Sonication-assisted Perfusion Decellularization of Whole Porcine Kidney

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***Abstract*—** Bioengineering of kidneys is a potential treatment option in addressing common problems such as incompatibility and shortage of donor organs. The first step in the kidney bioengineering process involves perfusion decellularization wherein the use of chemicals is considered the most preferred preparation method to date. However, the use of chemicals alone requires long treatment time and excessive chemical usage hence, in this study perfusion decellularization was enhanced by sonication treatment at varying sonication power (150, 200 and 250 W). Scaffolds produced were evaluated for cell removal and preservation of structural integrity. Results revealed that decellularization with sonication using 150 W, 200 W and 250 W required a treatment time of 24 h, 16 h, and 12 h respectively compared to the 28 h treatment time of decellularization without sonication. Cells were almost and completely removed as indicated by histological analysis. Meanwhile, preservation of renal structures such as glomerulus, tubules, and blood vessels were observed except for the kidney scaffolds produced from decellularization with 250 W sonication where minimal disruption of the glomerular basement membranes and thinning of blood vessels were observed. Overall, decellularization with 200 W of sonication power resulted in an acellular renal ECM scaffold and preserved ECM structure. It can also be concluded that the higher sonication power used, the shorter is the decellularization time needed to prepare a kidney scaffold thus reducing the amount of chemicals used.

***Keywords*—** porcine, kidney, perfusion decellularization, extracellular matrix (ECM), sonication

I. Introduction

Dialysis and kidney transplantation are currently the best available treatments for kidney failure. However, dialysis cannot replace the homeostatic and endocrine functions of the kidney while for transplantation, patients must tolerate the immunosuppressive medications for life to prevent organ rejection and the shortage of compatible donor organs are still the main problems to be solved. Thus, it is important to develop alternative strategies like the creation of transplantable bioengineered kidneys to address this problem.

A bioengineered kidney can be produced through decellularization - recellularization process. Decellularization of tissues and organs aims to remove cellular materials while preserving the native extracellular matrix (ECM) structure followed by recellularization with patient-specific cells to regenerate whole viable organs. Decellularization is classified into physical, chemical, and enzymatic methods [1]. Physical methods include lyophilization, repeated freeze-thawing, sonication, direct pressure, and agitation to disrupt and lyse the plasma membrane of the cells. In chemical methods, reagents such as solvents, detergents, and solutions are used to remove cellular components. Sodium dodecyl sulfate (SDS) is a chemical detergent that has the ability to lyse the cell membrane and its components while Triton X-100 serves as a cleaning detergent to remove toxic SDS residues. In enzymatic methods, proteases and nucleases are commonly used [2].

Perfusion decellularization is the most common method of decellularization. However, the use of chemical method alone requires long treatment time [3-6], repeated treatment cycles [7] and intensified washing steps [3, 8]. To solve this problem, the combination of physical and chemical methods is considered to shorten decellularization time and minimize chemical usage [9]. The reason is that the duration of treatment is reduced by the help of sonication with a continuous flow of chemical detergents. Sonication disrupts the nuclei and the cell membrane thus helping the chemical detergent efficiently remove cellular materials during perfusion decellularization. Sonication power has an effect on the efficiency of decellularization however a threshold sonication power must be observed not to adversely affect the structural integrity of the ECM.

Porcine kidneys will be decellularized in this study to produce a potential biological scaffold. Pig kidneys are a suitable model for renal studies as they share the same anatomy and functionality with that of a human kidney. Porcine kidney scaffold are better in characteristics as compared to rats [10] or monkeys [11] because it supports the attachment of cells which is an advantage to the recellularization process.

Previous studies confirmed the successful decellularization with the addition of sonication for tissues such as aorta [9, 12], skeletal muscle [13], larynx [14] and arteries [15]. No study thus far has provided results for whole kidney decellularization with the use of the combination of chemical perfusion and sonication. Hence, the aim of this study was to investigate the effect of sonication power (150 W, 200 W, and 250 W) on the perfusion decellularization of porcine kidney using Sodium dodecyl sulfate. Remaining cellular content and structural integrity were analyzed after the decellularization

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process.

II. MATERIALS AND METHODS

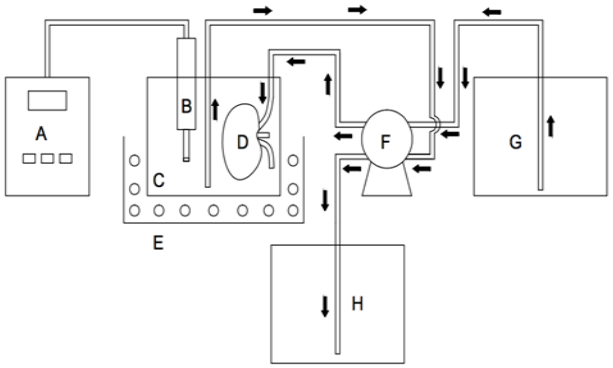

Fig. 1. Setup of the perfusion decellularization of porcine kidney with sonication.

A. Perfusion decellularization setup

The perfusion decellularization setup of porcine kidney with sonication consisted of three beakers (C, G, H) specifically for perfusion decellularization, chemical detergent, and waste solution, one peristaltic pump (F), cooler bath (E), sonicator (A) and sonication probe horn (B) as revealed in Fig. 1.

B. Procurement and preparation of porcine kidneys

Kidneys from female pigs were supplied by a local farmhouse. The porcine kidney was cannulated through its renal artery and then it was introduced to the perfusion decellularization system as shown in Fig. 1. Distilled water was introduced through the renal artery for 3-5 hours at a flow rate of 10 mL/min to remove the blood.

C. Sonication-assisted perfusion decellularization

The kidney was then sonicated following a cycle of two hours on and two hours off with a constant circulation of 1% Sodium dodecyl sulfate (SDS) at a flow rate of 10 mL/min. The cycle of sonication was repeated until the kidney turned white. The frequency was set at 20 kHz and the power was varied from 150 W, 200 W, and 250 W. During sonication, the cooling bath was used to maintain the solution temperature.

D. Washing of residual detergents

The decellularized kidney was then washed with 1% Triton X-100 for two hours at a flow rate of 10 mL/min and 1X Phosphate buffered saline (1XPBS) for three hours at a flow rate of 15 mL/min. The product scaffold was stored in 10% formalin for further analyses. A control was performed following the protocol for decellularization of porcine kidney but without sonication.

E. Histological and structural analyses

Samples from the cortical region of the native kidney and decellularized kidney were collected for analysis of cellular content removal and evaluation of ECM structure integrity using Hematoxylin and eosin (H&E) staining and scanning electron microscopy (SEM), respectively.

Fig. 2. Macroscopic evaluation of porcine kidneys before and after decellularization with varying sonication power applied and the control. The kidneys gradually turned translucent white with the progression of decellularization.

III. RESULTS AND DISCUSSION

Decellularized scaffold was successfully produced during the process as indicated by the change in its brown native color to translucent white. The change in color from brown to white provides evidence that the scaffold is already devoid of its cellular components. Fig. 2 shows the native kidney losing its natural color after decellularization with respect to different sonication powers applied.

Table 1. Duration and amount of chemical required for decellularization of porcine kidneys

Protocol	Time	1%SDS
No sonication	28 hours	17.4 L
150 W	24 hours	14.9 L
200 W	16 hours	10.1 L
250 W	12 hours	7.7 L

The control (decellularization without sonication) required 28 hours with 17.3 L of 1%SDS to completely remove the

cellular materials. Meanwhile, it only took 24 hours with 14.9 L of 1%SDS, 16 hours with 10.1 L of 1%SDS and 12 hours with 7.7 L of 1% SDS for decellularization with sonication power of 150 W , 200 W , and 250 W respectively to decellularize the kidney (Table 1). The efficient removal of cellular components might be because of the cavitation bubbles formed during sonication which destroys the cellular membrane and components which helps in the penetration of SDS detergent. In addition, the number of cavitation bubbles increases with increasing sonication power thus leading to decreased decellularization time [16-19].

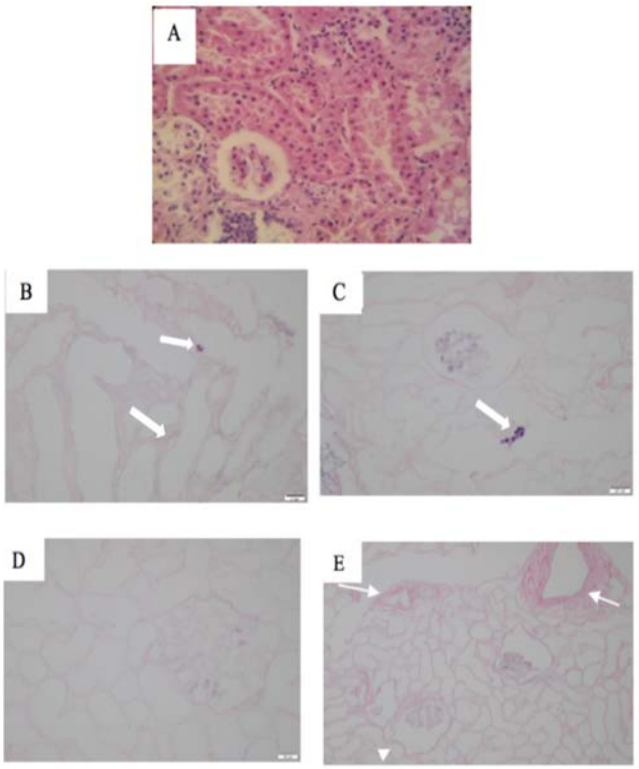

Fig. 3. H&E staining result of the native kidney (A), decellularized kidney with no sonication applied (B), decellularized kidney with 150 W sonication (C), decellularized kidney with 200 W sonication (D), and decellularized kidney with 250 W sonication (E).

Fig. 3 presents the Hematoxylin and eosin (H&E) stained images of native and decellularized kidneys. Percentage of cellular removal in the native kidney, control (without sonication), 150 W, 200 W, and 250 W were 0%, 99%, 99%, 100% and 100% respectively as scored and evaluated by a pathologist.

Aside from the removal of cellular content, the preservation of various renal structures can also be observed by H&E staining. The native ECM structure including glomerulus, tubular structure, and blood vessels were preserved in the control, 150 W, and 200 W. Meanwhile, in 250 W, minimal damage can be observed due to the thinning of blood vessels and minimal disruption of glomerular basement membranes. This might be because sonication enhanced the penetration of the detergent and the lysis of the cell membrane however, at a high setting or power, can destroy the integrity of the ECM [21-23].

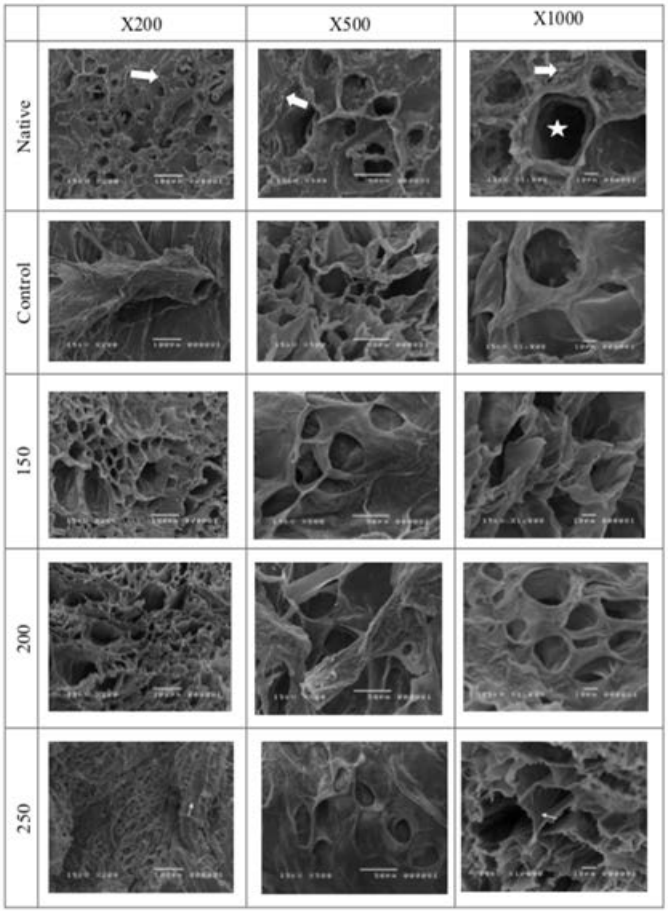

Fig. 4. SEM images of the native kidney (A), decellularized kidney with no sonication applied (B), decellularized kidney with 150 W sonication (C), decellularized kidney with 200 W sonication (D), and decellularized kidney with 250 W sonication (E).

The ECM structure of native and decellularized kidney was evaluated using Scanning electron microscope as presented in Fig. 4. The thick white arrows point at the cellular components, thin white arrows show the damage observed and the white star indicates the tubular structure at varying magnification (X200, X500, and X1000). Cells were found in the SEM image of the native kidney while these are absent in the decellularized kidney with no sonication applied and with sonication at 150 W , 200 W, and 250 W. In addition, the ultrastructure of the decellularized kidney without sonication and with sonication at 150 W and 200 W closely resembles that of the native kidney. On the contrary, holes were observed on the ECM structure of the decellularized kidney with 250 W sonication. The high acoustic cavitation of sonication produced causes strong mechanical stress which disrupts the ECM structure [13, 15, 20].

IV. CONCLUSION

The current study showed the successful decellularization of whole porcine kidneys with the addition of sonication. With 200 W of sonication power, the process was significantly enhanced. The resulting renal ECM scaffolds appear acellular and the native three-dimensional ECM structure was retained. Sonication can be used to enhance decellularization, however it must be noted that increased sonication power could offer minimal to adverse damage to the scaffolds. The results revealed in the study are quite encouraging, and this method might be considered a useful foundation for future decellularization procedures especially with the addition of sonication to the process. However, further studies need to be performed to evaluate more on the characteristics of the decellularized kidney that were prepared with the addition of sonication treatment to the decellularization process such as retention of structural proteins and growth factors. Overall, this study shows that the ultimate goal of providing a feasible and reliable therapy for kidney diseases through the bioengineering of kidneys is possible.

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