional bioprinting is novel technology that uses biomaterials to print the fragile and accurate organ layer according to specific anatomical positioning [1]. To date, most common printers use pneumatic or mechanical systems to produce continuous sections of material. Recently, we have seen some generation of tissue types, such as tissue-like organ skin [109,110], whole-organ heart [111] and bone [112]. Until now, early scientific developments to fabricate a whole-organ kidney have benefitted from informing patients on their disease. For example, 3D printing technology can be applied as teaching models of renal tumor localization [113,114]. As 3D printing becomes increasingly more accurate in modeling whole-organ kidney tissue, it may be used in morphological bioengineering applications. Surgical models may use printing technology to replicate real kidney tissue, presenting experienced urologists with tools for planning and training purposes [115,116].

Although there is still a long way to go before construction of a functional 3D kidney is feasible, Mu et al. [117] utilized methods of collagen fibrillogenesis in a liquid medium to construct 3D vascular networks using hydrogel, which enabled the replication of oxygen and nutrient diffusion in a nephron [117]. For the further clinical application of 3D printing of whole-organ kidney, there are some obstacles that must be overcome such as suitable biomaterials, correctly differentiated cell types and adequate supply of growth factors, with the aim of mimicking a native kidney tissue–like microenvironment [118]. Similar to 3D printing, four-dimensional (4D) printing is emerging as an interesting technique, but with the crucial difference that 4D printing produces products that are able to adjust themselves according to their surroundings, for example, a "dynamic" hydrogel ink against other static materials [119]. Four-dimensional printing may thus deliver complicated, clearly identified spatiotemporal anatomical details that may practically facilitate preoperative planning of surgical strategies and better replicate the structure of bioengineered kidney [120].

Conclusions

As the incidence of renal disease increases, the number of patients entering ESRD becomes greater and the demand for organ transplantation becomes a pressing issue, given the shortage of donor kidneys and the morbidity and mortality associated with lifetime immunosuppression. The first reported successful recellularization procedure was demonstrated by Ross et al. [28], and, recently successful recellularization of a kidney scaffold leading to functional tissue may hold promise for the future of kidney regenerative medicine [4,18,29]. However, there are still many obstacles to be overcome in kidney bioengineering, and protocols for decellularization and recellularizations must be further optimized for future clinical application, such as developing a suitable, systematic and reproducible

method for ensuring structural integrity of the ECM and vascular tree. In addition, the delivery of oxygen and nutrients is key for functional maintenance of highly metabolically active kidneys, and thus the bioreactor should be optimized according to organ size, the numbers of cells to be populated and an optimized ratio of oxygen to carbon dioxide. In addition, the bioreactor culture system should provide an environment capable of mimicking physiological conditions, which may include exposure to biophysical stimuli and shear stress [31]. Other issues including the selection of cell type, an understanding of each cell type's differentiation and proliferation, in addition to the culture environment necessary to support a kidney scaffold for the generation of complete single kidney capable of replicate native kidney function, requires substantial further research. In addition to the ultimate aim of reducing or even replacing organ transplantation, the use of engineered kidney scaffolds may have utility in drug toxicology in addition to augmenting drug development by personalized medicine [94]. Thus, the future holds optimism for several areas of clinical science, and we make look forward to fascinating scientific developments in many functional disciplines.

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References

[1] Peloso A, Katari R, Murphy SV, Zambon JP, DeFrancesco A, Farney AC, et al. Prospect for kidney bioengineering: shortcomings of the status quo. Expert Opin Biol Ther 2015;15:547–58.

[2] Evans RW, Manninen DL, Garrison LP Jr, Hart LG, Blagg CR, Gutman RA, et al. The quality of life of patients with end-stage renal disease. N Engl J Med 1985;312:553–9.

[3] Stel VS, Kramar R, Leivestad T, Hoitsma AJ, Metcalfe W, Smits JM, et al. Time trend in access to the waiting list and renal transplantation: a comparison of four European countries. Nephrol Dial Transplant 2012;27:3621–31.

[4] Allison SJ. End-stage renal disease: a bioengineered kidney with excretory function. Nat Rev Nephrol 2013;9:308.

[5] Ott HC, Matthiesen TS, Goh SK, Black LD, Kren SM, Netoff TI, et al. Perfusion-decellularized matrix: using nature's platform to engineer a bioartificial heart. Nat Med 2008;14:213–21.

[6] Uygun BE, Soto-Gutierrez A, Yagi H, Izamis ML, Guzzardi MA, Shulman C, et al. Organ reengineering through development of a transplantable recellularized liver graft using decellularized liver matrix. Nat Med 2010;16:814–20.

[7] Petersen TH, Calle EA, Zhao L, Lee EJ, Gui L, Raredon MB, et al. Tissue-engineered lungs for in vivo implantation. Science 2010;329:538–41.

[8] Badylak SF, Freytes DO, Gilbert TW. Extracellular matrix as a biological scaffold material: structure and function. Acta Biomater 2009;5:1–13.

[9] Hodde J, Record R, Tullius R, Badylak S. Fibronectin peptides mediate HMEC adhesion to porcine-derived extracellular matrix. Biomaterials 2002;23:1841–8.

[10] Hodde JP, Record RD, Liang HA, Badylak SF. Vascular endothelial growth factor in porcine-derived extracellular matrix. Endothelium 2001;8:11–24.

[11] Brennan EP, Reing J, Chew D, Myers-Irvin JM, Young EJ, Badylak SF. Antibacterial activity within degradation products of biological scaffolds composed of extracellular matrix. Tissue Eng 2006;12:2949–55.

[12] Soto-Gutierrez A, Yagi H, Uygun BE, Navarro-Alvarez N, Uygun K, Kobayashi N, et al. Cell delivery: from cell transplantation to organ engineering. Cell Transplant 2010;19:655–65.

[13] Bonandrini B, Figliuzzi M, Papadimou E, Morigi M, Perico N, Casiraghi F, et al. Recellularization of well-preserved acellular kidney scaffold using embryonic stem cells. Tissue Eng Part A 2014;20:1486–98.

[14] Caralt M, Uzarski JS, Iacob S, Obergfell KP, Berg N, Bijonowski BM, et al. Optimization and critical evaluation of decellularization strategies to develop renal extracellular matrix scaffolds as biological templates for organ engineering and transplantation. Am J Transplant 2015;15:64–75.

[15] Yu YL, Shao YK, Ding YQ, Lin KZ, Chen B, Zhang HZ, et al. Decellularized kidney scaffold-mediated renal regeneration. Biomaterials 2014;33:6822–8.

[16] Orlando G, Farney AC, Iskandar SS, Mirmalek-Sani SH, Sullivan DC, Moran E, et al. Production and implantation of renal extracellular matrix scaffolds from porcine kidneys as a platform for renal bioengineering investigations. Ann Surg 2012;256:363–70.

[17] Sullivan DC, Mirmalek-Sani SH, Deegan DB, Baptista PM, Aboushwareb T, Atala A, et al. Decellularization methods of porcine kidneys for whole organ engineering using a high-throughput system. Biomaterials 2012;33:7756–64.

[18] Nakayama KH, Batchelder CA, Lee CI, Tarantal AF. Decellularized rhesus monkey kidney as a three-dimensional scaffold for renal tissue engineering. Tissue Eng Part A 2010;16:2207–16.

[19] Orlando G, Booth C, Wang Z, Totonelli G, Ross CL, Moran E, et al. Discarded human kidneys as a source of ECM scaffold for kidney regeneration technologies. Biomaterials 2013;34:5915–25.

[20] Katari R, Peloso A, Zambon JP, Soker S, Stratta RJ, Atala A, et al. Renal bioengineering with scaffolds generated from human kidneys. Nephron Exp Nephrol 2014;126:119.

[21] Peloso A, Petrosyan A, Da Sacco S, Booth C, Zambon JP, O'Brien T, et al. Renal extracellular matrix scaffolds from discarded kidneys maintain glomerular morphometry and vascular resilience and retains critical growth factors. Transplantation 2015;99(9):1807–16.

[22] Wang Y, Bao J, Wu Q, Zhou Y, Li Y, Wu X, et al. Method for perfusion decellularization of porcine whole liver and kidney for use as a scaffold for clinical-scale bioengineering engrafts. Xenotransplantation 2015;22:48–61.
[23] Gilbert TW, Sellaro TL, Badylak SF. Decellularization of tissues and organs. Biomaterials 2006;27:3675–83.
[24] Kawasaki T, Kirita Y, Kami D, Kitani T, Ozaki C, Itakura Y, et al. Novel detergent for whole organ tissue engineering. J Biomed Mater Res A 2015;103:3364–73.
[25] O'Neill JD, Freytes DO, Anandappa AJ, Oliver JA, Vunjak-Novakovic GV. The regulation of growth and metabolism of kidney stem cells with regional specificity using extracellular matrix derived from kidney. Biomaterials 2013;34:9830–41.
[26] Burgkart R, Tron A, Prodinger P, Culmes M, Tuebel J, van Griensven M, et al. Decellularized kidney matrix for perfused bone engineering. Tissue Eng Part C Methods 2014;20:553–61.
[27] Lehr EJ, Rayat GR, Chiu B, Churchill T, McGann LE, Coe JY, et al. Decellularization reduces immunogenicity of sheep pulmonary artery vascular patches. J Thorac Cardiovasc Surg 2011;141:1056–62.
[28] Ross EA, Williams MJ, Hamazaki T, Terada N, Clapp WL, Adin C, et al. Embryonic stem cells proliferate and differentiate when seeded into kidney scaffolds. J Am Soc Nephrol 2009;20:2338–47.
[29] Song JJ, Guyette JP, Gilpin SE, Gonzalez G, Vacanti JP, Ott HC. Regeneration and experimental orthotopic transplantation of a bioengineered kidney. Nat Med 2013;19:646–51.
[30] Ott HC, Clippinger B, Conrad C, Schuetz C, Pomerantseva I, Ikonomou L, et al. Regeneration and orthotopic transplantation of a bioartificial lung. Nat Med 2010;16:927–33.
[31] Badylak SF, Taylor D, Uygun K. Whole-organ tissue engineering: decellularization and recellularization of three-dimensional matrix scaffolds. Annu Rev Biomed Eng 2011;13:27–53.
[32] Gilbert TW, Freund JM, Badylak SF. Quantification of DNA in biologic scaffold materials. J Surg Res 2009;152:135–9.
[33] Nagata S, Hanayama R, Kawane K. Autoimmunity and the clearance of dead cells. Cell 2010;140:619–30.
[34] Zheng MH, Chen J, Kirilak Y, Willers C, Xu J, Wood D. Porcine small intestine submucosa (SIS) is not an acellular collagenous matrix and contains porcine DNA: possible implications in human implantation. J Biomed Mater Res B Appl Biomater 2005;73:61–7.
[35] Crapo PM, Gilbert TW, Badylak SF. An overview of tissue and whole organ decellularization processes. Biomaterials 2011;32:3233–43.
[36] Sano MB, Neal RE 2nd, Garcia PA, Gerber D, Robertson J, Davalos RV. Towards the creation of decellularized organ constructs using irreversible electroporation and active mechanical perfusion. Biomed Eng Online 2010;9:83.
[37] Wainwright JM, Czajka CA, Patel UB, Freytes DO, Tobita K, Gilbert TW, et al. Preparation of cardiac extracellular matrix from an intact porcine heart. Tissue Eng Part C Methods 2010;16:525–32.
[38] Brown BN, Freund JM, Han L, Rubin JP, Reing JE, Jeffries EM, et al. Comparison of three methods for the derivation of a biologic scaffold composed of adipose tissue extracellular matrix. Tissue Eng Part C Methods 2011;17:411–21.
[39] Brown BN, Valentin JE, Stewart-Akers AM, McCabe GP, Badylak SF. Macrophage phenotype and remodeling outcomes in response to biologic scaffolds with and without a cellular component. Biomaterials 2009;30:1482–91.
[40] Dong X, Wei X, Yi W, Gu C, Kang X, Liu Y, et al. RGD-modified acellular bovine pericardium as a bioprosthetic scaffold for tissue engineering. J Mater Sci Mater Med 2009;20:2327–36.
[41] Guo SZ, Ren XJ, Wu B, Jiang T. Preparation of the acellular scaffold of the spinal cord and the study of biocompatibility. Spinal Cord 2010;48:576–81.
[42] Reing JE, Brown BN, Daly KA, Freund JM, Gilbert TW, Hsiong SX, et al. The effects of processing methods upon mechanical and biologic properties of porcine dermal extracellular matrix scaffolds. Biomaterials 2010;31:8626–33.
[43] Bolland F, Korossis S, Wilshaw SP, Ingham E, Fisher J, Kearney JN, et al. Development and characterisation of a full-thickness acellular porcine bladder matrix for tissue engineering. Biomaterials 2007;28:1061–70.
[44] Cebotari S, Tudorache I, Jaekel T, Hilfiker A, Dorfman S, Ternes W, et al. Detergent decellularization of heart valves for tissue engineering: toxicological effects of residual detergents on human endothelial cells. Artif Organs 2010;34:206–10.
[45] DeQuach JA, Mezzano V, Miglani A, Lange S, Keller GM, Sheikh F, et al. Simple and high yielding method for preparing tissue specific extracellular matrix coatings for cell culture. PLoS ONE 2010;5:e13039.
[46] Gui L, Chan SA, Breuer CK, Niklason LE. Novel utilization of serum in tissue decellularization. Tissue Eng Part C Methods 2010;16:173–84.
[47] Shupe T, Williams M, Brown A, Willenberg B, Petersen BE. Method for the decellularization of intact rat liver. Organogenesis 2010;6:134–6.
[48] Nakayama KH, Batchelder CA, Lee CI, Tarantal AF. Renal tissue engineering with decellularized rhesus monkey kidneys: age-related differences. Tissue Eng Part A 2011;17:2891–901.
[49] Tsuchiya T, Balestrini JL, Mendez J, Calle EA, Zhao L, Niklason LE. Influence of pH on extracellular matrix preservation during lung decellularization. Tissue Eng Part C Methods 2014;20:1028–36.
[50] Nakayama KH, Lee CC, Batchelder CA, Tarantal AF. Tissue specificity of decellularized rhesus monkey kidney and lung scaffolds. PLoS ONE 2013;8:e64134.
[51] Poornejad N, Frost TS, Scott DR, Elton BB, Reynolds PR, Roeder BL, et al. Freezing/Thawing without cryoprotectant damages native but not decellularized porcine renal tissue. Organogenesis 2015;11:30–45.
[52] Song JJ, Ott HC. Organ engineering based on decellularized matrix scaffolds. Trends Mol Med 2011;17:424–32.
[53] Freytes DO, Stoner RM, Badylak SF. Uniaxial and biaxial properties of terminally sterilized porcine urinary bladder matrix scaffolds. J Biomed Mater Res B Appl Biomater 2008;84:408–14.
[54] Gouk SS, Lim TM, Teoh SH, Sun WQ. Alterations of human acellular tissue matrix by gamma irradiation: histology, biomechanical property, stability, in vitro cell repopulation, and remodeling. J Biomed Mater Res B Appl Biomater 2008;84:205–17.
[55] Moreau MF, Gallois Y, Basle MF, Chappard D. Gamma irradiation of human bone allografts alters medullary lipids and releases toxic compounds for osteoblast-like cells. Biomaterials 2000;21:369–76.
[56] Sun WQ, Leung P. Calorimetric study of extracellular tissue matrix degradation and instability after gamma irradiation. Acta Biomater 2008;4:817–26.
[57] Rosario DJ, Reilly GC, Ali Salah E, Glover M, Bullock AJ, Macneil S. Decellularization and sterilization of porcine urinary bladder matrix for tissue engineering in the lower urinary tract. Regen Med 2008;3:145–56.

[58] Brown B, Lindberg K, Reing J, Stolz DB, Badylak SF. The basement membrane component of biologic scaffolds derived from extracellular matrix. Tissue Eng 2006;12:519–26.
[59] Qiu QQ, Leamy P, Brittingham J, Pomerleau J, Kabaria N, Connor J. Inactivation of bacterial spores and viruses in biological material using supercritical carbon dioxide with sterilant. J Biomed Mater Res B Appl Biomater 2009;91:572–8.
[60] Olivetti G, Anversa P, Rigamonti W, Vitali-Mazza L, Loud AV. Morphometry of the renal corpuscle during normal postnatal growth and compensatory hypertrophy. A light microscope study. J Cell Biol 1977;75:573–85.
[61] Kim IH, Ko IK, Atala A, Yoo JJ. Whole kidney engineering for clinical translation. Curr Opin Organ Transplant 2015;20:165–70.
[62] Rosines E, Sampogna RV, Johkura K, Vaughn DA, Choi Y, Sakurai H, et al. Staged in vitro reconstitution and implantation of engineered rat kidney tissue. Proc Natl Acad Sci U S A 2007;104:20938–43.
[63] Zambon JP, Magalhaes RS, Ko I, Ross CL, Orlando G, Peloso A, et al. Kidney regeneration: where we are and future perspectives. World J Nephrol 2014;3:24–30.
[64] Rosines E, Schmidt HJ, Nigam SK. The effect of hyaluronic acid size and concentration on branching morphogenesis and tubule differentiation in developing kidney culture systems: potential applications to engineering of renal tissues. Biomaterials 2007;28:4806–17.
[65] Humphreys BD, Valerius MT, Kobayashi A, Mugford JW, Soeung S, Duffield JS, et al. Intrinsic epithelial cells repair the kidney after injury. Cell Stem Cell 2008;2:284–91.
[66] Bonventre JV. Dedifferentiation and proliferation of surviving epithelial cells in acute renal failure. J Am Soc Nephrol 2003;14(Suppl. 1):S55–61.
[67] Nony PA, Schnellmann RG. Mechanisms of renal cell repair and regeneration after acute renal failure. J Pharmacol Exp Ther 2003;304:905–12.
[68] Al-Awqati Q, Oliver JA. The kidney papilla is a stem cells niche. Stem Cell Rev 2006;2:181–4.
[69] Angelotti ML, Ronconi E, Ballerini L, Peired A, Mazzinghi B, Sagrinati C, et al. Characterization of renal progenitors committed toward tubular lineage and their regenerative potential in renal tubular injury. Stem Cells 2012;30:1714–25.
[70] Bruno S, Camussi G. Isolation and characterization of resident mesenchymal stem cells in human glomeruli. Methods Mol Biol 2012;879:367–80.
[71] Franquesa M, Flaquer M, Cruzado JM, Grinyo JM. Kidney regeneration and repair after transplantation. Curr Opin Organ Transplant 2013;18:191–6.
[72] Sagrinati C, Netti GS, Mazzinghi B, Lazzeri E, Liotta F, Frosali F, et al. Isolation and characterization of multipotent progenitor cells from the Bowman's capsule of adult human kidneys. J Am Soc Nephrol 2006;17:2443–56.
[73] Uzarski JS, Xia Y, Belmonte JC, Wertheim JA. New strategies in kidney regeneration and tissue engineering. Curr Opin Nephrol Hypertens 2014;23:399–405.
[74] Ng SL, Narayanan K, Gao S, Wan AC. Lineage restricted progenitors for the repopulation of decellularized heart. Biomaterials 2011;32:7571–80.
[75] Baptista PM, Orlando G, Mirmalek-Sani SH, Siddiqui M, Atala A, Soker S. Whole organ decellularization—a tool for bioscaffold fabrication and organ bioengineering. Conf Proc IEEE Eng Med Biol Soc 2009;2009:6526–9.
[76] Lees JG, Lim SA, Croll T, Williams G, Lui S, Cooper-White J, et al. Transplantation of 3D scaffolds seeded with human embryonic stem cells: biological features of surrogate tissue and teratoma-forming potential. Regen Med 2007;2:289–300.
[77] Nussbaum J, Minami E, Laflamme MA, Virag JA, Ware CB, Masino A, et al. Transplantation of undifferentiated murine embryonic stem cells in the heart: teratoma formation and immune response. FASEB J 2007;21:1345–57.
[78] De Coppi P, Bartsch G Jr, Siddiqui MM, Xu T, Santos CC, Perin L, et al. Isolation of amniotic stem cell lines with potential for therapy. Nat Biotechnol 2007;25:100–6.
[79] Kusuma S, Gerecht S. Engineering blood vessels using stem cells: innovative approaches to treat vascular disorders. Expert Rev Cardiovasc Ther 2010;8:1433–45.
[80] Roomans GM. Tissue engineering and the use of stem/progenitor cells for airway epithelium repair. Eur Cell Mater 2010;19:284–99.
[81] Xu D, Wang F, Gu H, Wang J, Guo Q, Zhang Y, et al. Hybrid cells differentiate to hepatic lineage cells and repair oxidative damage. Cell Mol Biol Lett 2010;15:451–72.
[82] Montserrat N, Ramirez-Bajo MJ, Xia Y, Sancho-Martinez I, Moya-Rull D, Miquel-Serra L, et al. Generation of induced pluripotent stem cells from human renal proximal tubular cells with only two transcription factors, OCT4 and SOX2. J Biol Chem 2012;287:24131–8.
[83] Wang WW, Wang W, Jiang Y, Han GF, Lu S, Li G, et al. Reprogramming of mouse renal tubular epithelial cells to induced pluripotent stem cells. Cytotherapy 2013;15:578–85.
[84] Hong SG, Winkler T, Wu C, Guo V, Pittaluga S, Nicolae A, et al. Path to the clinic: assessment of iPSC-based cell therapies in vivo in a nonhuman primate model. Cell Rep 2014;7:1298–309.
[85] Song B, Smink AM, Jones CV, Callaghan JM, Firth SD, Bernard CA, et al. The directed differentiation of human iPS cells into kidney podocytes. PLoS ONE 2012;7:e46453.
[86] Lam AQ, Freedman BS, Morizane R, Lerou PH, Valerius MT, Bonventre JV. Rapid and efficient differentiation of human pluripotent stem cells into intermediate mesoderm that forms tubules expressing kidney proximal tubular markers. J Am Soc Nephrol 2014;25:1211–25.
[87] Araoka T, Mae S, Kurose Y, Uesugi M, Ohta A, Yamanaka S, et al. Efficient and rapid induction of human iPSCs/ESCs into nephrogenic intermediate mesoderm using small molecule-based differentiation methods. PLoS ONE 2014;9:e84881.
[88] Uzarski JS, Bijonowski BM, Wang B, Ward HH, Wandinger-Ness A, Miller WM, et al. Dual-purpose bioreactors to monitor noninvasive physical and biochemical markers of kidney and liver scaffold recellularization. Tissue Eng Part C Methods 2015;21(10):1032–43.
[89] Agarwal S, Loh YH, McLoughlin EM, Huang J, Park IH, Miller JD, et al. Telomere elongation in induced pluripotent stem cells from dyskeratosis congenita patients. Nature 2010;464:292–6.
[90] Dimos JT, Rodolfa KT, Niakan KK, Weisenthal LM, Mitsumoto H, Chung W, et al. Induced pluripotent stem cells generated from patients with ALS can be differentiated into motor neurons. Science 2008;321:1218–21.
[91] Moretti A, Bellin M, Welling A, Jung CB, Lam JT, Bott-Flugel L, et al. Patient-specific induced pluripotent stem-cell models for long-QT syndrome. N Engl J Med 2010;363:1397–409.
[92] Nguyen HN, Byers B, Cord B, Shcheglovitov A, Byrne J, Gujar P, et al. LRRK2 mutant iPSC-derived DA neurons demonstrate increased susceptibility to oxidative stress. Cell Stem Cell 2011;8:267–80.
[93] Yazawa M, Hsueh B, Jia X, Pasca AM, Bernstein JA, Hallmayer J, et al. Using induced pluripotent stem cells to investigate cardiac phenotypes in Timothy syndrome. Nature 2011;471:230–4.

[94] O'Neill AC, Ricardo SD. Human kidney cell reprogramming: applications for disease modeling and personalized medicine. J Am Soc Nephrol 2013;24:1347–56.
[95] Olson H, Betton G, Robinson D, Thomas K, Monro A, Kolaja G, et al. Concordance of the toxicity of pharmaceuticals in humans and in animals. Regul Toxicol Pharmacol 2000;32:56–67.
[96] Friedrich J, Seidel C, Ebner R, Kunz-Schughart LA. Spheroid-based drug screen: considerations and practical approach. Nat Protoc 2009;4:309–24.
[97] Kunz-Schughart LA, Freyer JP, Hofstaedter F, Ebner R. The use of 3-D cultures for high-throughput screening: the multicellular spheroid model. J Biomol Screen 2004;9:273–85.
[98] Pampaloni F, Reynaud EG, Stelzer EH. The third dimension bridges the gap between cell culture and live tissue. Nat Rev Mol Cell Biol 2007;8:839–45.
[99] Jang KJ, Suh KY. A multi-layer microfluidic device for efficient culture and analysis of renal tubular cells. Lab Chip 2010;10:36–42.
[100] Gao X, Tanaka Y, Sugii Y, Mawatari K, Kitamori T. Basic structure and cell culture condition of a bioartificial renal tubule on chip towards a cell-based separation microdevice. Anal Sci 2011;27:907–12.
[101] Jang KJ, Cho HS, Kang do H, Bae WG, Kwon TH, Suh KY. Fluid-shear-stress-induced translocation of aquaporin-2 and reorganization of actin cytoskeleton in renal tubular epithelial cells. Integr Biol (Camb) 2011;3:134–41.
[102] Jang KJ, Mehr AP, Hamilton GA, McPartlin LA, Chung S, Suh KY, et al. Human kidney proximal tubule-on-a-chip for drug transport and nephrotoxicity assessment. Integr Biol (Camb) 2013;5:1119–29.
[103] Shintu L, Baudoin R, Navratil V, Prot JM, Pontoizeau C, Defernez M, et al. Metabolomics-on-a-chip and predictive systems toxicology in microfluidic bioartificial organs. Anal Chem 2012;84:1840–8.
[104] Alp O, Zhang Y, Merino EJ, Caruso JA. Selenium effects on arsenic cytotoxicity and protein phosphorylation in human kidney cells using chip-based nanoLC-MS/MS. Metallomics 2011;3:482–90.
[105] Choucha-Snouber L, Aninat C, Grsicom L, Madalinski G, Brochot C, Poleni PE, et al. Investigation of ifosfamide nephrotoxicity induced in a liver-kidney co-culture biochip. Biotechnol Bioeng 2013;110:597–608.
[106] Maschmeyer I, Lorenz AK, Schimek K, Hasenberg T, Ramme AP, Hubner J, et al. A four-organ-chip for interconnected long-term co-culture of human intestine, liver, skin and kidney equivalents. Lab Chip 2015;15:2688–99.
[107] Wei Z, Amponsah PK, Al-Shatti M, Nie Z, Bandyopadhyay BC. Engineering of polarized tubular structures in a microfluidic device to study calcium phosphate stone formation. Lab Chip 2012;12:4037–40.
[108] Williamson A, Singh S, Fernekorn U, Schober A. The future of the patient-specific Body-on-a-chip. Lab Chip 2013;13:3471–80.
[109] Skardal A, Mack D, Kapetanovic E, Atala A, Jackson JD, Yoo J, et al. Bioprinted amniotic fluid-derived stem cells accelerate healing of large skin wounds. Stem Cells Transl Med 2012;1:792–802.
[110] Koch L, Kuhn S, Sorg H, Gruene M, Schlie S, Gaebel R, et al. Laser printing of skin cells and human stem cells. Tissue Eng Part C Methods 2010;16:847–54.
[111] Duan B, Hockaday LA, Kang KH, Butcher JT. 3D bioprinting of heterogeneous aortic valve conduits with alginate/gelatin hydrogels. J Biomed Mater Res A 2013;101:1255–64.
[112] Shor L, Guceri S, Chang R, Gordon J, Kang Q, Hartsock L, et al. Precision extruding deposition (PED) fabrication of polycaprolactone (PCL) scaffolds for bone tissue engineering. Biofabrication 2009;1:015003.
[113] Bernhard JC, Isotani S, Matsugasumi T, Duddalwar V, Hung AJ, Suer E, et al. Personalized 3D printed model of kidney and tumor anatomy: a useful tool for patient education. World J Urol 2015;doi:10.1007/s00345-015-1632-2.
[114] Stone L. Kidney cancer: a model for the masses—3D printing of kidney tumours. Nat Rev Urol 2014;11:428.
[115] Zhang Y, Ge HW, Li NC, Yu CF, Guo HF, Jin SH, et al. Evaluation of three-dimensional printing for laparoscopic partial nephrectomy of renal tumors: a preliminary report. World J Urol 2015;doi:10.1007/s00345-015-1530-7.
[116] Kusaka M, Sugimoto M, Fukami N, Sasaki H, Takenaka M, Anraku T, et al. Initial experience with a tailor-made simulation and navigation program using a 3-D printer model of kidney transplantation surgery. Transplant Proc 2015;47:596–9.
[117] Mu X, Zheng W, Xiao L, Zhang W, Jiang X. Engineering a 3D vascular network in hydrogel for mimicking a nephron. Lab Chip 2013;13:1612–18.
[118] Hsu CY, Lin F, Vittinghoff E, Shlipak MG. Racial differences in the progression from chronic renal insufficiency to end-stage renal disease in the United States. J Am Soc Nephrol 2003;14:2902–7.
[119] Bakarich SE, Gorkin R 3rd, Panhuis MI, Spinks GM. 4D printing with mechanically robust, thermally actuating hydrogels. Macromol Rapid Commun 2015;36:1211–17.
[120] Chae MP, Hunter-Smith DJ, De-Silva I, Tham S, Spychal RT, Rozen WM. Four-dimensional (4D) printing: a new evolution in computed tomography-guided stereolithographic modeling. principles and application. J Reconstr Microsurg 2015;31:458–63.