ASSESSING THE PERFORMANCE OF DECELLULARIZATION OF HUMAN TISSUES THROUGH MECHANICAL EVALUATION

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Introduction

Xenografts and allografts are usually crosslinked with glutaraldehyde, which implies cytotoxicity and tissue calcification [1]. An alternative preparation is the decellularization, which limits antigenicity while preserving the integrity of the extracellular matrix structure and thus its mechanical properties [2]. The aim of the present study is the evaluation of the efficacy of new decellularization protocols for human dermis and pericardium in preserving mechanical properties.

Methods

Human tissues were obtained from cadaver donors following Italian directives and with the proper informed consent. Two testing protocols were developed to evaluate the mechanical performance of the tissues, taking into account the availability of material from which to take samples in the two districts (which is much lower for the pericardium). Therefore, the dermis was uniaxially stretched until failure along multiple directions of sampling (size of specimens: 40×5 mm^2), while the pericardium was equibiaxially stretched up to 20% strain on cruciform specimens cut from 20×20 mm^2 patches using a custom tool. The thickness of the tissues was determined as the mean of three measurement taken with a thickness gauge (547–321, Mitutoyo, Lainate, Italy) in three points in the central region of each specimen. Uniaxial tensile tests permitted to evaluate the ultimate tensile strength (UTS), strain at break (ε_{UTS}) and elastic moduli in the toe region (E_{toe}) and in the linear region (E_{lin}) of human decellularized (hDD) versus non-treated dermis (control, hCD). Biaxial tensile tests were conducted to simultaneously explore the decellularization treatment effect – comparing human decellularized (hDP) and control (hCP) pericardium patches – and the tissue anisotropy. The E_{lin} was computed in the two directions. Being the direction of the fibers that compose the extracellular matrix unknown, the loading directions presenting the lowest and highest elastic moduli of each specimen were evaluated separately and will be referred as to D1 and D2 in the following. Three control patches and three decellularized patches obtained from three different donors were analyzed for each tissue.

Results

Despite the high inter-specimen variability, the hDD resulted significantly stiffer (Figure 1c) and more resistant in terms of UTS (Figure 1a) compared to the control tissue (p<0.05). Moreover, the ε_{UTS} decreased after decellularization (p<0.05, Figure 1a). The aforementioned properties obtained from the three dermis donors were significantly different (p<0.05). The results obtained from a single donor are shown in Figure 1a and 1c. In contrast, from biaxial tests, no significant differences were highlighted between hDP and hCP, neither along D1 nor along D2, and similar properties were obtained for all the donors (Figure 1b and 1d).

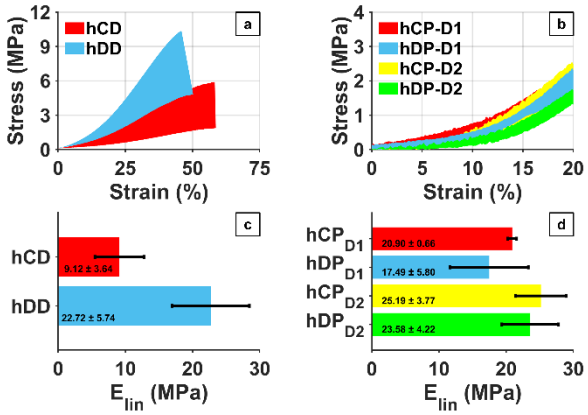

Figure 1: Dispersion of the stress vs. strain curves of human dermis (a) and pericardium (b); E_{lin} of dermis (c) and pericardium (d): treatment vs. control (mean ± SD).

Discussion

Previous findings confirmed the possibility of an increase of E_{lin} and UTS in decellularized tissue compared to native one [3]. Moreover, the dispersion of the results on hDD is very close to the variability range of the UTS and E_{lin} obtained from uniaxial tensile tests on AlloDerm®, a widely used acellular dermal matrix for soft tissue applications [4]. Inter-donor variability was expected considering the well-known variability in soft tissues. Nevertheless, the differences that emerged between hDD and hCD could be ascribed to the lack of control in the harvesting orientation and to the variability of fibers direction in the donors' back. On the other hand, although no differences were highlighted between hDP and hCP, the results might have been affected by the tissue anisotropy. Therefore, further studies should be conducted investigating the direction of the fibers in the extracellular matrix prior to testing.

References

1. Ma et al, Regen Biomater, 1(1):81–89, 2014
2. Montagner et al, Cell Tissue Bank, 2023
3. Bondioli et al, J Tissue Eng Regen Med, 8(4):325-336, 2014
4. Bottino et al, Dent Mater, 25(9), 1109–1115, 2009