Investigation of Hemocompatibility and Cytotoxicity of Decellularized Caprine pericardium for Tissue Engineering Applications

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The pericardium from xenogeneic source is a widely used biomaterial for tissue engineering applications. This study focuses on the hemocompatibility and in-vitro cytotoxicity of the decellularized caprine pericardium. The caprine pericardium was decellularized by sodium dodecyl sulfate (SDS) and Triton X-100. Hemolysis and plasma clotting assay was performed to understand the interaction of decellularized pericardium and whole blood. In-vitro cytotoxicity assay was performed with MG-63 cells to determine the wash-out residues present in the acellular matrix after the decellularization steps. Isolated platelet-poor plasma was incubated with an acellular matrix, and then $CaCl_2$ was added to initiate the plasma recalcification. The treatment with the combination of SDS and Triton X-100 resulted in an intact decellularized matrix and the absence of cell nuclei was confirmed through histological examination. The hemolysis rate of a decellularized matrix is found to be 2.1% and the matrix met the clinically acceptable implantation limit. The plasma recalcification time of the decellularized matrix is 244 seconds. Incorporating the washing steps in the decellularization process reduces the cytotoxic effect significantly.

Keywords: *Biomaterial, Decellularization, Extracellular matrix, Cytotoxicity*

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Introduction

- Pericardium derived from bovine and porcine is widely used for the design and fabrication of tissue-engineered bioprosthesis
- Glutaraldehyde is extensively used for the fixation and sterilization of tissue-based prostheses. Despite inactivation and capping immunological response, glutaraldehyde treatment has been associated with post-implant complications like calcification of bioprosthetic tissues
- Although developing scaffolds from caprine tissue is a safer option and reduce the risk of transmission of disease to human beings, caprine-derived biomaterials are less explored
- In vitro and in vivo studies are essential of any biomedical implants to determine the cytocompatibility and hemocompatibility

Work flow chart

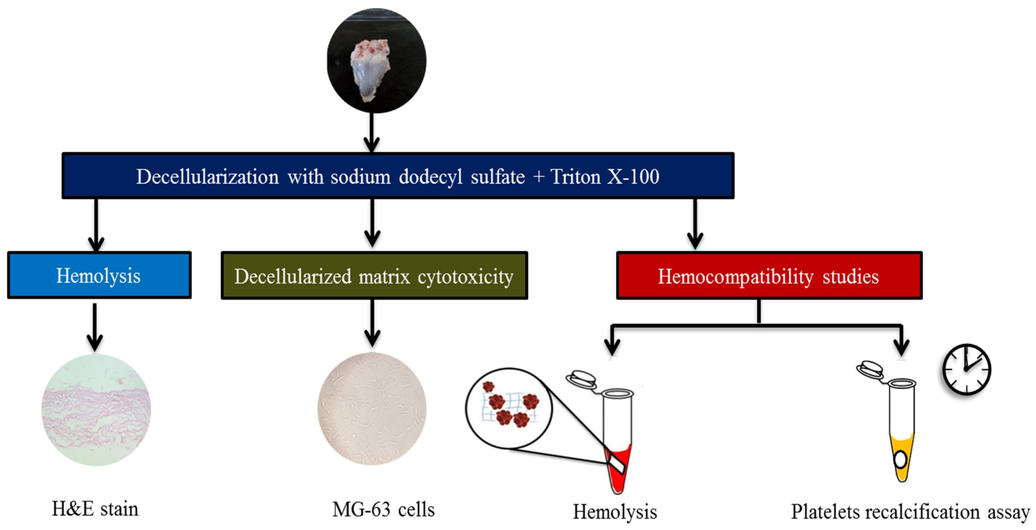

Figure 1. Work flow chart for the decellularized caprine pericardium for in vitro cytotoxicity and hemocompatibility study

Materials and methods

Decellularization

- Caprine pericardium decellularization was performed with the combination of sodium dodecyl sulfate (SDS) and Triton X-100

Histology

- Formalin-fixed paraffin-embedded tissue sections were stained by standard Hematoxylin–Eosin (H&E). In H&E staining, extracellular matrix appears pink in color, and cell nuclei appear blue

In vitro cytotoxicity

- The decellularized pericardium was immersed in a culture medium overnight at 4˚C in an orbital shaker, and the final washout medium of the decellularized matrix was tested against the MG-63 cell lines
- The MG-63 cell were seeded on 96 well plates at 30,000 cells/cm^2 overnight. Then the culture medium was replaced with a pre-warmed washout medium for 2 hr at 37 ˚C in a 5% CO_2 incubator. The cells were washed with PBS, and MTT assay was performed

Hemocompatibility study

- For hemolysis assay, the decellularized and native pericardium were incubated in the anticoagulated blood and after 1 hr at 37 ˚C. The mixture was centrifuged at 1000 rpm for 10 min after the incubation time, and at 545 nm, the absorbance of the supernatant was measured
- A_T is the absorbance of the diluted blood; A_N is the absorbance of PBS as negative control and A_P is the absorbance of 0.1 M of hydrochloric acid in diluted blood as a positive control

$$Hemolysis = \frac{[A_T - A_N]}{[A_P - A_N]} \times 100\%$$

- The platelet-poor plasma (PPP) isolated from whole blood and incubated with decellularized (n=6) and native (n=6) pericardium for 1 min at 37 ˚C. Then, plasma recalcification time was initiated by adding 0.25 M $CaCl_2$, and the time taken by the pericardium to clot is noted. For control, 0.9 % NaCl was used.

Result

Histology

The treatment with the combination of SDS and Triton X-100 resulted in an intact decellularized matrix and the absence of cell nuclei was confirmed through histological examination.

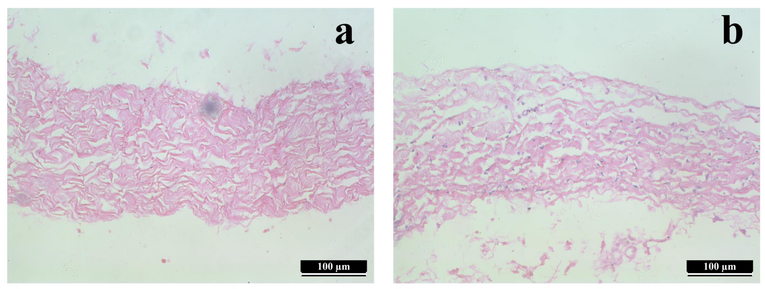

Figure 2. Images of decellularized and native tissue stained with H&E **(a)** SDS and Triton X-100; **(b)** Native pericardium; Magnification 400X, Scale bar- 100 µm

In vitro cytocompatibility

By incorporating the washing steps in the decellularization process reduces the cytotoxic effect in the decellularized matrix on the MG-63 cells

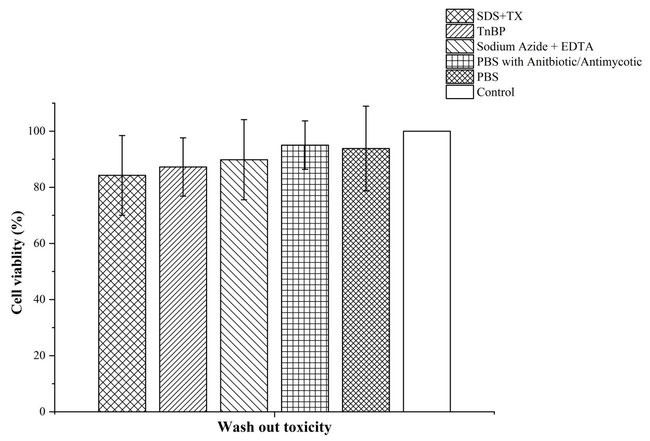

Figure 3. Wash out toxicity of decellularized matrix from each steps of decellularization protocols was tested on MG-63 cells

Hemocompatibility study

Hemolysis: The hemolysis rate of a decellularized matrix is found to be 2.1 % and the matrix met the clinically acceptable implantation limit

Platelet Poor Plasma: The plasma recalcification time of the decellularized matrix is 244 seconds which is similar to normal recalcification time

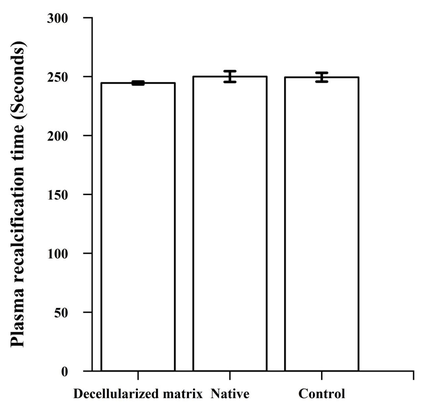

Table 1. Hemolysis percentage obtained for decellularized and native pericardium

Samples	OD at 540 nm	Hemolysis (%)
Decellularized	0.078 ± 0.017	2.16 ± 1.41
Native	0.093 ± 0.004	3.5 ± 0.45
Positive control	1.161	100
Negative control	0.054	-

Figure 4. Plasma recalcification time of decellularized, native and control samples

Conclusion

This research work demonstrated the matrix decellularized from caprine pericardium have hemocompatibility and biocompatibility with the in vitro cell culture model

References

Guo, G., Jin, L., Jin, W., Chen, L., Lei, Y., & Wang, Y. (2018). Radical polymerization-crosslinking method for improving extracellular matrix stability in bioprosthetic heart valves with reduced potential for calcification and inflammatory response. Acta biomaterialia, 82, 44-55