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Comparative analysis of two porcine kidney decellularization methods for maintenance of functional vascular architectures

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ABSTRACT

Kidney transplantation is currently the only definitive solution for the treatment of end-stage renal disease (ESRD), however transplantation is severely limited by the shortage of available donor kidneys. Recent progress in whole organ engineering based on decellularization/recellularization techniques has enabled pre-clinical *in vivo* studies using small animal models; however, these *in vivo* studies have been limited to short-term assessments. We previously developed a decellularization system that effectively removes cellular components from porcine kidneys. While functional re-endothelialization on the porcine whole kidney scaffold was able to improve vascular patency, as compared to the kidney scaffold only, the duration of patency lasted only a few hours. In this study, we hypothesized that significant damage in the microvasculatures within the kidney scaffold resulted in the cessation of blood flow, and that thorough investigation is necessary to accurately evaluate the vascular integrity of the kidney scaffolds. Two decellularization protocols [sodium dodecyl sulfate (SDS) with DNase (SDS + DNase) or Triton X-100 with SDS (TRX + SDS)] were used to evaluate and optimize the levels of vascular integrity within the kidney scaffold. Results from vascular analysis studies using vascular corrosion casting and angiograms demonstrated that the TRX + SDS method was able to better maintain intact and functional microvascular architectures such as glomeruli within the acellular matrices than that by the SDS + DNase treatment. Importantly, *in vitro* blood perfusion of the re-endothelialized kidney construct revealed improved vascular function of the scaffold by TRX + SDS treatment compared with the SDS + DNase. Our results suggest that the optimized TRX + SDS decellularization method preserves kidney-specific microvasculatures and may contribute to long-term vascular patency following implantation.

Statement of Significance

Kidney transplantation is the only curative therapy for patients with end-stage renal disease (ESRD). However, in the United States, the supply of donor kidneys meets less than one-fifth of the demand; and those patients that receive a donor kidney need life-long immunosuppressive therapy to avoid organ rejection. In the last two decades, regenerative medicine and tissue engineering have emerged as an attractive alternative to overcome these limitations.

In 2013, Song et al. published the first experimental orthotopic transplantation of a bioengineering kidney in rodents. In this study, they demonstrated evidences of kidney tissue regeneration and partial function restoration. Despite these initial promising results, there are still many challenges to achieve long-term blood perfusion without graft thrombosis. In this paper, we demonstrated that perfusion of detergents through the renal artery of porcine kidneys damages the glomeruli microarchitecture as well as peritubular capillaries. Modifying dynamic parameters such as flow rate, detergent concentration, and decellularization time, we were able to establish an optimized decellularization protocol with no evidences of disruption of glomeruli microarchitecture. As a proof of concept, we recellularized the kidney scaffolds with endothelial cells and *in vitro* perfused whole porcine blood successfully for 24 h with no evidences of thrombosis.

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1. Introduction

In the United States, approximately 1 million patients have end-stage renal disease (ESRD) with over 100,000 new diagnoses occurring every year [1]. Although hemodialysis has increased the survival rates of these patients, kidney transplantation remains the only curative treatment. Nearly 18,000 kidney transplants are performed in the United States each year; however, more than 100,000 patients are still awaiting a donor kidney [2]. Despite the efforts to increase the organ supply pool for transplantation, there is still a large gap between the number of organ donors and recipients [3]. The lack of transplantable allografts for ESRD treatment has prompted the development of bioengineered organs to overcome this limitation.

Recent progress in whole organ engineering techniques, based on decellularization of donor organs and recellularization of the resulting extracellular matrix, suggests that this approach may potentially be used for transplantation [4–8]. The decellularization process involves the removal of all the cellular components from a donor organ, leaving the structural components intact to serve as a scaffold for the bioengineering of a whole organ construct. Subsequently, the resulting acellular matrix is re-populated with organ-specific cells (“re-cellularization”) in order to augment and restore organ functionality. Decellularized and re-cellularized kidneys have been tested in pre-clinical *in vivo* studies with short term endpoints [9]. Previously, we developed a decellularization protocol that effectively removes cellular components from porcine kidneys [10]. We showed that decellularized kidney scaffolds retained no identifiable cellular components and preserved intact architecture, including the renal vasculature and essential extracellular matrix components [10]. To prevent clotting and maintain blood flow into the decellularized porcine kidney scaffold after implantation, endothelial cells were seeded on the vessel walls and histological evaluation confirmed a uniform re-endothelialization of major blood vessels and capillaries within the decellularized porcine renal scaffold [11]. In this study, CD31 antibody was conjugated onto the porcine scaffold blood vessels to improve the endothelial cell attachment. The heterotopic transplantation of the recellularized scaffold maintain vascular patency for up to 4 h in a pig model, but multi-day timepoints are still unattainable due to eventual thrombosis [12].

Based on the premise that detergents change the extracellular matrix (ECM) composition and invariably cause some degree of microarchitecture disruption, a possible explanation for the graft thrombosis could be a disruption of micro-vascular systems such as glomeruli and peritubular capillaries during the decellularization process [12]. Therefore, decellularization protocol optimization seems to be a rational strategy toward long-term patency following transplantation.

To optimize the decellularization protocol, proper evaluation of the decellularized porcine kidney scaffold vasculature is paramount. To date, several imaging methods, such as fluoroscopic angiography and CT, have been used to assess the vascular structures within the acellular kidney scaffold [9–11]. Such techniques effectively demonstrate macroscopic vascular patency; however, they do not provide reliable information regarding capillary structures. Histomorphological analysis also has been used to evaluate the structure of extracellular matrix [5,9–11]. However, the 2-dimensional microscopic image-based evaluation method not necessarily reflect the vascular structures within the scaffold. To address this critical issue, we utilized a vascular corrosion casting method [13], in which the kidney blood vessels are filled with a water-insoluble liquid polymer that polymerize under special conditions; after polymerization the remnant tissue is removed with sodium hydroxide. This technique can be used to analyze both morphology and architecture of blood vessels and capillaries [13,14] and therefore, could be a useful tool to predict vascular patency after implantation.

Using the vascular casting analysis, we attempted to optimize the decellularization protocol by assessing the effects of decellularization detergents and methods on vascular preservation [10]. The level of damage and disruption of renal specific microvasculatures, such as glomeruli and peritubular capillaries, was assessed in porcine kidneys decellularized with 0.25% SDS, 0.5% SDS, and Triton X-100, and compared with that of native kidney tissue. The perfusion of SDS regardless of its concentration caused glomeruli microarchitecture and peritubular capillaries disruption.

The decellularization protocol (Triton X-100 treatment) that showed the least vascular damage after decellularization was selected as a possible candidate for further examination. Furthermore, a combination strategy of the casting technique with the conventional analysis such as fluoroscopic angiogram and histological analysis was used to confirm that the optimized decellularization protocol preserves functional and intact microvasculature structures when compared with the previous decellularization protocol [10]. To validate whether kidney scaffolds decellularized with the optimized protocol would maintain vascular patency during implantation, *in vitro* porcine blood perfusion of recellularized scaffolds was performed and the vascular patency and graft thrombosis was compared with the controls.

2. Material and methods

2.1. Decellularization of porcine kidneys

Kidneys were harvested from adult Yorkshire pigs after euthanasia, which was performed in accordance with Institutional Animal Care and Use Committee (IACUC) guidelines. Each kidney was collected with an intact aorta and inferior vena cava to preserve the entire length of the renal artery and vein. Polycarbonate male Luer lock 1/8″ hose barb fittings (Cole Palmer Instrument Co., Chicago, IL, USA) were placed into the ends of the renal artery and vein and secured with Silk 0 suture. The kidneys were then connected to a high-throughput decellularization system as described previously [11], and perfused with 10 United States Pharmacopeia (USP) units/ml sodium heparin (Hospira Inc., Lake Forest, IL, USA) in PBS for 15 min at 0.75 l/h. After washing with heparin, the kidneys were decellularized with two previously developed decellularization protocols [10]. One protocol uses 0.5% sodium dodecyl sulfate (SDS)/DNase (SDS + DNase), and the other uses 1% Triton X-100/0.5% SDS (TRX + SDS). These protocols were used for further experiments (Table 1).

The 0.5% SDS/DNase (SDS + DNase) protocol was developed in our previous study [10]. Briefly, 0.5% SDS in PBS was perfused through the main renal artery at 12.5 ml/min for 36 h at room temperature. Following the detergent perfusion, kidneys were washed with PBS for 48 h to remove detergent residuals. Following the PBS perfusion, 500 ml of 0.0025 w/w% DNase solution (Sigma-Aldrich, St. Louis, MO, USA) and 10 mM magnesium chloride (Sigma-Aldrich) in PBS, at pH7.4, was recirculated through the kidneys overnight, followed by a final rinse with PBS for 1 h to flush out residual DNase and magnesium chloride.

In the alternative decellularization protocol with 1% Triton X-100/0.5% SDS (TRX + SDS), 1% Triton X-100 in deionized water was perfused through the renal artery at 5 ml/min for 36 h, followed by perfusion of 0.5% SDS in PBS for additional 36 h. Then, the perfused kidneys were washed with PBS for 72 h to remove detergent residuals and cell components. To evaluate the effects of decellularization on the glomerular microarchitecture and capillaries, two different protocols were used to produce acellular

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