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REVIEW

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Decellularized kidney matrix as functional material for whole organ tissue engineering

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ABSTRACT

Renal transplantation is currently the most effective treatment for end-stage renal disease, which represents one of the major current public health problems. However, the number of available donor kidneys is drastically insufficient to meet the demand, causing prolonged waiting lists. For this reason, tissue engineering offers great potential to increase the pool of donated organs for kidney transplantation, by way of seeding cells on supporting scaffolding material. Biological scaffolds are prepared by removing cellular components from the donor organs using a decellularization process with detergents, enzymes or other cell lysing solutions. Extracellular matrix which makes up the scaffold is critical to directing the cell attachment and to creating a suitable environment for cell survival, proliferation and differentiation. Researchers are now studying whole intact scaffolds produced from the kidneys of animals or humans without adversely affecting extracellular matrix, biological activity and mechanical integrity. The process of recellularization includes cell seeding strategies and the choice of the cell source to repopulate the scaffold. This is the most difficult phase, due to the complexity of the kidney. Indeed, no studies have provided sufficient results of complete renal scaffold repopulation and differentiation. This review summarizes the research that has been conducted to obtain decellularized kidney scaffolds and to repopulate the scaffolds, evaluating the best cell sources, the cell seeding methods and the cell differentiation in kidney scaffolds.

Keywords: Cell sources, Decellularization, Kidney scaffold, Recellularization

Introduction

Chronic kidney disease (CKD) currently remains a global problem (1), with a reported prevalence in adults, reaching approximately 8%-16% of the population worldwide. Despite recent advancements in improving detection and management, CKD has risen from 27th in 1990 to 18th in 2010 on the list of global causes of death. Current CKD treatment is based on renal replacement therapy consisting of peritoneal dialysis, hemodialysis and renal transplantation (2-4). The cost of dialysis treatments is a high percentage of the total of all medical costs, and it is foreseen that in less than 5 years, most public and private medical services will not be able to fund this treatment for an increasing patient population. Renal transplantation improves long-term survival (5), ameliorates quality of life (6, 7) and is cost-effective compared with long-term dialysis treatment (8). Unfortunately, the significant increase in the number of available kidneys from living donors and cadavers, including kidneys from old deceased donors (9), is not enough to meet the growing demand, resulting in prolonged waiting lists. Because of this disparity, many patients who need transplants do not receive them, causing increased mortality and morbidity.

Thus, innovative tissue engineering strategies to increase the pool of donated organs for kidney transplantation have recently been proposed. Over the past decade, novel techniques have been developed with the idea of generating new kidneys, starting from acellular whole kidney scaffolds that support cellular adhesion, growth and differentiation (10). Biological scaffolds are prepared removing all native cells while retaining the extracellular matrix (ECM), by a decellularization process (11) with detergents, enzymes or other cell lysing solutions perfused through the renal vasculature. The ECM consists of the proteins and growth factors, which are very important to direct cell attachment, survival, proliferation and differentiation (12).

The recellularization of the kidney scaffold is a difficult task due to the complexity of the organ, which contains more than 26 types of cells. Indeed, no studies have provided convincing results for complete renal scaffold repopulation and differentiation. However, the process of recellularization is still being

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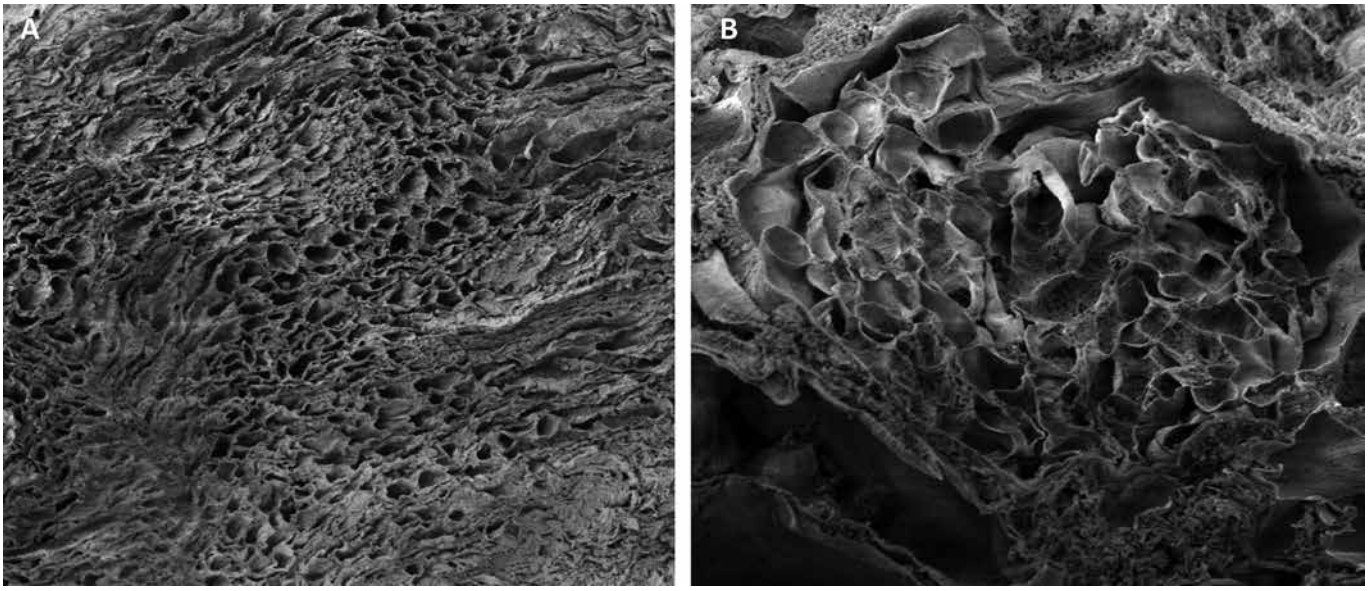

Fig. 1 - Scanning electron microscopy images of transverse sections of decellularized scaffold showing the complete removal of cellular material and the maintenance of well-organized 3-dimensional architecture in tubular regions (**A**) and glomerulus (**B**).

investigated using innovative strategies for cell seeding and for the choice of the cell source to repopulate the scaffold.

This review summarizes the research that has been conducted to obtain functional decellularized whole kidney scaffolds from animals or humans without adversely affecting the ECM, biological activity and mechanical integrity. Moreover, we will evaluate the choice of cell sources and the methods for cell seeding being used until now repopulate the scaffolds. Cell differentiation in repopulated kidney scaffolds will also be discussed.

Kidney decellularization to obtain an ECM

One of the most promising tissue engineering technologies is the fabrication of whole organ bioscaffolds, through the process called decellularization, followed by repopulation of the scaffold with new cells. During decellularization, which may involve chemical, physical or enzymatic treatments, all cellular components including immunogenic proteins are cleared from the tissue. Importantly, this process allows preservation of the innate 3-dimensional architecture and biochemical composition of the ECM. A major advantage in using native renal scaffold for kidney regeneration is the production of an ECM template with the intact mixture of structural and functional molecules which are highly tissue-specific. After removal of cellular components, decellularized scaffolds retain not only the fibrous proteins, but also soluble signals including cytokines, growth factors and chemokines that are able to influence cell adhesion, migration, proliferation and differentiation (13, 14).

Currently, the most efficient method to decellularize parenchymal kidney whole organs is the delivery of decellularization solutions through the vasculature. The chemical reagents, mainly detergents or acids, have the ability to disrupt cell membranes, thus removing native cells (15). Several research groups have reported successful generation of kidney scaffolds from different mammalian organisms, including humans.

Rat

In 2009, Ross et al first reported the production of a rat renal scaffold (16). The decellularization protocol consisted of the continuous perfusion of ionic and nonionic detergents and enzymatic solutions through the vasculature for 5 days. This study showed that the ionic detergent sodium deoxycholate (SDC) did not clear enough cellular debris, while the more robust sodium dodecyl sulfate (SDS) solution consistently produced completely acellular scaffolds with the preserved 3-dimensional framework of the ECM (16).

This work paved the way for the establishment of several protocols to optimize the rodent kidney decellularization procedure. To overcome the previously described time-consuming perfusion method (16), our group simplified the rat kidney decellularization protocol by shortening the duration of organ decellularization by using SDS alone as lysis buffer (17). Histological evaluation revealed that cell removal was already complete after 17 hours of perfusion with SDS detergent under physiological pressure. In this study, we documented the preservation of the 3D architecture of blood vessels, glomeruli and tubuli by scanning electron microscopy (SEM) analysis (Fig. 1), as well as the integrity, patency and connection of the vascular network. Moreover, the expression pattern of ECM proteins (collagen IV, laminin and fibronectin) was similar to that of native kidney ECM (17).

For a systematic evaluation of rodent kidney scaffold preparation, Caralt and colleagues (18) developed histological scoring systems to compare 3 different strategies used for rat kidney decellularization (1% Triton X-100, 1% Triton X-100 with 0.1% SDS and 0.02% trypsin-0.05% EGTA with 1% Triton X-100). Histological analysis showed that treatment with Triton X-100 alone resulted in incomplete cell removal in some

regions of the scaffold. All areas in the kidney scaffold were efficiently decellularized by both Triton X-100 with SDS and trypsin-EGTA with Triton X-100. However, trypsin treatment caused deterioration of renal scaffold structure and growth factor loss. The most effective outcome was obtained with the Triton X-100 with SDS protocol, which resulted in effective cell removal together with a well-preserved ECM structure and composition and the highest preservation of matrix-bound growth factors(18).

Very recently, He et al (19) compared the success in generation of whole rat kidney ECM bioscaffolds through arterial perfusion of SDS in different combinations of concentration and perfusion times (4 and 8 hours). The best results were achieved after perfusion for 4 hours with 0.125% SDS, a far lower concentration of SDS than that used in other studies on whole rat kidney decellularization (20, 21). Histological and immunohistochemical evaluation showed that this improved protocol was at least as effective in removing cellular components as the others, with more efficient preservation of sulfated glycosaminoglycans (sGAG) and growth factors (19).

Another study evaluated the effects of cryostorage (3 months) on renal tissue structure and compared the decellularization of cryostored and freshly isolated rat kidneys. SEM analysis demonstrated preservation of the native ultrastructure both in scaffolds generated from freshly isolated kidneys and in scaffolds obtained from cryostored ones. The immunohistochemical evaluation documented that there was no significant loss of sGAG, collagen and laminin in cryopreserved-decellularized kidneys (22). Based on these observations, cryopreserved kidneys are a valuable alternative for the production of functional renal ECM scaffolds (22).

Rhesus monkey

Rhesus monkey kidneys have been proposed by Nakayama et al (23) as an ideal platform for investigating the role of ECM scaffold in the field of kidney bioengineering. Sections of kidney taken from macaques in various age groups (fetal, infant, juvenile and adult) were treated with either 1% (v/v) SDS or Triton X-100. SDS at 4°C was the most effective detergent in removing cellular material and preserving native architecture and ECM composition. These findings demonstrate that decellularized sections of kidneys of all age groups provide a natural ECM with adequate structural and biochemical properties (23). In addition to ECM structural proteins, the scaffolds retained several growth factors and antimicrobial proteins, which may be beneficial for in vitro culture of the recellularized organ as well as for downstream host integration (24). Interestingly, it has been demonstrated that the age of the donor is a critical factor in repopulation efficiency (25).

Pig

Thanks to their similarity to the human, pig kidneys may represent an invaluable source for ECM scaffolds for clinical use (26). Indeed, pigs are known as the most common animal source of clinical xenografts and are considered a clinically relevant animal model to verify the feasibility of applying the decellularization technology to human-size organs. In this field, Sullivan and coworkers evaluated the effectiveness of 0.25 and 0.5% SDS and 1% Triton X-100 in the decellularization of adult porcine kidneys with a high-throughput apparatus (27). This study showed that 0.5% SDS was the most effective detergent in removing cellular material. The native structure of the renal scaffold, including the kidney vasculature and the components of ECM, was preserved after the decellularization procedure (27).

Another study evaluated the ability of acellular renal ECM to withstand physiological blood pressure without extravasation (10). Successfully decellularized porcine kidneys maintained intact ECM architecture and vascular tree. After implantation, unseeded scaffolds were easily reperfused and sustained blood pressure, without extravasation. However, extensive thrombosis of the vascular tree was evident 2 weeks after implantation, demonstrating massive activation of the coagulation cascade in the absence of the endothelial layer (10).

In a comparative study, Fischer et al (28) developed a scoring system as an attempt to standardize ECM quality assessment. Porcine kidney tissues were decellularized by immersion in 3 detergents (Triton X-100, SDS or SDC) at different temperatures. Application of the scoring system showed that decellularization with 1% SDS at 4°C resulted in a better preservation of ECM structure and composition, while 1% SDC at 4°C showed higher cell attachment and viability. The proposed scoring system allows intrastudy and interstudy evaluation of decellularization strategies.

As the detergents currently used to generate acellular scaffolds could be detrimental to the microstructural elements of renal tissue, some investigators have made efforts to minimize the undesired effects of detergent treatment. Poornejad et al (29) combined physical and chemical steps in an improved method to efficiently remove native cells with minimal damage to ECM. Freezing then thawing, increase in flow rate, exposure to osmotic shock, and low concentrations of SDS were strategies used to decrease detergent treatment time to a total of 5 hours, resulting in preservation of microstructure and 99% DNA removal (29).

Humans

Human kidneys are the ideal source of renal ECM, and the application of human scaffolds for tissue engineering may be more clinically compatible than kidneys derived from other species. This idea has supported the use of discarded kidneys for transplantation as a platform for organ engineering and regeneration (30, 31). The first study in which the decellularization strategy was applied to discarded human kidneys was reported in 2013 (32). In this study, the SDS-based decellularization protocol was used to obtain acellular scaffolds, while preserving the innate ECM architecture and biochemical properties. Significantly, the compliance of the innate vascular network was preserved in the human renal ECM scaffolds (32). A further study demonstrated that the decellularization strategy preserved the 3-dimensional conformation of the native glomerulus (33). Resin casting and pulse-wave measurements documented the retention of the microvascular morphology and morphometry, and physiological function of human renal ECM. Moreover, growth factors including vascular endothelial growth factor and its receptors were preserved within the matrices (33).

The effective removal of intracellular components satisfies preclinical procedures allowing us to obtain decellularized scaffolds ready for cell reseeding, which is discussed in the next section. However, the clinical feasibility of decellularization methods cannot be extended to damaged organs, because the damage due to disease or age can negatively affect decellularization or ECM structure. Thus, further investigations are needed regarding decellularization methods and their applicability to different types of donors.

Cell sources for recellularization of kidney ECM

Recellularization of the resulting kidney acellular matrix is the most complex phase of the tissue engineering process. The selection of cell sources to repopulate the 3-dimensional ECM scaffold is of great importance in creating a functional bioengineered organ. For complete organ regeneration, the reconstruction of parenchyma, vasculature and support structures is also essential. Ideally, the cells used for kidney recellularization should be patient-derived to potentially eliminate immune rejection following implantation, and they should be easy to expand, well characterized and fully functional in their new environment. Many different cell types, including renal epithelial and endothelial cells, embryonic stem cells (ESCs) and, more recently, adult-derived induced pluripotent stem cells (iPSCs) have been used for kidney recellularization.

Renal epithelial cells

Primary adult cells have been evaluated for recellularization, and renal-specific cell sources are critical components for kidney recellularization. In 2013, Song et al (20) reported a method for recellularization of whole rat kidneys by infusion of human umbilical venous endothelial cells via the renal artery and rat neonatal kidney cells via the ureter creating a transrenal pressure gradient. The preservation of glomerular, tubular and vascular structure was observed, and after approximately 4 days in culture, epithelial and endothelial cells engrafted in appropriate epithelial and vascular compartments providing the anatomic basis for renal function (20).

Recently, Caralt et al (18) reported the recellularization of whole rat kidneys with an immortalized human renal cortical-tubular epithelial cell line seeded through the renal artery with high pressure. They showed that high pressure caused the cells to pass through the vessel walls where they formed tubular structures. The cells were not found within arterioles, suggesting that cells were translocated out of microcirculation into peritubular space (18).

Abolbashari et al (34) isolated and expanded primary porcine renal cells for the repopulation of an acellular kidney scaffold. Reepithelialization of the porcine scaffold was performed by multiple injections of primary porcine renal cells into the kidney parenchyma. They demonstrated cell attachment and organization into appropriate renal tubular structures with limited function of the renal cells compared with a native kidney (34). However, this method is not appropriate for the reconstruction of complete cortical and medullar structure of a whole porcine kidney.

Ideally, epithelial cells represent an autologous source for recellularization, but they cannot provide all of the cell types needed to repopulate the kidney scaffold. Moreover, the limited passage number achievable in vitro does not allow us to obtain the cell expansion required for clinical applications.

Endothelial cells

The long-term in vivo success of recellularized organs is due to vascular patency. The absence of endothelial cells on the vasculature of organ scaffolds causes significant thrombosis even with anticoagulation (10, 35). Therefore, acellular kidney scaffold must be completely recellularized before implantation, and endothelial cells are the primary cell types used for this purpose. Recently, Ko et al (36) described an endothelial cell seeding approach for reendothelialization of whole pig kidneys. They also demonstrated that conjugation of endothelial cell–specific antibodies to the vascular walls favored endothelial cell adhesion to the vessel wall and extended vascular patency of the implanted scaffold. In this study, reendothelialized vessels maintained patency over a period of 4 hours, and thus, a longer-term study must be performed.

In another recent study, Song et al (20) utilized primary endothelial cells along with neonatal kidney cells for recellularization. After 3-5 days of perfused organ culture, endothelial cells lined the vascular channels throughout the entire scaffold cross-section.

Efficient recellularization of kidney scaffold vasculature to obtain functional long-term endothelialization has yet to be demonstrated. Moreover, setting up new methods for the isolation and expansion of autologous endothelial cells with the aim of generating an adequate numbers of cells is under investigation.

Embryonic stem cells (ESCs)

ESCs are pluripotent stem cells derived from the inner cell mass of the blastocysts. These cells are characterized by the ability to renew themselves and the capability of generating all of the renal cell types, depending on culture conditions (37, 38). Thus, the differentiation of ESCs can be induced through the contact of seeded cells with ECM proteins. The first attempt at kidney scaffold recellularization with ESCs was reported in 2009 by Ross et al (16). They manually perfused murine ESCs through the renal artery and the ureter, and observed that infused cells repopulated mainly small vessels, arterioles and glomeruli. In a similar study, we infused mouse ESCs in a rat renal scaffold via the renal artery (17). Seeded cells were uniformly distributed in the vascular network, but only occasionally reached peritubular capillaries.

Batchelder et al (39) perfused decellularized rhesus monkey kidneys with human ESCs via the renal artery and via the ureter followed by short culture periods (<5 days). They studied a custom bioreactor to allow constant scaffold perfusion to optimize kidney recellularization. Analysis of morphology demonstrated that cells were observed in vascular or tubular lumens of medulla but not in outer cortical tubules or glomeruli (39).

Recently, we published a second paper where we evaluated strategies of cell seeding to obtain the recellularization of all of the compartments of the scaffold with mouse ESCs (35). Our data demonstrated that mouse ESCs infused via the renal artery repopulated glomerular capillaries, as previously shown

(17), but they did not reach peritubular capillaries and veins. Therefore, we infused the cells through the renal vein showing that some cells arrived into the peritubular capillaries, but they did not reach the glomerular capillaries because of their retrograde direction. To recellularize the tubular compartment, we also infused ESCs into the ureter, while applying a negative pressure outside the scaffold. A small number of cells arrived in the tubular structures with a spread distribution into the scaffold. Thus, we evaluated the degree of recellularization obtained when infusions were by artery, vein and ureter. However, also under these conditions, cells were not uniformly distributed in the cortical and medullar regions. Finally, we recellularized kidney scaffolds by infusing mouse ESCs via the renal artery with a higher number of cells and at a higher pressure. Cell distribution was slightly better into the tubules, likely due to the braking of blood vessel membranes and to the consequent translocation of cells from the vessels into the peritubular spaces. In our paper, we concluded that kidney recellularization with ESCs did not result in great cell deposition throughout the entire volume of the acellular scaffolds (35).

Beside the disadvantages of the difficulty of repopulating the whole scaffold with mouse ESCs that we reported, the use of ESCs is also limited by ethical issues because of the destruction of embryos, as well as because of teratoma formation in vivo (40).

Induced pluripotent stem cells (iPSCs)

iPSCs are pluripotent stem cells derived from the transfection of 4 transcription factors in somatic cells (41). Like ESCs, they are able to self-renew and differentiate. In 2015, Caralt et al (18) used human iPSC-derived endothelial cells to repopulate the vasculature of rat kidney scaffolds combined with renal cortical tubular epithelial cells. The iPSC-derived endothelial cells were injected through the renal artery and incorporated into vascular structures, well distributed throughout the kidney. Renal cortical tubular epithelial cells were infused via renal artery under high pressure, which caused the translocation of the cells from the arterial circulation into peritubular structures.

In 2016, Du et al (42) repopulated a mouse kidney scaffold manually through the renal artery with human iPSC-derived PAX2-positive renal progenitor cells and endothelial cells. The recellularized kidneys were implanted subcutaneously in SCID mice for up to 12 weeks. At the end of the study, the authors observed that glomeruli were repopulated with renal progenitor cells only in the presence of endothelial cells. These results suggest that endothelial cells cocultured with renal progenitor cells improved the repopulation of the scaffold. Moreover, glomerular units were shown to be functional in vitro using a bioreactor set-up with urea and creatinine clearance and albumin reabsorption (42).

iPSCs have a great potential for regenerative medicine, although, like ESCs, the risk of teratoma formation exists. However, iPSCs have many of the advantages of ESCs without the ethical controversy.

Cell seeding strategies

The reintroduction of various cell types in a location-specific manner is one of the major hurdles in the field of kidney bioengineering. For complete organ regeneration, the parenchyma, vasculature and supporting components must be reestablished prior to implantation. Different recellularization strategies have been proposed with the ultimate goal of reconstructing a functional tissue.

Slices of acellular tissue

In the attempt to develop strategies to recellularize acellular scaffolds, it is important to understand how the structure and composition of the ECM affect cell function. To address this question, one strategy is the repopulation of slices of acellular renal tissue.

In 2011, sections of decellularized rhesus monkey kidneys were recellularized using layering of explants from different age groups (fetal, juvenile, adult) or seeding with fetal renal cell fractions. Cells were able to infiltrate and organize in the scaffold ECM. However, the greatest repopulation was achieved with scaffolds from the youngest donors, and with seeding of mixed fetal renal aggregates, suggesting that the age of the donor is a critical factor in repopulation efficiency (25). A further study from the same group investigated cell differentiation of human ESCs in sections of acellular kidney (24). Immunohistochemistry of the repopulated ECM slices demonstrated that seeded cells assumed epithelial tubule phenotypes and expressed kidney-associated genes, which were not present in human ESCs before seeding. This study pointed out that decellularized scaffolds were able to direct human ESC differentiation by physically shaping cells into tissue-appropriate structures and phenotypes (24).

Kidney ECM was also selected as a model to study how tissue matrix from 3 different specialized regions affects the function of kidney stem cells (KSCs) (43). After culture on papilla ECM, KSCs exhibited lower proliferation, higher metabolic activity, and cell morphology differences compared with KSCs that were cultured on slices of cortex and medulla ECM. These data indicate that region-specific ECM can be used as an effective substrate for studying stem cells in vitro (43).

Perfusion

The optimal recellularization technique to generate a functional kidney would be perfusion of the whole organ with cell suspension through the vasculature and/or the collecting system, followed by organ culture in a specialized bioreactor.

Numerous studies have proposed these seeding methods. Firstly, Ross et al (16) manually injected murine ESCs through the renal artery and ureter of a rat kidney scaffold. The seeded cells attached to the ECM, populated and proliferated within the glomerular, vascular and tubular structure (16). In similar studies, we reported the repopulation of acellular kidney scaffolds by infusing murine ESCs through the renal artery with a syringe pump (17). Seeded cells were uniformly distributed in the vascular network and glomerular capillaries, but they only occasionally reached peritubular capillaries and did not occupy the tubular lumen, as expected (17).

To improve cell engraftment in renal parenchyma, Song et al (20) introduced endothelial cells through the renal artery and neonatal kidney cells through the ureter while a negative pressure gradient was applied to the organ chamber.

This approach ensured cell dispersion throughout the kidney parenchyma (20). An alternative method proposed the perfusion of renal cortical tubular epithelial cells into the kidney parenchyma through the renal artery following by a high flow pressure for 15 minutes before reducing the flow to a physiological rate. Seeded cells translocated out of the arterial circulation into peritubular space, leading to 50% coverage of the renal area at 24 hours (18).

Very recently, our group compared the potential of different recellularization procedures with murine ESCs (35). The injection of cells in the renal artery resulted in repopulation of vascular structures up to glomerular capillaries, but cells rarely reached the venous circulation. Thus, we estimated the potential for repopulating the scaffold by retrograde cell infusion into the renal vein. Some cells occupied the peritubular capillary bed, but only a small fraction of the scaffold was repopulated (35). When murine ESCs were injected through the ureter, with negative pressure applied, they arrived to the tubular structures in the cortex, but only in a small volume fraction. In addition, we also assessed the degree of repopulation achieved by combining the 3 infusion routes (renal artery, vein and ureter) (Fig. 2). Our results show that this strategy improved cellular delivery, but the repopulation was limited to focal areas in the cortex and in the medullary volume (35). Finally, we assessed the effect of the high-pressure perfusion protocol during cell seeding. This procedure resulted in a better repopulation of the scaffold, compared with the protocols previously proposed. However, our data showed that injected cells proliferated in the first 2 days after seeding, but this phenomenon was significantly reduced after 7 days (35).

The overall conclusion is that, independently of the perfusion and cell seeding procedure selected, it is unlikely that perfusion of an acellular scaffold with cells expanded in vitro will reproduce an adequate number of cells and structures comparable to the native organ. This remains a major limitation in this field of research.

Multiple injections

An alternative protocol for renal scaffold repopulation is the delivery of cells into the cortical region of the renal parenchyma using a needle. Direct injection into kidney parenchyma allows better control over the recellularization area and distribution of cells, compared with the perfusion technique. This recellularization strategy was applied in a study in which primary renal cells were injected directly into different areas of the renal parenchyma of acellular porcine renal scaffolds, resulting in good repopulation. Seeded cells formed tubule-like structures that expressed markers typical of renal tubules and demonstrated normal renal functions (34). This protocol could be useful for better repopulation of renal parenchyma, which is nonuniformly seeded by antegrade or retrograde perfusion. However, it may cause damage to the renal tissue and does not allow the endothelialization of renal vasculature that is necessary for the success of transplantation.

Cell differentiation on kidney ECM

Studies of recellularization of the kidney ECM have not produced completely repopulated renal scaffold, due to the

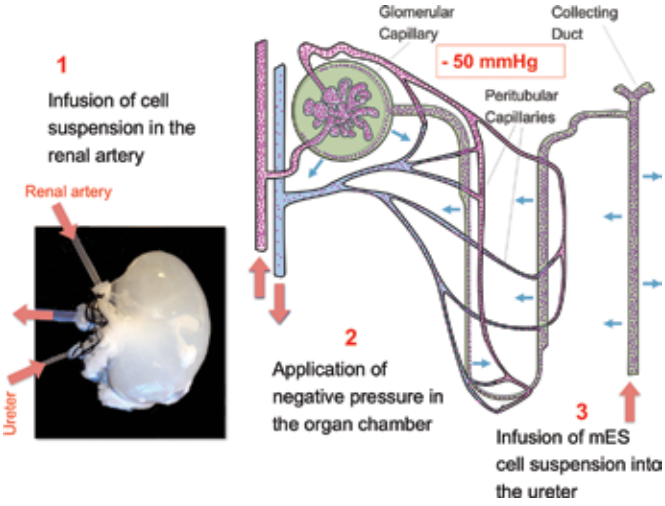

Fig. 2 - Schematic representation of different recellularization procedures via the renal artery, the renal vein and the ureter; mES = murine embryonic stem.

complexity and diversity of the components and structures of the kidney. Furthermore, studies to understand the influence of the ECM on cell migration and differentiation are important to achieve the complete kidney repopulation. Ross et al (16) firstly reported that murine ESCs seeded into the artery of kidney scaffolds and cultured without exogenous growth factors lost their embryonic phenotype. The cells acquired Pax-2 and Ksp-cadherin expression, cell adhesion proteins normally expressed in distal nephron tubular cells at a late developmental stage. The same research group demonstrated that mouse ESCs infused into the scaffold through the renal artery lined the basement membrane and were induced from the matrix to differentiate into endothelial lineages positive for endothelial markers BSLB4 lectin and vascular endothelium growth factor receptor-2 (21). In similar studies, we showed that mouse ESCs seeded into rat kidney scaffolds through the renal artery started to express endothelial markers such as Tie-2 and CD31 in glomerular capillaries (17). Moreover, the seeded cells progressively lost expression of the marker of stemness they had in 2D culture, while acquiring expression of neuronal cell adhesion molecule (NCAM), a marker of mesoderm precursors. With this study we demonstrated that 3D ECM induced ESCs to alter their phenotype and to differentiate into a mesendodermal lineage (17).

A clinically relevant model of kidney tissue engineering is the rhesus monkey. Nakayama et al (24) showed that human ESCs seeded onto sections of decellularized rhesus monkey kidney formed tubular structures and expressed markers of tubular cells such as dipeptidase 1 and heparan sulfate 6-O-sulfotransferase 1 (24). This study highlights the role of the ECM in inducing differentiation of pluripotent cells into specific kidney structures without adding exogenous growth factors. Also Batchelder et al (39) confirmed the role of the ECM of rhesus monkey kidneys in directing renal lineage differentiation without cytokine or growth factor stimulation. Human ESCs seeded in whole kidney scaffolds or on sections of kidney scaffolds progressively up-regulated renal lineage markers over time, expressing genes of renal progenitor, proximal

tubule, endothelial and collecting duct cells, as shown by quantitative real-time polymerase chain reaction (qPCR) and immunohistochemistry (39).

Petrosyan et al (44) have described the ability of human kidney scaffold to differentiate human amniotic fluid stem (AFS) cells toward renal lineage, as demonstrated by the induction of the expression of Wilms tumor WT-1, podocalyxin-like 2, glial cell-derived neurotrophic factor (GDNF), LMX1B, nephrin and synaptopodin 2-like indicators of mature renal cells (44).

These data underline the role of the kidney scaffold in regulating cell fate and cell renewal or differentiation. Future studies to define optimal scaffolds, 3-dimensional culture conditions and differentiation protocols will be important to define functional tissues to replace diseased or damaged kidneys.

Conclusions

In the last few years, a number of studies have been published on new technologies in regenerative medicine for the kidney. Particularly, these investigations were based on decellularized ECM scaffolds as a potential tool in the field of tissue engineering and regenerative medicine. We have summarized the steps that are needed to achieve kidney regeneration by ECM decellularization and cell repopulation. However, results reported so far suggest that we are still in the early stages of research aimed at regenerating the kidney and that the processes tested so far need to be greatly improved. The protocols concerning decellularization reach almost 100% cell removal with successful preservation of the 3-dimensional architecture and biochemical composition of the ECM structures. But, recellularization of the scaffolds is the most complex and challenging stage due to the numerous differentiated cells that populate the kidney. A large number of cells, including a great variety of cell types, are required for an adequate repopulation of the kidney scaffold. No studies have provided sufficient results in terms of kidney scaffold repopulation with an adequate cell type and number, and/or with an appropriate cell differentiation. Although seeding methods have been implemented, the most hopeful results were obtained with multiple seeding techniques, several cell infusions and different cell types. Based on the results obtained thus far, we conclude that renal tissue engineering is in its very early stages and the production in the laboratory of functional kidneys will not be ready soon. However, these studies demonstrate that it is possible in the laboratory to use functional acellular renal ECM for cell seeding under dynamic flow conditions to help understand kidney cell biology, cell differentiation and remodeling. In addition, these experimental set-ups may be useful to obtain new evidence regarding the mechanisms responsible for kidney pathophysiology.

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References

1. Schieppati A, Remuzzi G. Chronic renal diseases as a public health problem: epidemiology, social, and economic implications. Kidney Int Suppl. 2005;68(98):S7-S10.
2. Ronco C. Continuous renal replacement therapy: forty-year anniversary. Int J Artif Organs. 2017;40(6):257-264.
3. Mann BS, Manns BJ, Barnieh L, et al. Peritoneal dialysis: a scoping review of strategies to maximize PD utilization. Perit Dial Int. 2017;37(2):159-164.
4. Jassal SV, Krahn MD, Naglie G, et al. Kidney transplantation in the elderly: a decision analysis. J Am Soc Nephrol. 2003;14(1): 187-196.
5. Wolfe RA, Ashby VB, Milford EL, et al. Comparison of mortality in all patients on dialysis, patients on dialysis awaiting transplantation, and recipients of a first cadaveric transplant. N Engl J Med. 1999;341(23):1725-1730.
6. Dew MA, Switzer GE, Goycoolea JM, et al. Does transplantation produce quality of life benefits? A quantitative analysis of the literature. Transplantation. 1997;64(9):1261-1273.
7. Anil Kumar BN, Mattoo SK. Organ transplant & the psychiatrist: an overview. Indian J Med Res. 2015;141(4):408-416.
8. Hutton J. The economics of immunosuppression in renal transplantation: a review of recent literature. Transplant Proc. 1999;31(1-2):1328-1332.
9. Kute VB, Trivedi HL, Vanikar AV, et al. Deceased donor renal transplantation from older donors to increase the donor pool. Int J Artif Organs. 2012;35(9):663-670.
10. Orlando G, Farney AC, Iskandar SS, et al. Production and implantation of renal extracellular matrix scaffolds from porcine kidneys as a platform for renal bioengineering investigations. Ann Surg. 2012;256(2):363-370.
11. Baptista PM, Orlando G, Mirmalek-Sani S-H, et al. Whole organ decellularization: a tool for bioscaffold fabrication and organ bioengineering. Conf Proc IEEE Eng Med Biol Soc. 2009;2009:6526-6529.
12. Fedecostante M, Onciu OG, Westphal KG, Masereeuw R. Towards a bioengineered kidney: recellularization strategies for decellularized native kidney scaffolds. Int J Artif Organs. 2017;40(4):150-158.
13. Sebinger DD, Ofenbauer A, Gruber P, Malik S, Werner C. ECM modulated early kidney development in embryonic organ culture. Biomaterials. 2013;34(28):6670-6682.
14. Hussein KH, Park K-M, Lee Y-S, et al. New insights into the pros and cons of cross-linking decellularized bioartificial organs. Int J Artif Organs. 2017;40(4):136-141.
15. Gilbert TW, Sellaro TL, Badylak SF. Decellularization of tissues and organs. Biomaterials. 2006;27(19):3675-3683.
16. Ross EA, Williams MJ, Hamazaki T, et al. Embryonic stem cells proliferate and differentiate when seeded into kidney scaffolds. J Am Soc Nephrol. 2009;20(11):2338-2347.
17. Bonandrini B, Figliuzzi M, Papadimou E, et al. Recellularization of well-preserved acellular kidney scaffold using embryonic stem cells. Tissue Eng Part A. 2014;20(9-10):1486-1498.
18. Caralt M, Uzarski JS, Iacob S, 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(1):64-75.
19. He M, Callanan A, Lagaras K, Steele JA, Stevens MM. Optimization of SDS exposure on preservation of ECM characteristics in whole organ decellularization of rat kidneys. J Biomed Mater Res B Appl Biomater. 2017;105(6):1352-1360.

20. 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(5):646-651.
21. Ross EA, Abrahamson DR, St John P, et al. Mouse stem cells seeded into decellularized rat kidney scaffolds endothelialize and remodel basement membranes. Organogenesis. 2012;8(2):49-55.
22. Chani B, Puri V, Sobti RC, Jha V, Puri S. Decellularized scaffold of cryopreserved rat kidney retains its recellularization potential. PLoS ONE. 2017;12(3):e0173040.
23. 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(7): 2207-2216.
24. Nakayama KH, Lee CCI, Batchelder CA, Tarantal AF. Tissue specificity of decellularized rhesus monkey kidney and lung scaffolds. PLoS ONE. 2013;8(5):e64134.
25. 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(23-24): 2891-2901.
26. Salvatori M, Peloso A, Katari R, et al. Semi-xenotransplantation: the regenerative medicine-based approach to immunosuppression-free transplantation and to meet the organ demand. Xenotransplantation. 2015;22(1):1-6.
27. Sullivan DC, Mirmalek-Sani S-H, Deegan DB, et al. Decellularization methods of porcine kidneys for whole organ engineering using a high-throughput system. Biomaterials. 2012;33(31):7756-7764.
28. Fischer I, Westphal M, Rossbach B, et al. Comparative characterization of decellularized renal scaffolds for tissue engineering. Biomed Mater. 2017;12(4):045005.
29. Poornejad N, Momtahan N, Salehi AS, et al. Efficient decellularization of whole porcine kidneys improves reseeded cell behavior. Biomed Mater. 2016;11(2):025003.
30. Katari R, Peloso A, Zambon JP, et al. Renal bioengineering with scaffolds generated from human kidneys. Nephron, Exp Nephrol. 2014;126(2):119-124.
31. Gifford S, Zambon JP, Orlando G. Recycling organs: growing tailor-made replacement kidneys. Regen Med. 2015;10(8):913-915.
32. Orlando G, Booth C, Wang Z, et al. Discarded human kidneys as a source of ECM scaffold for kidney regeneration technologies. Biomaterials. 2013;34(24):5915-5925.
33. Peloso A, Petrosyan A, Da Sacco S, et al. Renal extracellular matrix scaffolds from discarded kidneys maintain glomerular morphometry and vascular resilience and retains critical growth factors. Transplantation. 2015;99(9):1807-1816.
34. Abolbashari M, Agcaoili SM, Lee M-K, et al. Repopulation of porcine kidney scaffold using porcine primary renal cells. Acta Biomater. 2016;29:52-61.
35. Remuzzi A, Figliuzzi M, Bonandrini B, et al. Experimental evaluation of kidney regeneration by organ scaffold recellularization. Sci Rep. 2017;7:43502.
36. Ko IK, Abolbashari M, Huling J, et al. Enhanced re-endothelialization of acellular kidney scaffolds for whole organ engineering via antibody conjugation of vasculatures. Technology. 2014;2(03):243-253.
37. Morizane R, Monkawa T, Itoh H. Differentiation of murine embryonic stem and induced pluripotent stem cells to renal lineage in vitro. Biochem Biophys Res Commun. 2009;390(4): 1334-1339.
38. Kim D, Dressler GR. Nephrogenic factors promote differentiation of mouse embryonic stem cells into renal epithelia. J Am Soc Nephrol. 2005;16(12):3527-3534.
39. Batchelder CA, Martinez ML, Tarantal AF. Natural scaffolds for renal differentiation of human embryonic stem cells for kidney tissue engineering. PLoS ONE. 2015;10(12):e0143849.
40. Fong C-Y, Gauthaman K, Bongso A. Teratomas from pluripotent stem cells: a clinical hurdle. J Cell Biochem. 2010;111(4): 769-781.
41. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell. 2006;126(4):663-676.
42. Du C, Narayanan K, Leong MF, et al. Functional kidney bioengineering with pluripotent stem-cell-derived renal progenitor cells and decellularized kidney scaffolds. Adv Healthc Mater. 2016;5(16):2080-2091.
43. ONeill 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(38):9830-9841.
44. Petrosyan A, Orlando G, Peloso A, et al. Understanding the bioactivity of stem cells seeded on extracellular matrix scaffolds produced from discarded human kidneys: a critical step towards a new generation bio-artificial kidney. CellR4. 2015; 3(1):e1401.