Effect of sonication power on perfusion decellularization of cadaveric porcine kidney

Sreypich Say[1], *Nathaniel* Dugos[1*], *Susan* Roces[1] and *John Martin* Mondragon[1]

[1]Department of Chemical Engineering, Gokongwei College of Engineering, De La Salle University, 2401 Taft Avenue, Manila 1004, Philippines

Abstract. Kidney problems rank 7th among the top 10 causes of mortality among Filipinos. One of the potential future treatment options is the use of bioengineered kidney. The preparation of scaffolds is the first step in kidney bioengineering and perfusion decellularization using chemicals is considered the most preferred preparation method to date. However, the use of chemicals alone requires long treatment time hence, in this study perfusion decellularization is to be enhanced by sonication treatment at varying sonication power. Decellularization was carried out by perfusing the kidney with 1% SDS and was subjected to sonication treatment with a 2h sonication and 2h rest cycle. The cycle is repeated until the kidney is clear and transparent. Washing using 1% Triton X-100 and 1x PBS then follows to remove residual SDS. The extent of cell removal was determined by H&E staining. The results showed that decellularization with sonication using 150W, 200W and 250W required a treatment time of 24h, 16h and 12h respectively compared to the 28h treatment time of decellularization without sonication. The result clearly shows that with higher sonication power, the shorter is the decellularization time needed to prepare a good kidney scaffold.

1 Introduction

End-stage renal disease (ESRD) is a rising medical concern worldwide. According to the National Kidney and Transplant Institute (NKTI) in 2017, kidney disease ranks 7th among other diseases leading to death among Filipinos [**17**]. The most common treatments are dialysis and transplantation but these are very costly and offers disadvantages. In the case of dialysis, it needs to be performed regularly [**20**] while shortage of organs available and the incompatibility of the kidneys are notable in transplantation treatments [**9**].

Tissue engineering and regenerative medicine provides a novel technique to solve ESRD by engineering a new kidney that is specific for each patient to avoid immunorejection and limited organ supply [**7, 19**]. The technique is composed of two stages which are the decellularization and recellularization process. Decellularization process is a technique used to remove the cellular content from the donor organ to minimize immunorejection [**19,23**]. Meanwhile, recellularization is the process of repopulating the decellularized organ (scaffold) with patient stem cells [**7, 24**].

Decellularization may be done by chemical, physical and enzymatic methods [**18**]. Perfusion is the most common and preferred chemical method. Sodium dodecyl sulfate (SDS) was reported to be an effective chemical to remove cellular components. On the other hand, physical methods include sonication, freezing and agitation [**12**]. Enzymatic method makes use of Trypsin, Deoxyribonuclease (DNase) and Ribonuclease (RNase) [**8**]. The successfully-prepared scaffold has to follow certain requirements such as preserved extracellular matrix (ECM), and completely or near completely removed cellular materials [**6**].

Decellularization of porcine kidney using chemical method alone requires a long time to remove cells, repeated treatment cycles and washing procedure [**10, 13, 15, 21,25**]. To address this problem, the combination of chemical and physical method (sonication) was used in the study. This is due to the formation of cavitation bubbles during sonication that aids in the penetration of the chemical detergent by denaturing the cellular membrane resulting in the faster removal of cellular debris. Suitable sonication power is needed because too high sonication power might adversely damage the ECM structure while too low sonication power will not aid in cellular removal [**2-4, 12,14, 22,23**].

The objective of this study was to investigate the effect of sonication power (150W, 200W and 250W) on the decellularization of porcine kidney using Sodium dodecyl sulfate (SDS) with sonication. Remaining cellular content was analyzed after the decellularization process.

2 Materials and methods

2.1 Perfusion decellularization system

The perfusion decellularization of porcine kidney with sonication consisted of three beakers (C, G, H)

* Corresponding author: nathaniel.dugos@dlsu.edu.ph

specifically for perfusion decellularization, chemical detergent, and waste solution, one peristatic pump (F), cooler bath (E), sonicator (A) and sonication probe horn (B) (See **Fig. 1**).

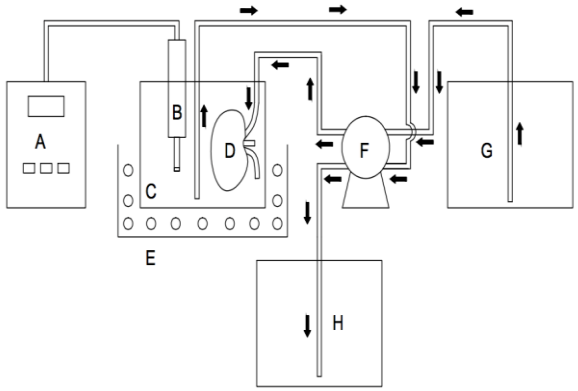

Fig. 1. Perfusion decellularization of porcine kidney with sonication setup.

Porcine female kidneys were supplied by Foremost Farms, Incorporated, Philippines. The porcine kidney was cannulated through its renal artery and then it was introduced to the perfusion decellularization system as shown in Figure 1. Distilled water was introduced through the renal artery for 3-5 hours at flow rate of 10mL/min to remove blood. Kidney is then sonicated with two hours on and two hours off cycle with a constant circulation of 1% Sodium dodecyl sulfate (SDS) at a flow rate of 10mL/min. The cycle of sonication was repeated until the kidney turned white. The frequency was set at 20kHz and the power was varied from 150W, 200W and 250W. During sonication, the cooler bath was used to maintain the solution temperature. It was then washed with 1% Triton X-100 for two hours at a flow rate of 10mL/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 analysis. A control was performed following the protocol for decellularization of porcine kidney but without sonication.

Cellular content was analyzed using Haematoxylin and eosin (H&E) staining.

3 Results and discussion

Decellularized scaffold was succesfully produced during the process as evident by the change in brown native color to white or transparent. **Fig. 2** shows the native kidney lost its native cellular color after decellularization with respect to different sonication powers applied. The control (decellularization with 1% SDS alone) set-up required 28 hours to completely remove cellular material. Meanwhile, it only took 24 hours, 16 hours and 12 hours for decellularization with sonication power of 150W, 200W and 250W respectively to decellularize the kidney. The faster removal of cellular material might be because of the cavitation bubbles formed during sonication which destroys cellular membrane and cellular components thus helping in the penetration of SDS. In addition, the number of cavitation bubbles increased with increasing sonication power thus leading to decreased decellularization time [**1,5,11, 16**].

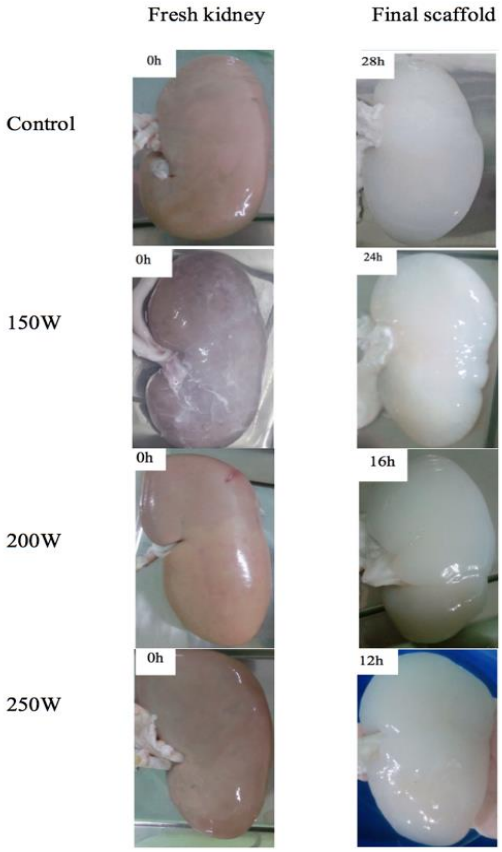

Fig. 2. Macroscopic evaluation of porcine kidneys before and after decellularization with varying sonication power applied and the control. The kidneys gradually turned translucent white with the progression of the decellularization process.

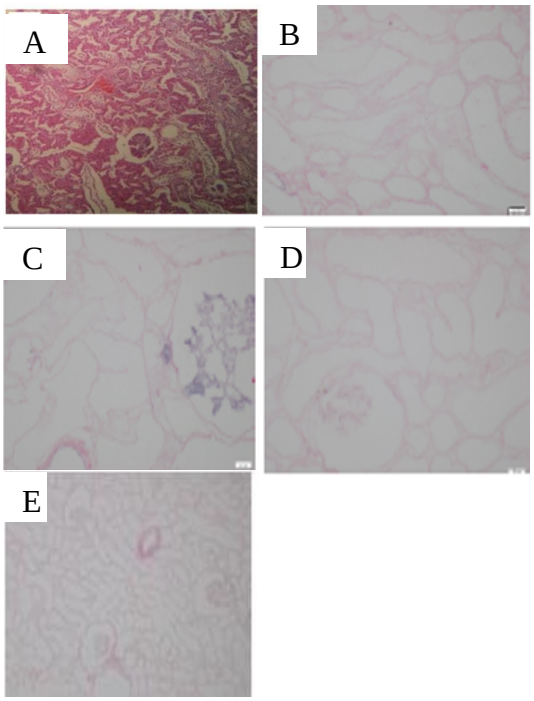

Fig. 3. Histological analysis of native (A), control (B), 150W(C), 200W(D) and 250W (E) through H&E staining. The glomerular structure, tubular organization and the nucleus are clear.

Fig. 3 shows the Hematoxylin and eosin (H&E) stained images of native and decellularized kidneys. Percentage of cellular removal of native kidney, control (without sonication), 150W, 200W and 250W 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, 150W and 200W. Meanwhile in 250W, minimal damage can be observed due to the thinning of blood vessels and minimal disruption of glomerular basement membranes.

4 Conclusion

The results of the current study showed the successful decellularization of porcine kidneys with the aid of sonication. The resulting renal ECM scaffolds maintained their structure and appears devoid of cells. The best sonication power setting to achieve both 100% cellular removal and preservation of the ECM structure in the renal scaffold is 200W. This indicates that the combination of perfusion of 1% SDS with 200W of sonication power in decellularization procedure could be of good choice for future studies due to its efficiency in cell removal while maintaining the native-like state of the ECM structure and with shorter decellularization time. In conclusion, sonication can be used to prepare decellularized scaffolds with the least amount of time. However, further studies need to be conducted to fully understand the effects of sonication in decellularization of whole organs such as the kidney.

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