Inter-species Variation in the Decellularization of Aortic Valves

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Statement of Purpose: Due to reduced immunogenicity and excellent hemodynamic function, decellularized valves hold great potential as valve substitutes and as a scaffold for the tissue engineered heart valve. However, regulatory guidelines require pre-clinical evaluation of candidate valve substitutes in the ovine model. This is problematic for strategies in which the ultimate product is human tissue, due to the species mismatch between the candidate valve and recipient animal. Pre-clinical evaluations in sheep necessitate the use of allogeneic valves. Therefore, it is critical to evaluate the efficacy of a given decellularization (decell) process for both ovine and human tissue. The purpose of this present study was to investigate the mechanical and biochemical effects of decell on the human valve leaflet and elucidate any differences in decell in the ovine model.

Methods: Cryopreserved (cryo) human aortic valves, with research authorization and not usable clinically, were obtained from a commercial tissue bank, and ovine aortic valves were obtained under approved IACUC protocols. Valves were decellularized using a multi-detergent, endonuclease, and osmotic shock protocol. The process was identical for human and ovine valves and automated to eliminate operator induced variability. The quasi-static mechanical behavior and the stress relaxation behavior of leaflet samples were evaluated under biaxial loading conditions as described previously[1]. Human (n = 9) and ovine samples (n = 9) were loaded to equibiaxial tensions 60 and 30 N/m, respectively. Leaflet morphology (H&E staining) and double stranded DNA (dsDNA) was analyzed to confirm decell. Concentrations of elastin, collagen, and sulfated glycosominoglycans (sGAG) within the leaflet were measured colorimetrically (n = 8-9). Data evaluated using parametric (Student's t-test) and non-parametric (Mann-Whitney) statistical methods.

Results: Histologic acellularity (not shown) and an absence of detectable dsDNA confirmed adequate decell (Table 1). A significant reduction in elastin content was observed in the human leaflets following decell, but not in the ovine leaflets (Table 1). Both species presented a significant reduction in sGAG concentration (Table 1); however, sGAG removal was more extensive in ovine tissue. Planar biaxial testing revealed significant differences following decell. There was a statistically significant increase in the areal strain of ovine leaflets; however, this effect was not observed in human leaflets (Figure 1). Stress relaxation was significantly different between cryo and decell ovine leaflets in the radial (42±3.2% cryo, 35±2.2% decell, $p < 0.05$) and circumferential (42±6.3% cryo, 35±4.6% decell, $p < 0.05$) directions. However, stress relaxation of the human leaflets only revealed a significant difference in the radial direction (37±2.0% cryo, 34±1.9% decell, $p < 0.05$), but not the circumferential (35±4.1% cryo, 33±4.7% decell, $p = 0.33$).

Table 1: Concentrations of various compounds showing mean ± SD.
***significance within species, p < 0.05.**
****non-parametric data showing median (range).**

	Human		*Ovine*	
	Cryo	**Decell**	**Cryo**	**Decell**
Elastin (µg/mg)	6.5 ± 1.4*	4.7 ± 1.7*	6.7 ± 1.4	6.6 ± 1.5
sGAG (µg/mg)**	2.02 (1.2)*	0.75 (0.6)*	1.30 (3.2)*	0.14 (1.1)*
Collagen (µg/mg)**	19.0 (6.5)	22.3 (27.2)	25.6 (9.1)	28.1 (21.6)
Tot. Protein (µg/mg)	160 ± 47	167 ± 67	153 ± 46	133 ± 64
dsDNA (ng/mg)	71 ± 21*	< 8.0E-6	81 ± 41*	< 8.0E-6

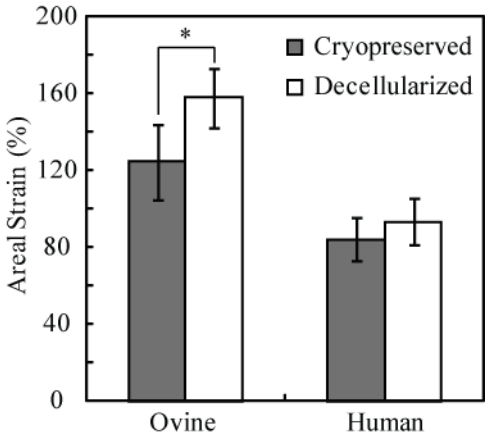

Figure 1: Areal strain under equibiaxial loading (* indicates significance between groups, p < 0.05)

Conclusions: The effect of decell on the mechanical behavior was more pronounced in the ovine leaflet, compared to human. Collagen is the primary structural protein responsible for high strain loading. However, collagen concentrations remained similar following decell, indicating the process may have caused structural changes to the collagen in ovine leaflets[2]. Inter-species differences observed in stress relaxation were likely the result of more substantial sGAG removal in the ovine leaflet. The results of this study have implications towards the clinical translation of decell heart valves in that a process optimized for human tissue may have more substantial effects on ovine tissue (i.e., the required pre-clinical animal model), requiring costly and time consuming design iterations. Future work will investigate the species-specific effects of decell on associated arterial tissues, as the conduit portion is typically implanted in conjunction with the valve leaflets.

References:

1. Converse GL. Acta Biomater. 2012; 8(7): 2722-2729.
2. Liao J. Biomaterials. 2008; 29(8): 1065-1074