IMPACT OF SUPERCRITICAL CARBON DIOXIDE AS A DECELLULARIZATION AGENT FOR AORTIC TISSUE

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Introduction

Valvular heart disease is growing globally due to an increase in the aging population, leading to more valve replacement surgeries.[1] The use of both mechanical and biological prothesis have reported risks (drawbacks) [2,3], thus new devices aimed at restoring normal tissue function through bioengineered matrices are essential. Scaffolds deriving from decellularized tissues have been used with varying degrees of success for human applications. However, most current protocols are long and use harsh chemicals with a negative impact on the extracellular matrix (ECM) mechanical performance and bioactivity [4]. This work aims to study the potential of supercritical carbon dioxide (scCO2) decellularization to preserve the original aortic tissue bifunctionality, in a faster and more efficient manner.

Methods

Experiments were performed on aortas of 6-months pigs, frozen after collection. The cellular materials were removed from tissues by scCO2 decellularization process. Samples were tested to evaluate the decellularized ECM via hematoxylin–eosin (H&E) staining and DNA quantification. The mechanical properties were analysed via uniaxial tension testing using a texturometer. The samples' structure and morphology were characterized using SEM and histological analysis. Biocompatibility are ongoing by direct contact assays using Human Dermal Fibroblasts.

Results

The sc-CO_2 decellularization protocol to produce a decellularized matrix was first optimized and validated through H&E staining and DNA quantification (Fig 1a, b, c). Among the several protocols tested, the tissue resulting from a combination of batch followed by semi-continues sc-CO_2, was able to produce the desired structures, with increased ultimate tensile strength and young's modulus of aortic valve while the elongation at break was inferior, when compared to untreated sample (Fig 1i).

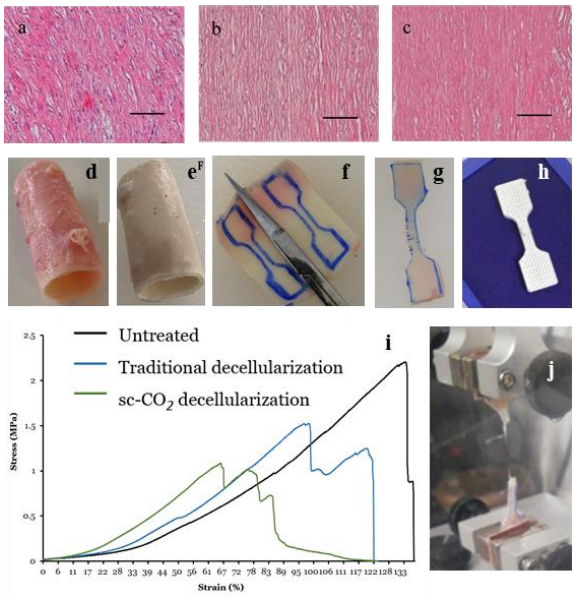

Figure 1. H&E staining of Aorta Samples: (a) Untreated; (b) traditional treatment (c) and sc-CO2 treated (Scale bar 100 µm). Preparation of aorta for uniaxial testing: (d) untreated specimen; (e) scCO2 treated; (f) longitudinally cut with stamped template;(g) cut dumbbell sample (h) with the 3D printed mold. (i) Stress-strain profile of aorta samples. (j) resulting fractured tissue specimen

Discussion

Supercritical fluid decellularization was able to completely remove cellular material that was embedded within the aorta matrix, with a more compact and rigid structure than untreated specimens and less degraded fibers than traditional treated aortas.

References

1. Clark, M. A. et al, *Circ. Cardiovasc. Qual. Outcomes* 5:697–704 (2012).
2. Yount, K. W. et al, *J. Card. Surg*. 37:1224–1229 (2022).
3. Maganti, K., et al. *Mayo Clin. Proc*. 85:483 (2010).
4. Duarte, M. M. *et al. Mater. horizons* **9**, 864–891 (2022).

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