Have we hit a wall with whole kidney decellularization and recellularization: a review

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Have we hit a wall with whole kidney decellularization and recellularization: A review

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Abstract

The purpose of organ decellularization is to remove all cellular components whilst preserving the extracellular matrix (ECM). It has been hypothesized that this decellularized ECM can be used as a scaffold for the development of personalized bioengineered kidneys by repopulating it with patient-derived cells. The renal artery, vein, and ureter are most frequently used for whole kidney repopulation. Cell perfusion through the artery and vein enables revascularization of decellularized kidneys. However, adequate repopulation of the epithelial compartment remains unattainable. Although it has become unlikely that recellularized whole kidneys will be the solution to reduce donor organ shortages within the foreseeable future, advances made within the field of whole organ decellularization and recellularization have paved the way for alternatives that actually may help to solve these shortages. This includes ex vivo refurbishment and personalization of discarded donor organs during machine perfusion.

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Introduction

Kidney transplantation is at present the only definitive treatment for patients with end stage renal disease (ESRD), significantly improving the quality of live compared to dialysis [1]. While the incidence of ESRD is increasing, donor organs are of limited availability [2]. As an alternative to donor organs, bioengineered kidneys are of major interest. Different approaches for kidney regeneration are being studied, including kidney decellularization and recellularization, blastocyst complementation, kidney organoids, bioartificial kidneys, and xenotransplantation [3].

Organ decellularization aims to remove all cellular components by perfusing detergents, enzymes, or other cell-disrupting solutions through the vascular network whilst preserving the extracellular matrix (ECM). The resulting decellularized ECM (dECM) — an acellular scaffold that can be repopulated in vitro — holds several advantages over artificial scaffolds. These include appropriate macro and micro structures to support physiological function, an intact vascular tree, and tissue- and location-specific cues [4]. This makes that dECM should not solely be seen as a construct that provides structure to cells. It actively interacts with them guiding cell adhesion, migration, and differentiation [5,6].

Feasibility of decellularization has been shown for most organs and tissues derived from rodents, large animals, and humans [7,8]. Current challenges have shifted from decellularization to cellular repopulation of decellularized scaffolds. Whereas recellularization of tissues such as vasculature, trachea and muscle can be achieved by surface attachment or cell injection into the interstitial space, these methods are of limited use for kidney recellularization due to the organ's complex anatomy.

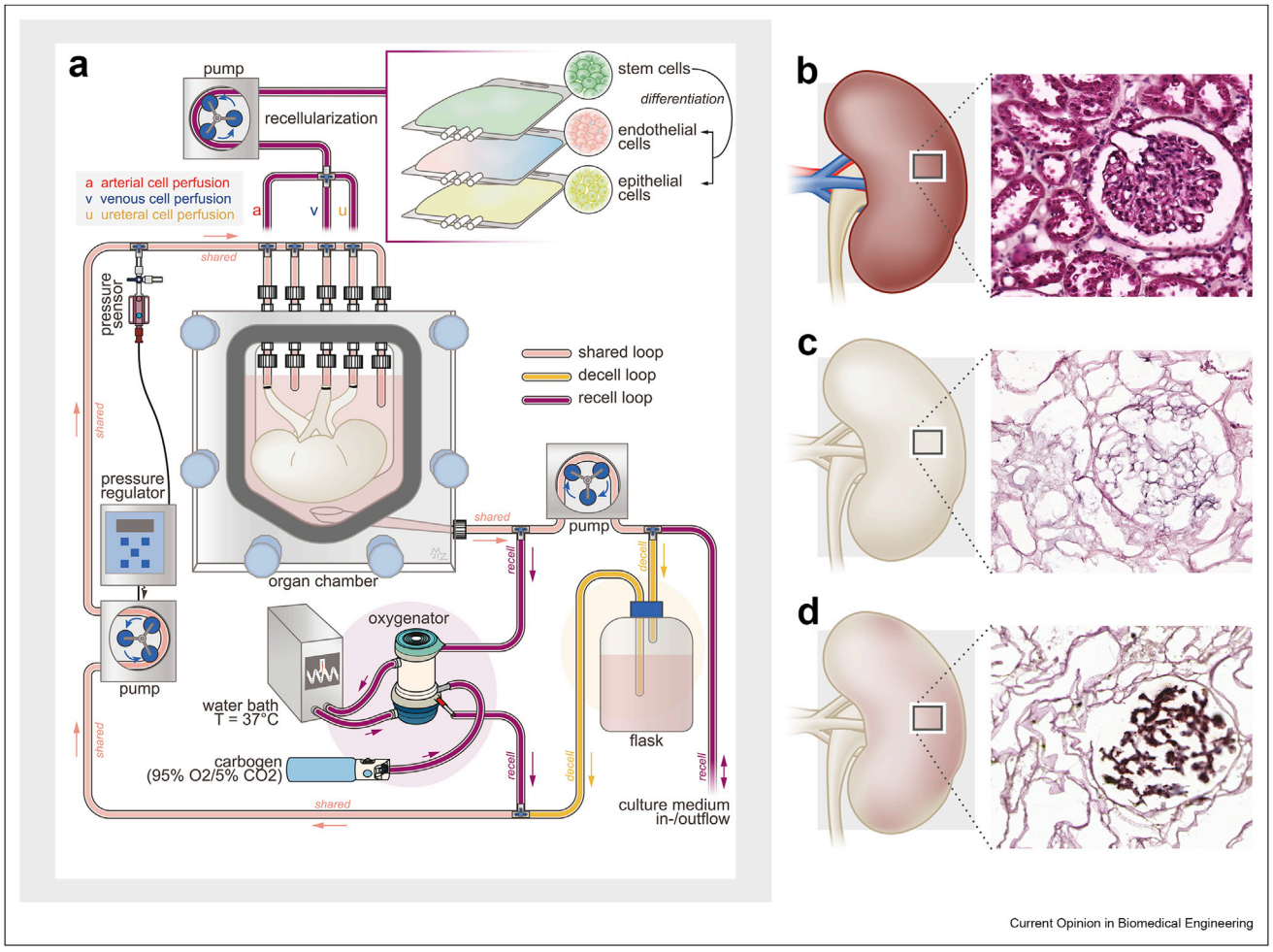
The aim of this review is to provide a toolbox for whole kidney recellularization with a primary focus upon the parts of the nephron that can be repopulated following recellularization. Since the quality of the dECM determines the success of recellularization, key aspects of whole kidney decellularization are discussed first. Next, different repopulation methods (i.e., arterial, venous, and ureteral cell perfusion) for whole kidney engineering are compared. To conclude, the potential of whole organ recellularization for the development of a functional bioengineered kidney is discussed.

Decellularization

Cell removal

Perfusion-based decellularization of organs enables the fabrication of organ-derived acellular scaffolds (Figure 1c).

Figure 1



Concept of whole kidney engineering.(**a**). Set up of the decellularization and recellularization system. By integrating the components needed for decellularization, recellularization and kidney culture in one system risk of contamination is reduced. (**b**). Microscopic appearance of a normal kidney. (**c**). Microscopic appearance of a decellularized kidney. Cells and cellular material have been removed during perfusion-based decellularization whilst ECM structure has been preserved. (**d**). Microscopic appearance of a recellularized kidney. Following repopulation via the renal artery and vein cells can be found lining the vasculature down to the capillary bed, including the glomerular capillaries.

However, to produce such a scaffold two problems are encountered. First all cells have to be solubilized in a manner that is minimally disruptive to the surrounding ECM. Next, these cellular remnants have to be removed from the scaffold. To this end, the vasculature is used to distribute decellularization agents homogenously throughout the scaffold, followed by extensive washing steps to remove cellular remnants. Figure 1 shows a perfusion system that integrates all components needed for both decellularization and recellularization.

Although there is no consensus on the use of specific agents, detergents form an essential part of most decellularization protocols. A literature overview of reported protocols for human-scale whole kidney decellularization is provided in Table 1. Sodium dodecyl sulfate (SDS) is considered the most potent detergent for removing cellular material [8]. Although enzymatic digestion with trypsin has been used for whole rodent kidney decellularization it has not yet been reported for whole porcine and human kidneys [9]. Removal of the cellular content is essential because retention of cell membrane epitopes or DNA within the scaffold invokes adverse immune responses upon in vivo application [10,11]. Although quality criteria for the assessment of decellularized constructs have yet to be fully defined by regulatory expert bodies [10], the following minimal criteria have been proposed: a) <50 ng dsDNA per mg ECM dry weight, b) <200 bp DNA fragment length, and c) absence of visible nuclear material in tissue sections [7].

Table 1

Overview of human-scale whole kidney decellularization protocols. Although human-scale decellularized kidney scaffolds will be needed for clinical translation most groups have investigated decellularization of rodent kidneys [37]. Below an overview is provided of the decellularization protocols used for the generation of human-scale acellular kidney scaffolds. The rationale behind the use of specific agents has been reviewed elsewhere [8].

Ref.	Pre treatment	Decellularization protocol				Post treatment	Total duration
		Agents (*concentration*)	Duration exposure	Flow rate (mL/min)	Pressure (mmHg)		
Whole porcine kidney decellularization							
[12,41,55]	Heparinized PBS; freeze (–20 °C);	**Pr1**. Cycles of 0.5 M NaCl in diH_2O (30 min), SDS (0.5%, 30 min), and diH_2O (30 min); **Pr2**. SDS (0.5%)	12 h	10–50	<80	diH_2O	2.5 d
[13]	Heparinized PBS	**Pr1**. SDS (0.5%)/DNase; **Pr2.** Triton X-100 (1%)/ NH_4OH (0.1%), and SDS (0.5%)	**Pr1**.: 1.5 d **Pr2**.: 3 d	**Pr1**.: 12.5 **Pr2**.: 5	–	DNase and 10 mM $MgCl_2$ in PBS; PBS	**Pr1**.: 4 d **Pr2**.: 6 d
[24,56]	Heparinized PBS	SDS (1.0%); Triton X-100 (1.0%)	5 d; 1 d	–	60	PBS	12–14 d
[35,36]	Heparinized PBS	SDS (0.5%)	1.5 d	12.5	–	DNase; PBS	4 d
[57]	diH_2O	SDS (NR)	2 d	12	–	PBS	7.5 d
[58]	Heparinized PBS	SDS (1%)	12 h	100	–	PBS	1.5 d
[59]	Heparinized PBS	**Pr1**. SDS (0.5%); **Pr2**. SDS (0.25%); **Pr3**. Triton X-100 (1%)/ NH_4OH (0.1%)	1.5 d	12.5	–	DNase and 10 mM $MgCl_2$ in PBS; PBS	4 d
[60]	Heparinized PBS; diH_2O;	SDS (1%); Triton X-100 (1%)	28 h; 2 h	10	–	PBS	5 d
[61]	Freeze (–20 °C); diH_2O;	SDS (1%)/Triton X-100 (1%)/PAA (1%)/SDC (1%)	18 h	15	–	PBS	1 d
[62]	Heparinized PBS	SDS (0.1%)	13 h	25	–	PAA (0.1%); PBS	1.5 d
[63]	Heparinized PBS	SDS (0.5%)	3 d	10	–	diH_2O	4 d
[64]	diH_2O	SDS (0.25, 0.625, 1%) with sonication (0, 60, 120 W); Triton X-100 (1%)	2–19 h; NR	15, 30, or 45 mL/min	–	PBS	NR
Whole human kidney decellularization							
[15]	PBS	SDS (0.5%) (*in both artery and ureter*)	2 d	12	–	DNase; PBS	7.75 d
[19]	Heparinized PBS	SDS (1%)/DNase in PBS; Triton X-100 (1%) in diH_2O	5 d; 1 d	–	75	PBS	12 d
[24,56]	Heparinized PBS	SDS (1.0%); Triton X-100 (1.0%)	5 d; 1 d	–	60	PBS	12-14 d
[65]	diH_2O	SDS (0.5%) (*in both artery and ureter*)	2 d	12	–	PBS	7.5 d

diH_2O, distilled water; SDS, sodium dodecyl sulfate; PBS, phosphate buffer saline; NH_4OH, ammonium hydroxide; PAA, peracetic acid; SDC, sodium deoxycholate.

ECM preservation

The success of recellularization depends on the capacity of a decellularized scaffold to support and instruct the seeded cells [12]. It has become evident that there is a delicate balance between cell removal and preservation of ECM components and microvascular structures [9,13–15]. Maintaining intact and functional microvascular structures such as glomeruli and peritubular capillaries depends on detergent choice and concentration, flow rate, and decellularization time [13]. The same applies for preservation of glycosaminoglycan's (GAGs) and growth factors (GFs), which are considered main drivers of location-specific scaffold instructiveness [6,14–16].

Interestingly, studies that have reported tissue specific differentiation of pluripotent stem cells (PSCs) or PSC-derived renal progenitor cells generally used milder decellularization protocols (i.e., 0.1% SDS instead of 1.0% SDS) [6,16]. Although it should be noted that they looked at passive decellularization, successful perfusion-based decellularization of rat kidneys has also been reported for 0.1% SDS [14]. Moreover, these studies reported reduced GF content post decellularization raising the question whether partial loss of GAGs and GFs is inevitable or can be averted by further optimizing decellularization protocols. Conversely, some publications have demonstrated preservation or even increases in the number of GAGs and GFs after decellularization [14,15,17]. However, these latter outcomes should be questioned given that it is biologically implausible that the amount of GAGs or GFs has increased following decellularization. The quantitative comparison of GAGs and GFs between normal and decellularized kidney is difficult since normal tissue contains both ECM and cells whereas dECM solely contains the ECM. The reported increases in GAG and GF content could hence also be the result of normalization [18]. Substituting GAGs and GFs by pre conditioning decellularized kidneys might be a solution to correct for the partial loss. This method has been reported for acellular human kidneys prior to hiPSC-derived ECs seeding [19].

Another point that has to be addressed is that ECM composition changes with age and disease conditions, which subsequently can influence cell behavior upon repopulation [20,21]. Therefore, especially when using human kidneys for whole kidney engineering — the ECM condition has to be taken into account, making human-scale scaffolds from young animals a more attractive starting point for repopulation.

In sum, it can be stated that researchers face a "decellularization paradox". Organs have to be exposed to protocols that are sufficiently aggressive to remove all cellular material to make the scaffold eligible for transplantation. But this should occur in a manner that minimizes irreversible damage to the ECM components and structure, all in service of improving recellularization outcomes. Contrary, recellularization outcomes such as cell attachment and viability are seldom included in the evaluation of decellularization protocols [22].

Recellularization

General concept of kidney recellularization

The next step is to repopulate decellularized kidney scaffolds with patient-derived cells to bioengineer personalized kidneys in the laboratory. The primary functions of a kidney are the maintenance of fluid balance by filtration and reabsorption and the excretion of waste products. These functions are the result of a complex interplay between the interstitial space and endothelial and epithelial compartments. Cells have to be reintroduced into decellularized scaffolds to repopulate these compartments.

Cell type

Given the number of cells needed to repopulate decellularized human kidneys, choosing the right cell source is paramount. Whereas for repopulation of the vascular tree approximately 150 million endothelial cells (ECs) are needed [19], hundreds of billions of epithelial cells will be needed to repopulate the surrounding dECM [23]. Potential cell types vary from induced pluripotent stem cells (iPSCs) toward more adult renal cells such as renal cortical tubular epithelial cells. By perfusing ECs through the renal artery and rat neonatal kidney cells through the ureter of decellularized rat kidneys, Song et al. were the first to have developed a rudimentary kidney [24]. While this study provided proof-of-concept for rodent kidneys, repopulating the epithelial compartment of human-scale decellularized scaffolds with sufficient cells to achieve adequate cell coverage poses a major problem [23]. Table 2 provides an overview of the studies that looked at human-scale whole kidney repopulation.

Using stem cell-like populations for whole kidney engineering holds two advantages over more adult cells. First, fewer cells will be needed given their ability to proliferate following recellularization. Second, preservation of location specific signals embedded within glomerular, tubular, and vascular structures have been hypothesized to guide stem cell differentiation and maturation toward the many different cell types present within the adult kidney [24–27]. However, repopulation of renal dECM with pluripotent stem cells (PSCs) showed limited differentiation toward the renal lineage at best [16,25–27]. Protocols have been established for the development of renal progenitor cells from PSCs [28–30]. Culture of hiPSCs-derived mesodermal precursor cells on human renal dECM resulted in development of tubular- and blood vessel-like structures [6]. After 14 days, functionality of these tubular-like cells was shown with an electrolyte reabsorption assay.

Table 2

Overview of studies reporting repopulation of decellularized human-scale whole kidney scaffolds. One of the main hurdles for human-scale repopulation of decellularized kidney scaffolds is acquiring sufficient cell numbers. Hundreds of billions of cells will be needed to repopulate human-scale kidneys [23]. This hurdle is also reflected by the low number of groups that reported repopulation of these constructs.

Ref.	Pre seeding treatment	Recellularization protocol				Outcomes
		Cell types	Seeding route	Cell number	Post seeding conditions	
Whole porcine kidney repopulation						
[13]	Conjugated with CD31 antibody	MS1 ECs	Arterial cell perfusion	3.0×10^8	Static (1.5×10^8) and perfusion (1.5×10^8) seeding steps; **3 day** perfusion up to 20 ml/min	Perfused with heparinized whole porcine blood for 24 h after 3 d bioreactor culture. In SDS/Triton-X100 decellularized kidneys vascular patency was observed.
[36]	Conjugated with CD31 antibody	MS1 ECs	Arterial cell perfusion	1.0×10^8	Static (0.5×10^8) and perfusion (0.5×10^8) seeding steps; **3 day** perfusion up to 20 mL/min	In vivo transplantation showed occlusion of vascular network after 2 h. CD31 Ab conjugation prior to revascularization prolonged preservation of vascular patency up to 4 h (*time of explantation*).
[41]	2 h priming with DMEM with 10% FBS	MDCK epithelial cells	**Pr1**.: arterial cell perfusion **Pr2**.**&3**.: ureteral cell perfusion	6.0×10^8	**Pr1**.: high flow perfusion (80 mL/min) immediately after seeding for 30 min **Pr2.**: high flow perfusion (40 mL/min) immediately after seeding for 30 min **Pr3**.: vacuum (–40 mmHg) during seeding; **All: 7 day** perfusion at 2–10 mL/min	Lower cell concentrations during seeding result in more homogenous distribution. (Optimum: 4–5 $x10^6$/mL) **Pr1**.: only few cells in glomerular region due to washout following high flow perfusion. **Pr2**.: cells remained within tubules of the medulla with few reaching the cortex region. **Pr3**.: homogenous distribution. Medulla: majority of tubules repopulated. Cortex: some cells in Bowman's capsules. Function at cellular- and construct-levels was not assessed.
[35]	24 h priming with renal cell media[a]	Primary renal cells	Cortical injection	4.0×10^8	Injections: *upper pole, 2*.5 mm *between sites,* 5 mm *depth, 5 million cells/site;* Static for 30 min; **28 day** perfusion at 10 mL/min	Renal (tubule-like) structures by d7 and maintained for 28 days. Function of cells following repopulation on a cellular level shown up to d14 (*sodium uptake, hydrolase activity, erythropoietin production*). Function at construct-level was not assessed.
Whole human kidney repopulation						
[19]	Overnight priming with VEGF and Ang1 in EC-SFM	hiPSC-derived ECs	Venous and arterial cell perfusion	60×10^6 (V) and 70×10^6 (A)	Vacuum (–30 mmHg) during seeding; **24 h** perfusion at 20 mL/min	ECs in ±80% of glomeruli, with 3.8% of the glomerular area CD31+ (*normal human kidney: 6.7%*). Perfused with recalcified human whole blood for more than 20 min. Massive clotting in decellularized kidneys within 5 min.

ECs, endothelial cells; Ab, antibody; FBS, fetal bovine serum; EC-SFM, human endothelial serum free medium.

[a] Composed of 1:1 mixture of the following media: one part keratinocyte serum free media containing 2.5% fetal bovine serum (FBS), 1% penicillin–streptomycin (P/S) solution, 0.4% ITS (insulin, transferrin, selenium) liquid media, and supplements for keratinocyte serum free media (epidermal growth factor, bovine pituitary extract), and one part DMEM high glucose media containing 10% FBS and 1% P/S solution [35].

Likewise, 2D culture of renal dECM with kidney derived adult stem cells resulted in differentiation toward both epithelial and vascular cells when cultured in basal medium [31]. However, these results are based upon in vitro cultures where the cells were pipetted upon dECM slices. Repopulation of 3D acellular kidneys requires substantial mechanical challenges to overcome.

Preservation of location-specific cues in the dECM theoretically enables phenotypic specification toward cell types that were previously present at a given location. For example, renal vasculature comprises diverse populations of endothelial cells with each population supporting specific functions like filtration of blood plasma or reabsorption and excretion of water and solutes [32]. The perivascular ECM is known to play an important role on phenotypic changes of ECs [33]. Interestingly, Ciampi et al. showed that fenestrated endothelium was present in glomerular capillaries whilst no fenestrae were seen in vascular capillaries following recellularization of whole rodent kidneys with hiPSC-derived ECs [34].

Thus, tissue-specific differentiation and location-specific phenotypic changes are seen when renal dECM is repopulated. These findings highlight the true value of using decellularized scaffolds. When organs have been properly decellularized they are not merely carriers for cells, but actively instruct cell fate. Future research should focus upon conditioning the dECM with specific growth factors prior to repopulation [16,19] or adding these factors to the culture medium following repopulation with renal progenitor cells to promote cell attachment, differentiation, and phenotypic specification.

Routes for whole kidney repopulation

Whereas recellularization of less complex tissues can be achieved by surface attachment or cell injection into the interstitial space, these methods are of limited use for whole organ recellularization, especially for organs as complex as the kidney. One group reported cell injection to repopulate a quadrant of an acellular porcine kidney scaffold [35]. However, adequate cell coverage can only be achieved following cell injections performed at small distances of each other, making this method laborious at best.

A unique feature of the kidney is that the scaffold can be reached through three ports of entry: the vascular tree through the renal artery and vein and the tubular compartment through the ureter. This makes that perfusion-based recellularization has been the most reported method. It should be noticed that the interstitial compartment is still not reached with these methods (see Figure 2), but one could postulate a dynamic remodeling of this compartment from the other recellularized compartments. Each perfusion-based approach has its own advantages and limitations, which are discussed below.

Vascular cell perfusion

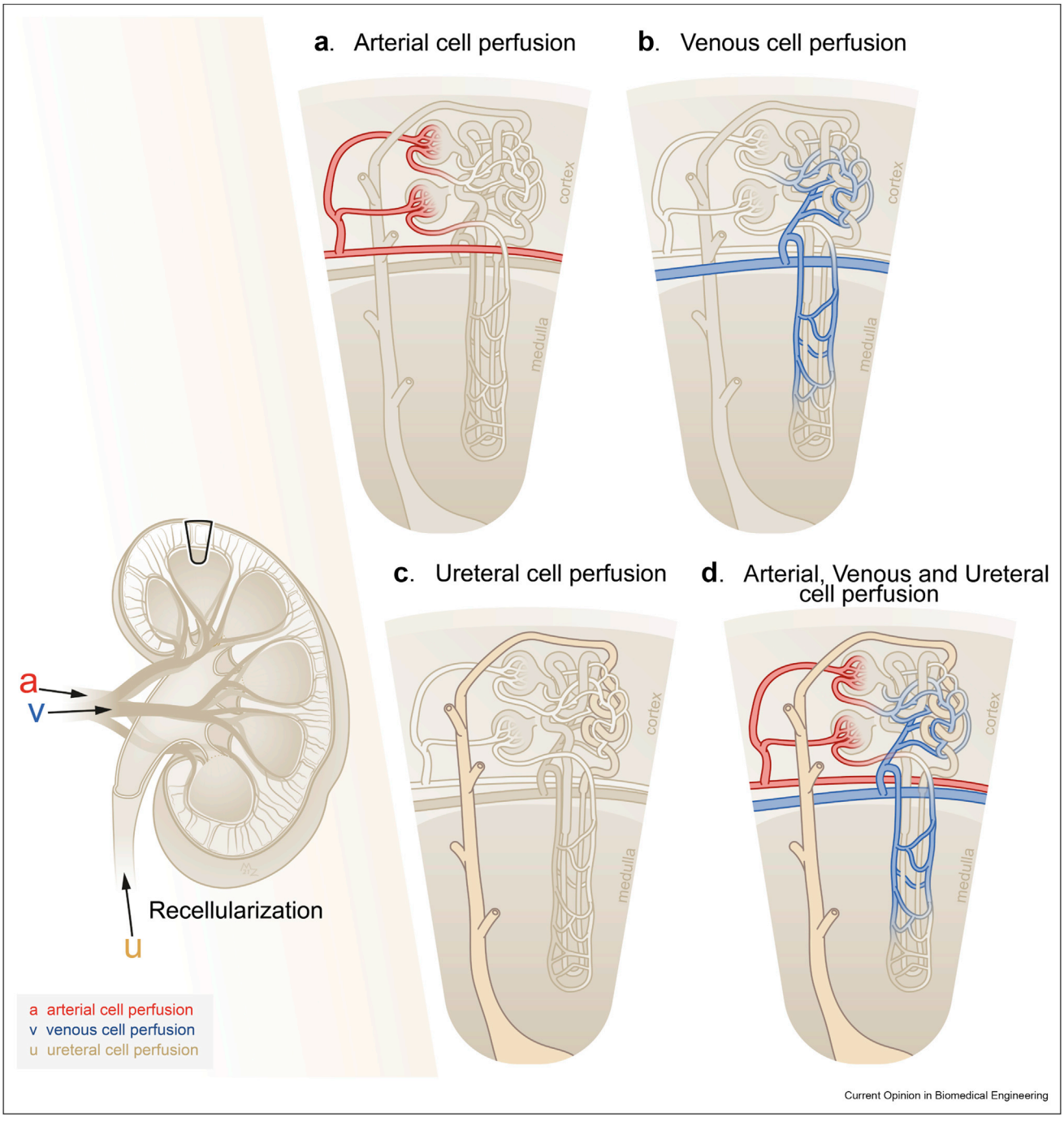
The entire vascular tree of decellularized kidneys, including both the glomerular and peritubular microcirculation, has been repopulated with endothelial cells [19]. Figure 2 shows the areas of the vasculature that are reached following arterial and venous cell perfusion. During arterial perfusion, cells move through the branches of the arterial tree and accumulate at the glomerular level, with 70%–86% of glomeruli repopulated [24,34]. Due to low flow velocities in the microcirculation cell clusters tend to form within the capillary bed they first encounter [23]. This coincides with cells rarely being seen in peritubular capillaries following arterial perfusion and in glomerular capillaries following venous perfusion. As a consequence, revascularization of both microcirculations and the arterial and venous vascular tree has only been reported following adjuvant arterial and venous perfusion [19,34].

Complete revascularization is imperative prior to any in vivo application given that thrombosis will occur if the vascular wall is not completely covered with endothelium [19]. This is also reflected by the limited success of in vivo revascularization of acellular kidney scaffolds [23]. Song et al. were first to attempt transplantation of a recellularized rodent kidney construct but did not report the graft survival time [24]. Ko et al. were first to transplant revascularized porcine kidney constructs [36]. However, loss of vascular patency only a few hours after transplantation makes long-term transplantation an objective that has yet to be achieved [36,37].

The question remains whether the poor outcomes following transplantation are the result of harsh decellularization protocols, incomplete revascularization, or both. During ex vivo heparinized whole blood perfusion it has been shown that Triton/SDS-treated kidneys maintained vascular patency for 24 h whilst SDS/DNase-treated kidneys showed several sites of blood extravasation [13]. In addition, thrombosis has been reported for revascularized kidneys in areas where ECs detached from the vascular wall [13,36]. This poses a problem since cell coverage appears to decrease when constructs are cultured for longer periods of time [19,23].

Thus, while revascularization of decellularized scaffolds in principle is possible, acquiring, and maintaining adequate endothelial coverage and preventing detachment of ECs remain bottlenecks. Interestingly, preloading of decellularized scaffolds with VEGF has been shown to improve endothelial cell adhesion in decellularized human kidneys [19].

Figure 2



Whole kidney engineering toolbox: Localization of cells following cell perfusion through the renal artery, vein and ureter. (**a**). Arterial cell perfusion results in antegrade repopulation of the arterial vasculature down to the glomerular capillaries, with 70%–86% of glomeruli repopulated [24,34]. (**b**). Venous cell perfusion results in retrograde repopulation of the venous vasculature down to the peritubular capillaries. Due to the low flow velocities in the microcirculation cell clusters tend to form within the capillary bed that they first encounter [23]. Glomeruli are solely present within the cortex, whilst peritubular capillaries are present in both cortex and medulla. This explains why with arterial perfusion cells mainly localize within the cortex (i.e., glomerular capillary bed) [19,23,40] whilst cells are seen in both cortex and medulla following venous perfusion (i.e., peritubular capillaries) [19,23]. (**c**). Ureteral cell perfusion results in retrograde repopulation of the renal pelvis, collecting ducts and nephrons up to the distal tubules. Repopulation of the nephron can be improved when a vacuum is maintained within the bioreactor during cell perfusion. (**d**). Although combining arterial, venous and ureteral cell perfusion results in improved repopulation of the nephron, several areas remain out of reach. Especially the tubular system is difficult to repopulate (i.e., loop of Henle and proximal tubules). Additionally, cells remain within the vascular and tubular lumen following cell perfusion and rarely translocate to the surrounding ECM.

Vascular perfusion has also been attempted for repopulation of the epithelial compartment with stem cells and primary epithelial cells [14,23,38,39]. However, this approach is impaired by the vascular basement membrane that is preserved during decellularization [11,13,19,25]. Consequently, this limits cell extravasation to the extent that most cells are retained within the vascular lumen [19,27,38,40]. Thus, cells literally hit a wall when attempting epithelial repopulation through the vasculature.

Ureteral cell perfusion

As for vascular perfusion, the same problem is encountered when cells are perfused into the ureter. Since the tubular basement membrane is preserved [24], cells remain within the tubular lumen [39]. Additionally, cells rarely reach the proximal tubules and Bowman's capsule during ureteral perfusion [26,41]. This is probably the result of decreasing flow velocities when cells move further through the ureter into the renal pelvis, collecting ducts and the individual tubules of each nephron. Likely resulting in a near-stagnant flow by the time the cell solution reaches the loop of Henle/proximal tubule. This poses a major problem given that for renal filtration, the vascular and epithelial compartment have to meet at Bowman's capsule.

Parenchymal repopulation: Methods to promote cell extravasation

Intact basement membranes limit the effective epithelial repopulation of decellularized constructs. To overcome this limitation, two methods have been described to promote extravasation of cells into the perivascular and peritubular space.

When recellularized constructs are perfused via the renal artery with high pressures directly after cell seeding, translocation of cells out of the microcirculation into the perivascular space is seen [9,42,43]. This high-pressure perfusion likely relies on focal disruptions that are made within the basement membrane through which cells move into the perivascular and peritubular space [23,34]. Translocation of cells from the tubular lumen into the peritubular space has not been reported following high-pressure perfusion through the ureter [41]. In addition, high-pressure ureteral perfusion did not improve distribution of cells throughout the length of the nephron with most of the cells being retained in the tubules of the medulla and few reaching the cortex region [41].

Alternatively, extravasation of cells can be improved when cells are infused when a vacuum is maintained in the bioreactor [19,23,24,41,42]. A vacuum of −40 mmHg did not cause macroscopic or microscopic tissue damage or leakage of cells [24]. Additionally, distribution of cells throughout the nephron was improved with some of the cells reaching the proximal tubule and Bowman's capsule [41,42].

In conclusion, decellularized kidneys can be fully revascularized when combining arterial and venous cell perfusion. Repopulation of the epithelial compartment can be seen as the biggest obstacle for whole kidney engineering. This compartment can only be reached by damaging the integrity of the vascular and tubular wall. Acquiring adequate cell coverage in the epithelial compartment seems unattainable.

Perspective

New direction for whole kidney engineering

Graft rejection after transplantation remains the main obstacle for long-term graft survival. Endothelial cells within donor organs are first and foremost exposed to the recipient's immune system due to the expression of surface molecules such as HLA [44]. Allo-recognition results in endothelial activation leading to, among others, activation of the complement system, transmigration of leukocytes across the endothelium and changes in vascular structure [45,46]. The different forms of graft rejection involve different graft vessels but are principally mediated by the host's adaptive immune system [46].

Approaches to reduce graft immunogenicity have been reported including cloaking of the graft endothelium with a thin layer of ECM proteins [47] and by genetically silencing MHC transcripts [48,49]. Alternatively, we propose the replacement of the donor graft vasculature by recipient-derived endothelium as a strategy to personalize donor organs and prevent or reduce graft rejection. Machine perfusion provides the setting during which these modifications can be made ex vivo. At present, human kidneys have been perfused ex vivo up to 24 h at normothermic temperatures (i.e., 37 °C) [50,51].

Replacement of a cell compartment during ex vivo organ perfusion has been pioneered for damaged airway epithelium in discarded human lungs [52,53]. During continuous vascular perfusion the epithelial compartment was exposed to mild detergents via the trachea followed by repopulation with pulmonary airway epithelial cells.

Theoretically, replacement of donor ECs with recipient endothelium will conceal the underlying allogeneic epithelium from the recipient's circulating immune system. Central to this approach will be partial decellularization with mild decellularizing agents. By using low concentrations of decellularizing agents and limiting the duration of exposure the vascular wall can be selectively targeted, whilst the underlying epithelial compartment is preserved. This can be performed during ex vivo machine perfusion. Interestingly, Cohen

et al. recently showed that partial decellularization of the vasculature for rodent and porcine kidneys, and revascularization in rodent kidneys is feasible, leading to engineered endothelial chimeric (xenogeneic) donor organs [54]. Although the investigators aimed to keep "the rest of the organ viable and functional during devascularization and revascularization", information on survival of the graft epithelium is unfortunately absent. In addition, the approach reported for revascularization is not transferrable to human organs due to a 1.5h period of warm ischemia to allow cell attachment and the absence of an oxygen carrier during normothermic machine perfusion. While interesting as a proof-of-concept study, further research is needed to address the translational potential of this approach.

Conclusion

Establishing a vasculature with sufficient EC coverage is one of the largest challenges faced to transplant whole organs. Cell perfusion through the renal artery and vein enables revascularization of decellularized kidneys. Alternatively, adequate repopulation of the epithelial compartment remains unattainable, even when applying techniques to promote cell extravasation from the vascular and tubular lumen. Thus, it can be concluded that human-scale whole kidney decellularization and recellularization will not be the solution for the donor organ shortages that we are currently faced with. Although an in vitro engineered patient-derived kidney remains out of reach, reaching toward the unreachable has enabled many steps forward. Interesting alternatives have started to present themselves — including refurbishment and personalization of discarded donor organs during ex vivo organ perfusion — that are potentially capable to reduce donor organ shortages within the foreseeable future.

Author contributions

M.J.A.d.H and **F.M.R.W**: Conceptualization, Writing — Original Draft; **M.A.E** and **T.J.R**: Conceptualization, Writing — Review & Editing, Supervision, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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* of special interest
** of outstanding interest

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