Original Article

Development of an Experimental Model of a Decellularized Kidney Scaffold by Perfusion Mode and Analyzing the Three-dimensional Extracellular Matrix Architecture by Edge Detection Method

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Abstract

Renal transplant is treatment of choice for the patients with end stage renal disease. The kidney transplants are expensive and there are risks of immunological and infectious complications. We planned to develop an *in vitro* decellularized kidney scaffold model using sheep kidney. Kidney decellularization was carried out by perfusing chemical detergents such as sodium dodecyl sulfate (SDS), SDS and trypsin, and SDS and ethylenediaminetetraacetic acid solvent solution. Complete kidney was decellularized in 5 days by perfusing various chemical detergents in time-dependent intervals. Histological finding revealed the complete removal of cellular material in various regions of renal corpuscle, distal convoluted tubules, other cortex and medulla region. Details of interlobular veins and arteries were seen through naked eyes after trypan blue dye injection. We used edge detection technique for developing a three-dimensional (3-D) image (Image J software) for nephrological vasculature constructed of decellularized kidney scaffold specimen. This technique opens a gateway for the whole organ decellularization by perfusion technology and further imaging of its 3-D extracellular matrix texture by edge detection technique software.

Keywords: *Histology, Image J, kidney decellularization, perfusion, trypan blue*

Introduction

The kidney is the main excretory and osmoregulatory organ in mammals. It is highly complex organ of the body. Physiologically, the kidney is subjected to various stress and oxidative impairment,[1] which further leads to various renal diseases leading to chronic kidney diseases.[2,3] However, permanent failures of both the kidneys need an organ transplant. It is a great challenge for the clinicians and patients to get a matched (human leukocyte antigen [HLA]) donor for kidney transplant.[4] The donated and matched (HLA) transplanted kidneys have risk of virus infections transmission (HIV, HCV, and HBC).[5] In absence of proper registry, it is assumed that nearly 3 lakh renal patients need dialysis or renal transplant. The dialysis and renal transplant are very expensive and the medical cost is difficult to be bear by the patient and their families.[6]

Hence, with this aim in our mind, it was thought to develop a model of decellularized kidney scaffold (bioprosthesis) at *in vitro* condition. Limited work has been done on decellularization of kidney scaffolds and recovery of renal cells (pelvis) and endothelial cell lining has been shown after implantation.[7] However, poor retention of cells has been a problem.

Biological origin kidney scaffolds (extracellular matrix [ECM]) maintain the physical characteristics similar to native kidney after decellularization. They are also known to remodel to a normal kidney.[8] Kidney perfusion and decellularized scaffolds would be significant to study its suitability as three-dimensional (3-D) tissue-engineered prostheses at *in vitro* condition.[9] Hence, the study was designed to develop a decellularized biological kidney scaffold by perfusion methodology composed of perfusing various grades of chemical detergent which leaves an ECM. We further used the ImageJ software (online https://imagej.nih.gov/ij/) [Oracle America, Inc., 500 Oracle Parkway, Redwood City, CA 94065] for construction of decellularized nephrological vasculature organization

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for with and without trypan blue dye perfusion. We also studied the ECM of decellularized kidney scaffold by edge detection method (ImageJ).[10]

Methods

Collection of sheep kidney

Institutional ethics committee approval was obtained for the collection of sheep kidney. Sheep kidney was immediately collected after slaughtering and scarifying the animal from local abattoir under clean condition. The kidney was collected in a sterile phosphate-buffered saline (PBS) and transported to the RMS-Biomedical laboratory, Indian Institute of Technology (IIT) Hyderabad.

Processing of kidney for perfusion

Collected kidney was cleaned and rinsed with sterile PBS, and adrenal glands and fat tissues were removed. Peritoneum tissue was removed and kidney was rinsed in sterile PBS. Finally, renal artery, renal vein, and ureter were clearly visible (located) [Figure 1]. The renal artery traced was cannulated with vein cannula (syringe needle) and clamped using an artery forceps. Care was taken for not puncturing or damaging the renal artery. The kidney was kept suspended in the beaker, and intravenous connector was connected to the vein cannula (syringe needle) [Figure 2].[11]

Decellularization of kidney by perfusion method using chemical detergent

The renal artery was cannulated and perfused with 500 ml normal saline under the influence of the gravity. Nearly 1% SDS solution was flushed through the cannulated renal artery by perfusion method. About sixty drops were dripped per minute and 500 ml of 1% SDS was perfused in the kidney in first 2 h (1st day). Further, kidney was perfused for another 10 h and the kidney was kept in 1% SDS detergent overnight at 4°C (1st day). Next day, the renal perfusion was preceded by PBS followed by ionic 1% SDS flushing. Perfusion drip and flow rate were reduced to ten drops per minute drips; this was done with continuous flow for about 8 h. Further drip flow was increased for PBS flushing the renal cortex and medulla region canals. Again 1% SDS was flushed through renal artery under perfusion and drip flow rate was maintained for about 10 drops per minute (2nd day).[9] The same procedure was repeated for overnight incubation in 1% SDS detergent and PBS-1X flushing (3rd day). Further combination of 1% SDS and 1X trypsin (1:1) solution was perfused through renal artery with increase in drip flow rate from thirty drops per minute (4th day). The perfused kidney was incubated overnight in 1% SDS solution and further perfused with 1% SDS and 1% EDTA (1:1) combined solvent solution (5th day).[9] The drip flow rate was increased to sixty drops per minute and followed by PBS-1X equivalent drip flow rate. Final perfusion and flushing were carried out by flow of sterile water (5th day).[9] This procedure was repeated for 2 times or cycles (n = 2).

Histological investigation for kidney scaffolds

The perfused kidney was further fixed in 10% formaldehyde solution and processed for paraffin wax-embedded blocks. Blocks were trimmed onto sections of 5 μ on rotary microtome. The transverse section (TS) of perfused kidney was stained for hematoxylin and eosin (HE) staining for localization cellular and tissue histological details[12] and another set was stained for elastin and collagen analysis by the Verhoeff–van Gieson method (EVG).[13]

Trypan blue dye injection for detection of vasculature and ImageJ

The decellularized kidney capsule was further injected and perfused with 1% trypan blue for localization of the kidney scaffold architecture including venules, arterioles and smaller veins and arteries.[10] To distinguish between the trypan blue dye perfused and nonperfused kidney scaffold, we performed some ImageJ software analysis[11] and further observed the comparable minute details for the organization of renal vasculature architecture and texture.

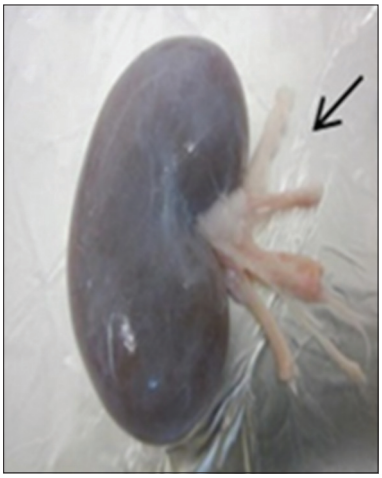

Figure 1: Sheep Kidney

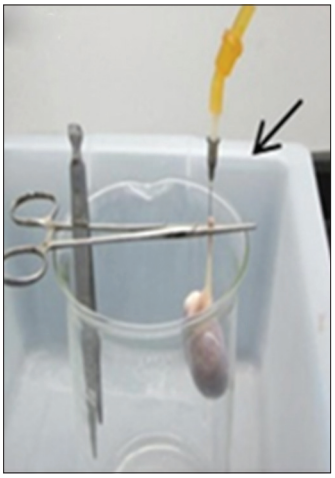

Figure 2: .Perfusion set up

Results

Processing and decellularized kidney scaffold development by perfusion

The perfusion of normal saline and 1% SDS lead to alterations in appearance of the kidney (2h). Therefore, this lead to change in coloration from dark reddish-brown [Figure 1] to grayish. Further, perfusion (8h) lead to translucent cortical (cortex) capsule [Figure 3]. After 12 h of ionic perfusion in cortical and medullary region of the kidney mosaic patches were developed; this indicates the dislodging of the cortex and medullar cellular cell matrix [Figure 4]. Perfused kidney starts showing complete translucent nature [Figure 5] (1% SDS and 1X trypsin) by 12hrs. Appearance of venules and other vasculature [Figure 6] was clearly seen. Entire whitish translucent (opaque) [Figure 7] scaffold along with vasculature branches (venules) was clearly demarcated. The sterile water was perfused through the decellularized kidney scaffold (renal artery) for 2 h. The kidney scaffold developed showed various morphological texture changes from dark-reddish brown to grayish to absolute white translucent in 5 days.

Histological investigation for decellularized kidney scaffolds

HE staining and histological investigation revealed the absence of cuboidal columnar epithelial cells in the cortex and medulla area after decellularization by perfusion methods. TS of kidney scaffolds clearly showed the absence of the cellular material in cortex region of kidney that is renal corpuscle (glomerulus knot, Bowman's capsule [BC], proximal collecting tubule [PCT]) whereas loop of Henle was very difficult to observe under microscope. Distal collecting tubule (DCT) could be seen in the medulla region and showed opening in collecting tubules (CTs). Stroma region could be seen in between the PCT and DCT regions. Stroma region was completely devoid of mesangial cells. No abnormality and shrinkage of ECM was observed after decellularization [Figure 8]. Glomerulus showed ghost outline clearly for decellularized kidney scaffolds [Figure 8], and EVG staining showed fragmentation in elastic lamina and vessel [Figure 9].

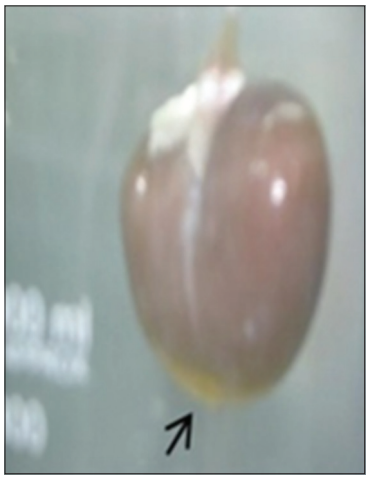

Figure 3: Perfusion showed translucent appearance at cortex curvature

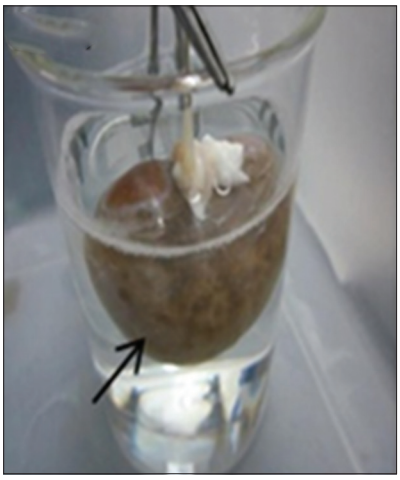

Figure 4: Mosiac patches in cortex and medulla

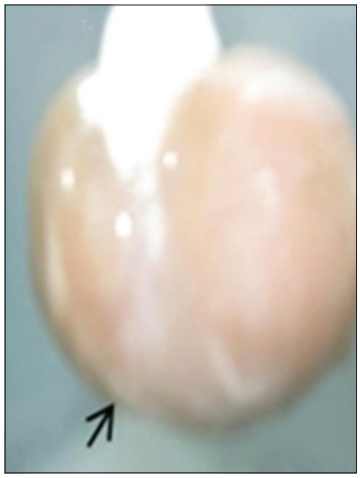

Figure 5: Perfused kidney with 1%SDS+1XTrypsin translucent

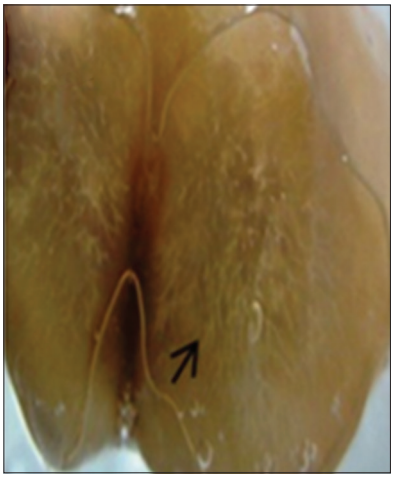

Figure 6: Translucent vasculature

Dye injection for scaffold architecture and ImageJ

The entire renal and nephrological vasculature was observed easily with translucent scaffold of the kidney capsule. Clearly interlobular-lobular arterioles and venules were seen, but their arrangement is much complexed in cortical and medullary region which was assessed with the ImageJ software [Figure 10] and edge detection technique. We have tried to rebuild the lobular arrangements within the decellularized kidney scaffolds, and found that the arrangement was much more intermingled from cortex to medullary region. The arrangement of the vasculatures or else was impossible to visualize even though the scaffold was translucent [Figure 10]. The flow of trypan blue through various renal cortex and medulla including the venules and smaller veins could be easily observed morphologically [Figure 11] through translucent decellularized kidney scaffold. We used ImageJ software and edge detection method to understand the fundamental fabrication or architecture of the kidney scaffold and their renal vasculature.

We further attempted to find out whether dye perfused kidney scaffolds gave better visualization of interlobular junctions and renal vasculature than without dye [Figure 11]. We again used the same method of ImageJ software. It was observed that trypan blue dye perfusion gave better visualization of renal and nephrological vasculature (details) through normal observation, as well as it provided us intact mode of ECM texture with preservation of renal and nephrological network organization along with glomerular opening in the cortical area [Figure 12] in comparison to without dye perfusion [Figure 10]. Finally, we compared the edge detection method (ImageJ) images for dye perfused and nondye perfused for observation of renal vasculature in scaffold capsule; it was clearly established that dye perfused scaffold showed very

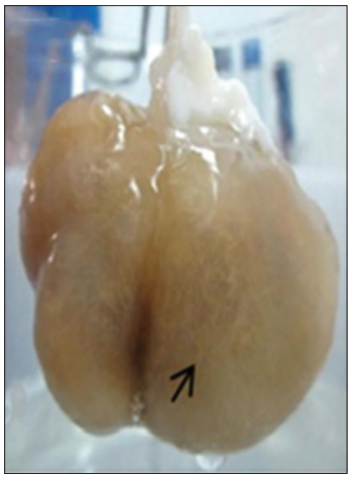

Figure 7: Translucent opaque & branches of venules, arterioles

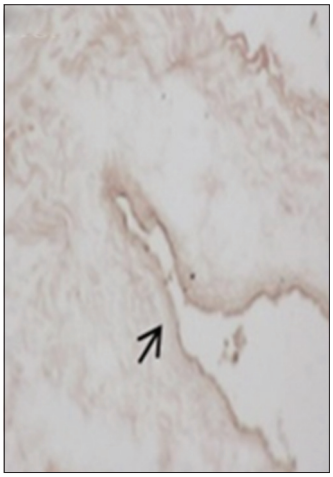

Figure 9: EVG staining

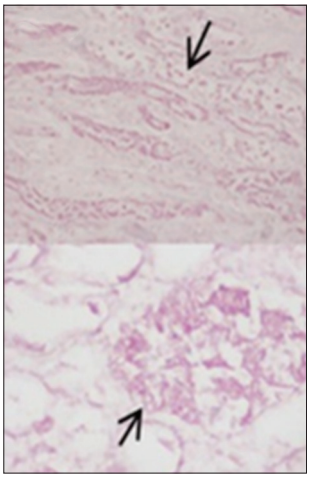

Figure 8: PCT, loop of Hensle, DCT and urine collecting tubule can be seen

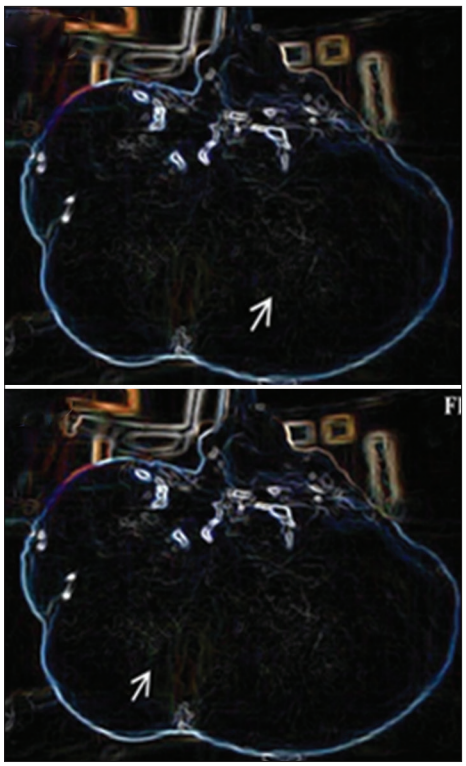

Figure 10: Image-J Kidney without dye perfusion

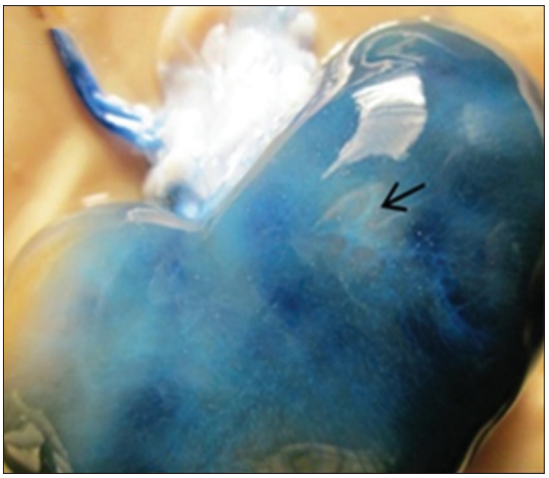

Figure 11: Trypan blue perfusion

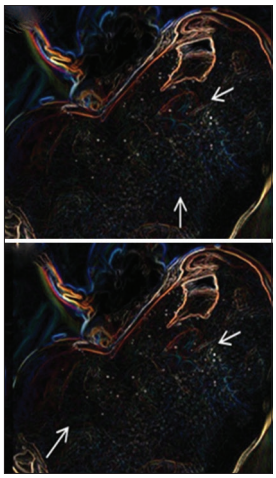

Figure 12: Trypan blue image J picture

minute details [Figure 11] in comparison to without dye perfusion [Figure 10].

Discussion

Kidney is the most important excretory and osmoregulatory organ of the mammalian system. It is extremely complex and has varieties of cells assembled in its tissue matrix architecture. Kidneys contain mainly cuboidal columnar epithelial cells in its phenotype. It has to perform many functions including urine formation and maintaining of salt and water balance in the body. Kidney region is divided into various areas such as cortex and medulla. The cortex region is composed of renal corpuscle while medullary region has distal convoluted tubule (DCT) and CTs, which further proceed to renal pelvis region. In our research study, we have developed a model of decellularized sheep kidney scaffolds by perfusion method using various chemical detergents at various time intervals. In our findings, it is clear that using 1% SDS along with combination of 1% SDS and 1% trypsin and 1% SDS and 1% EDTA leads to complete decellularization of kidney in 5 days under sterile condition.

Histological investigation about cellular architecture in relation to ECM provides exact cellular arrangement within the tissue. It clearly defines the type of cells, their behavior, and its phenotype when stained by specific dyes. Histological investigation by HE staining supports the process of decellularization of whole organ and is possible by perfusing of various chemical detergents such as SDS, trypsin, and EDTA. Histochemistry (HE staining) for histological structural details explains the dislodging and absence of cells in the cortex and medulla region of the kidney leaving behind the ECM scaffolds of renal corpuscle (glomerulus knots, BC, proximal convoluted tubule [PCT]) in the cortex area whereas loop of Henle and DCT in the medulla region. However, one of the limitations about sectioned and stained histological tissue is that it provides 2-D analysis under the microscope.

Some mosaic patches were seen after 12 h of 1% SDS in the entire kidney [Figure 4]. After 12 h of 1% SDS and 1% trypsin, perfusion details of kidney vasculature were seen through naked eyes. This clearly demarcates the process of decellularization. It was interesting to study the vasculature details of the decellularized kidney scaffold and this was done by perfusing a biological safe trypan blue dye routinely used for cell culture. Perfusion of 1% trypan blue dye through the renal artery in decellularized kidney scaffolds demonstrated the details of tree-like renal and nephrological vasculature of venules and arterioles [Figure 10]. After dye injection, details of renal artery, renal pelvis, and renal vein along with other interlobular structures such as arterioles and venules scaffolds were observed easily. ImageJ Software analysis revealed us the excellent renal vasculature construct after trypan blue perfusion [Figure 11] and clearly emphasized that renal structural and functional details along with ECM were preserved excellently after decellularization.

This model suggests that the kidney scaffolds models can be developed at *in vitro* condition by perfusing various cell dislodging chemical detergents and various pathology diseased models can be developed and can be studied in 3-D tissue-engineered kidney scaffolds and even analyzed by 3-D construction. The reason for selection of sheep kidney as a model for developing scaffolds was that sheep kidney is similar in structure and function as compared to native human kidney, irrespective of size and weight. Even pigs, rats, and other mammals have been tried for transplants and regeneration, but the chances of zoonotic infection and diseases in these animals are more in relation to sheep.

This method can generate a cost-effective 3-D tissue-engineered viable functioning kidney scaffolds in time-bound situations, and further, its structural and functional units such as renal and nephrological vasculature in the cortex and medulla regions can be visualized by ImageJ for 3-D construction.

Conclusion

This study concludes that kidney scaffold bioprosthesis can be developed by perfusion technique using combination of various chemicals at different time intervals and ImageJ software can be used to construct the 3-D ECM vasculature. This also confers an optimistic hope for autologous 3-D scaffold development for tissue engineering a kidney and their transplants to patients.

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Conflicts of interest

Nil.

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