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Supercritical Carbon Dioxide Sterilised Decellularised Porcine Pulmonary Heart Valves: Biological and Biomechanical Characterisation.

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Introduction

Decellularised pulmonary valve roots are a promising regenerative solution for reconstruction of the right ventricular outflow tract in young patients. Application of a robust terminal sterilisation process would enhance the sterility assurance level, improve logistics, and expedite clinical translation. Supercritical carbon dioxide ($ScCO_2$), combined with oxidizers, may provide the ideal sterilisation conditions for decellularised biological scaffolds[1,2]. The aim of this study was to compare the biological and biomechanical properties of decellularised pulmonary valve roots (dPHV), with dPHV exposed to $ScCO_2$ sterilisation cycles.

Methods

Low concentration detergent decellularised porcine pulmonary valves (n=6 in each group) were stored in phosphate buffered saline (PBS controls), exposed to $ScCO_2$ cycles whilst immersed in PBS ($ScCO_2$-wet), or exposed to $ScCO_2$ cycles without immersion ($ScCO_2$-dry). Histological analysis of the samples included Haematoxylin and Eosin, Picrosirius Red-Millers, and Movat's Pentachrome. Presence of collagen type IV and fibronectin was detected by immunohistochemistry. Uniaxial tensile testing was used to determine the dPHV wall and leaflet material biomechanical properties, in the radial and circumferential direction, differential scanning calorimetry (DSC) to determine thermal stability of collagen, and colorimetric assays to quantify collagen and GAG content.

Results

Other than compression of the $ScCO_2$-dry samples, there were no differences observed within the histoarchitecture and microscopic structures of $ScCO_2$-wet and $ScCO_2$-dry groups, or collagen and GAG content, in comparison to the dPHV controls. There was a significant reduction in the thermal stability of the $ScCO_2$-wet and $ScCO_2$-dry treated dPHVs, and a significant increase in the ultimate tensile strength of $ScCO_2$-dry leaflets (radial direction). Significant increases in the elastin phase slope were determined in the circumferential direction of ScCO2-dry (wall and leaflet) and $ScCO_2$-wet (wall only), as well as in the axial direction of $ScCO_2$-dry (wall and leaflet) and $ScCO_2$-wet (leaflet only).

Conclusions

With further process optimisation, $ScCO_2$-wet remains a promising sterilisation option for decellularised pulmonary valve roots. The role of the oxidising additives in the detected thermal stability and material property changes requires further investigation, as does the capacity for cellular repopulation.

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