Optimization of Pericardium Decellularization Method for Tissue Engineering Applications

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Abstract

Pericardium tissue is widely used for the development of pericardial patches, and bioprosthetic heart valves. The purpose of this study is to understand the impact of chemical-based decellularization protocols on the microstructure and biomechanical properties of the caprine pericardium. In order to achieve the objective, two different decellularization procedures was designed, first based on anionic detergent (protocol A) and the second with combinations of anionic detergent and non-ionic surfactants (protocol B). The effectiveness of decellularization procedure was evaluated by histological analysis. Further, uniaxial experiments were performed in the decellularized pericardium tissue and compared the response with native tissue. This study shows that the treatment of caprine pericardium tissue with the combination of anionic detergent and non-ionic surfactants helps to obtained decellularized tissue with intact extracellular matrix.

Introduction

- The pericardium scaffold from the xenogeneic origin contains viable cells that induce humoral immune responses [1]
- To minimize the complications associated with the xenogeneic pericardium, the tissue specimens are treatment with glutaraldehyde before clinical applications
- The glutaraldehyde fixation process causes significant changes in the biomechanical behavior of the pericardium tissue and also induces calcifications and immunological response [2]
- The purpose of the decellularization approach is to remove cells from the pericardium tissue and thereby cap immune response
- Sodium dodecyl sulfate (SDS), Triton X-100, sodium deoxycholate and tri-n-butyl phosphate are chemicals typically used for decellularization of the pericardium tissue

Materials and Methods

- Caprine hearts (n=6) with pericardium sac were obtained from a local abattoir and pericardium were isolated
- The two different decellularization procedures was designed, first based on anionic detergent (protocol A) and the second with combinations of anionic detergents and non-ionic surfactants (protocol B)
- The anionic detergent used in this study is SDS and non-ionic surfactant used is Triton X-100
- The decellularized tissue were sectioned and stained with hematoxylin & eosin (H&E) in order to differentiate cell nuclei and extracellular matrix
- To investigate the biomechanical behavior of decellularized pericardium tissue, uniaxial tensile tests were performed

Work flow of decellularization process

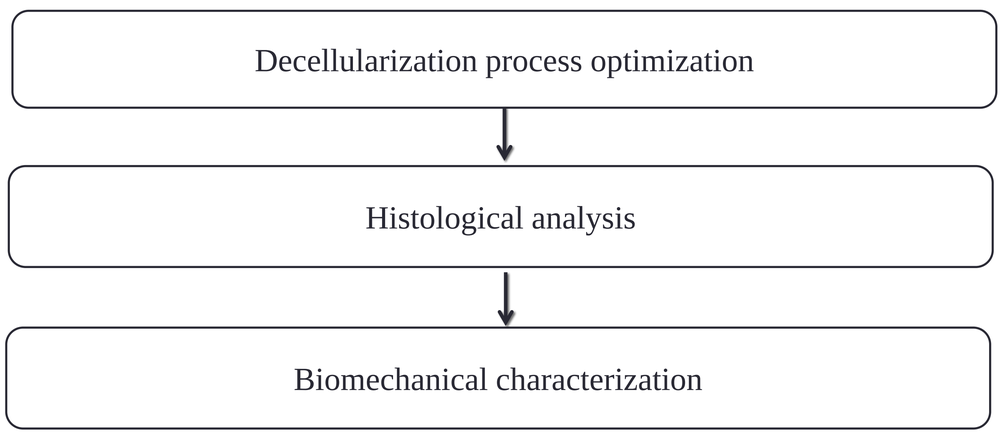

Figure 1: Work flow of decellularization process

Process optimization

Figure 2: Process optimization

Results and Discussion

- **Histological analysis**

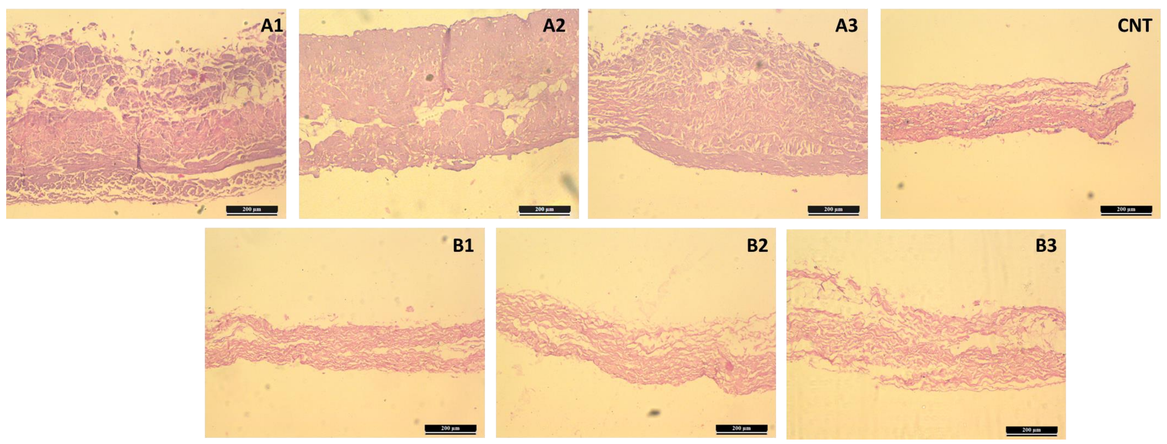

Figure 3: Images of hematoxylin & eosin stained pericardium tissues. A1-A3 (SDS 1, 0.5, 0.25%), CNT (native), B1-B3 (SDS 0.5% and Triton X-100 0.5%, SDS 0.5% and Triton X-100 0.25%, SDS 0.25% and Triton X-100 0.5%). Scale bars: 200µm. The H&E staining shows complete absence of cell nuclei in decellularized pericardium tissue .

- **Biomechanical characterization**

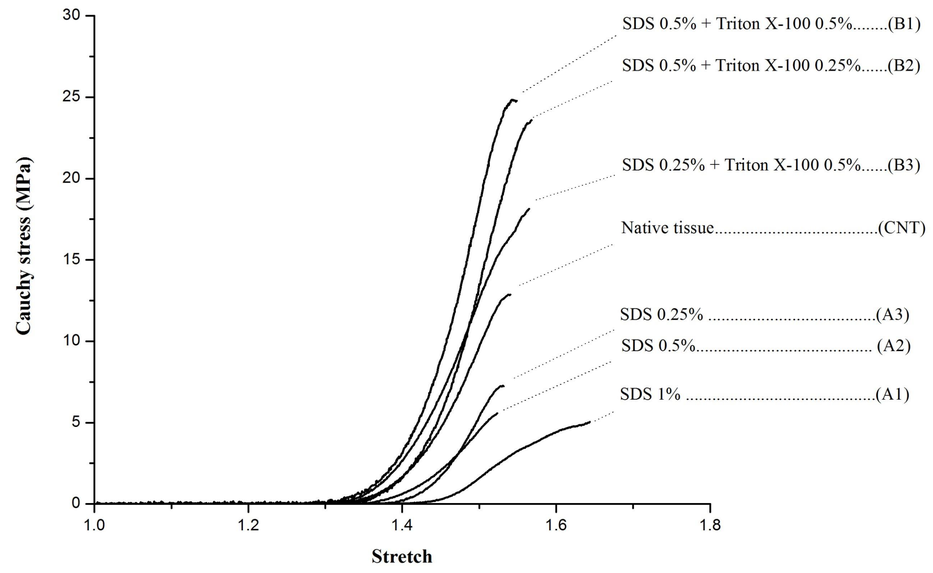

Figure 4: Biomechanical response of representative native and decellularized pericardium tissue.

- Histological images indicate both the protocols A and B removes the mesothelial cells from the pericardium tissue
- In group A protocol, after treatment with various concentration of SDS, there is increase in thickness of the pericardium tissue
- From B1 and B2 protocol, we observed an intact pericardium architecture with complete removal of cells. Whereas, microarchitectural damage was observed in B3 protocol
- The stiffness of the pericardium tissue increased after treatment with the combination of SDS and Triton X-100 and decrease in stiffness were observed in pericardium tissue treated with SDS

Conclusion

- The decellularization process completely removes the mesothelial cells from the pericardium tissue
- Among the two different decellularization protocols, combination of SDS and Triton X-100 (B1 and B2) shows better decellularization with less ECM damage
- These decellularization approach can be further used for the construction of bioprosthetic heart valves and other biomedical application

Acknowledgements

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References

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