Biomimetic Proteoglycans Increase the Indentation Modulus Of the Porcine Aortic Valve Leaflet Spongiosa

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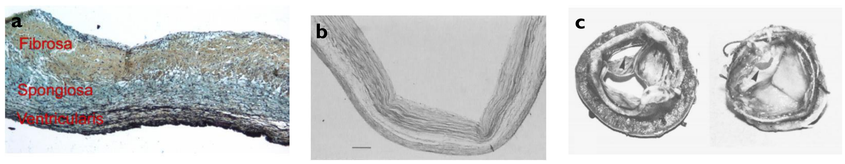
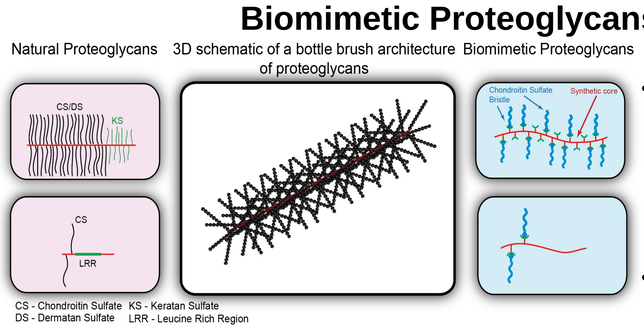
Introduction

Bioprosthetic heart valves (BHVs) are a valuable option for patients and clinicians for the operative management of valve disease, which affects about 2.5% of the US population[1]. The primary disadvantage of BHVs compared to mechanical valves is their poor durability[2], which has been attributed to deterioration of the prosthetic extracellular matrix (ECM) and the resulting aberrant flexural behavior of the prosthesis leaflets[3]. The proteoglycan (PG) and hyaluronan-rich middle layer of the leaflet, the spongiosa, is normally responsible for mediating shear stresses between the outer layers of the leaflet and is particularly susceptible to these changes[4]. Novel biomimetic proteoglycans (BPGs), enzymatically resistant synthetic versions of PGs[5,6], have been shown to augment the mechanical properties of cartilage in animal models of osteoarthritis and stress urinary incontinence[7,8]. In porcine aortic valve leaflets, BPGs increase the tissue glycosaminoglycan content and indentation modulus of the spongiosa without interfering with standard tissue cross-linking protocols.

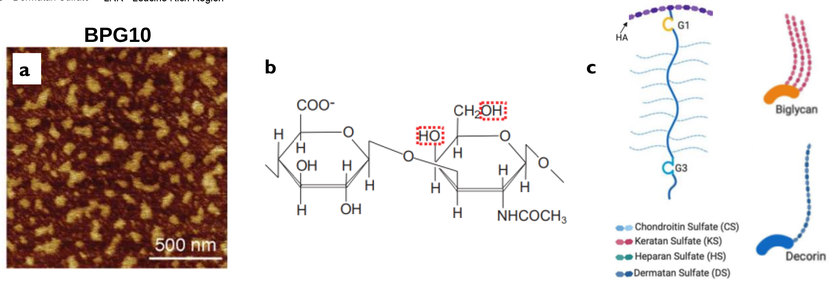


A) The spongiosa mediates shear produced by tension in the ventricularis and compressive stresses in the fibrosa[9] B) Loss of the spongiosa results in marked buckling of the leaflet[3] eventually leading to C) macroscopic tearing of the prosthesis leaflets[10] (L; tear at the leaflet base, R; tear in the leaflet body)

Biomimetic Proteoglycans



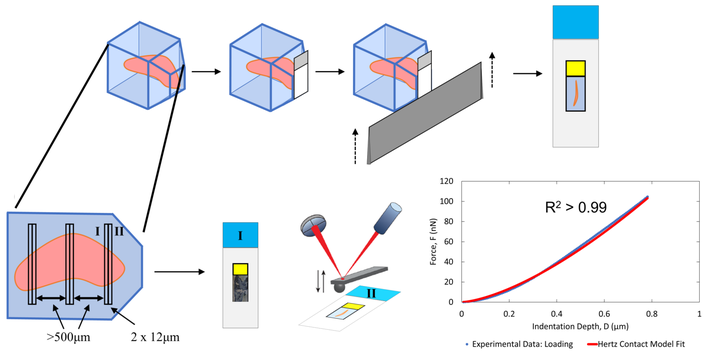
- Natural chondroitin sulfate (CS) bristles coupled to an enzymatically resistant synthetic polymer core[5,6]
- BPG10: ~160kDa



A) AFM imaging of BPG10[5] B) Chondroitin sulfate (CS) disaccharide composed of N-acetyl-D-galactosamine and D-glucuronic Acid C) Schematic representation of proteoglycans found in the porcine aortic valve leaflet[11]

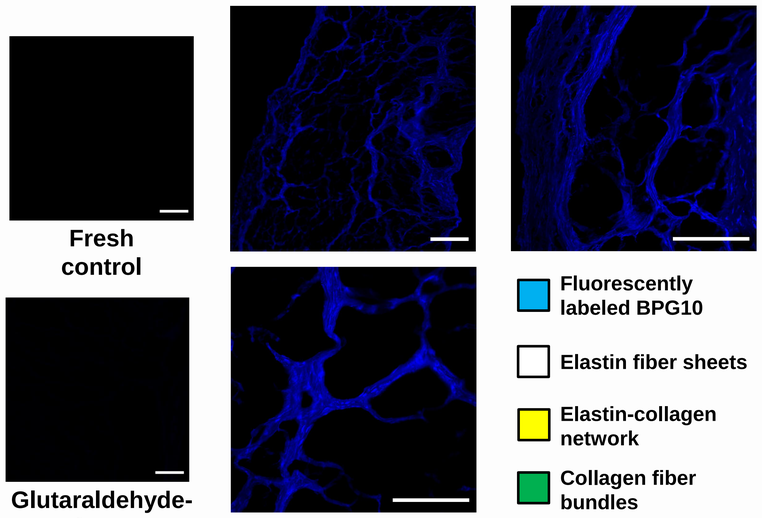
Experimental Methods

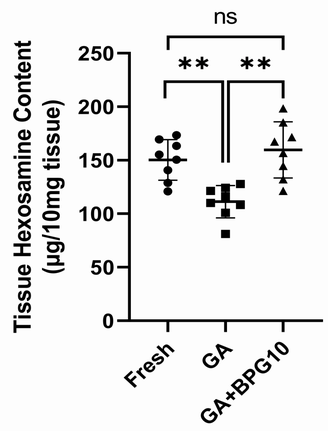
- Fresh porcine aortic valves were were harvested and were fixed in 0.6% glutaraldehyde (GA) with and without BPG10
- Leaflets were dissected and embedded for Kawamoto's film-assisted cryosectioning[12]
- Histology-guided AFM[13] was performed on paired 12 µm sections (labeled I and II below) – section I was stained with modified Movat Pentachrome to differentiate the spongiosa from the fibrosa and ventricularis and section II was evaluated using AFM
- Leaflet glycosaminoglycan content was measured by hexosamine acid assay
- The thermal denaturation temperature (T_d) of the fixed tissue was measured by differential scanning calorimetry on hydrated tissue samples



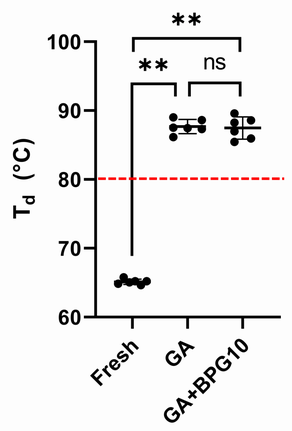
Results and Discussion

BPG10 distributes throughout the leaflet and increases tissues hexosamines without compromising collagen crosslinking stability



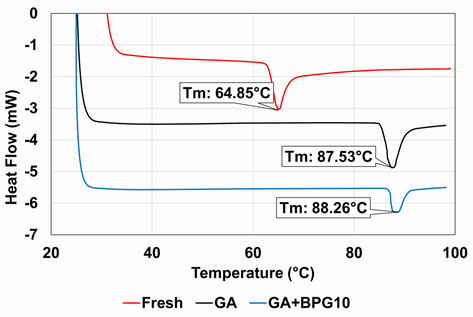


Mann-Whitney U-test, $p^{**} < 0.01$, $p^{ns} > 0.4$, N = 8



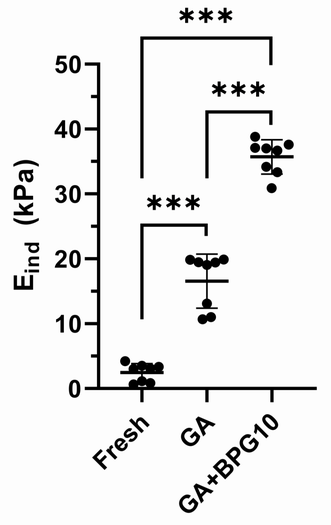
Mann-Whitney *U*-test, $p^{**} < 0.01$, $p^{ns} > 0.6$, N = 6

- Fluorescently labeled BPG10 distributes throughout the leaflet and demonstrates strong affinity for fibrous mesostructures
- Preferential interactions between BPG10 and elastin – similar to the supportive role biglycan, decorin, and versican play in elastin microfibril organization – may account this distribution
- Incorporation of BPG10 into the fixation solution resulted in a hexosamine content, a proxy for GAG content, after seven days similar to that of fresh tissue
- Glutaraldehyde crosslinking significantly increases the collagen denaturation temperature (T_d) of the tissue and is not compromised by addition of BPG10

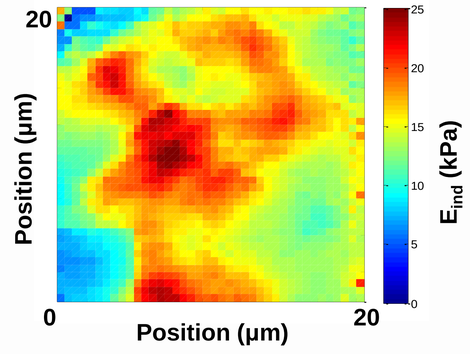


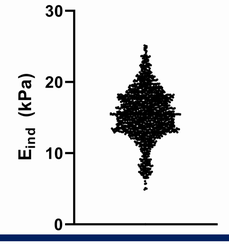
BPG10 increases the indentation modulus of the leaflet spongiosa

- Left: Scatter plot showing mean ± SD effective indentation moduli (E_{ind}) of the leaflet spongiosa in each experimental group. Each point represents a unique animal.
  Right: E_{ind} heat map of a fresh leaflet spongiosa, each pixel represents a unique indentation
- BPG10 increases the E_{ind} of the spongiosa beyond the strength conferred by glutaraldehyde crosslinking
- Heat map shows micromechanical heterogeneity, possibly attributable to molecular structures including collagen-elastin bundles, hyaluronan-versican complexes, and interstitial cells
- Further characterization is needed to discern the effect of BPG10 on long term prosthetic durability and performance



Mann-Whitney *U*-test, $p^{***} < 0.001$
Mean ± SD
N = 8





E_{ind} = 15.46 kPa ± 3.603
N = 1

Conclusions

- Demonstrated for the first time molecular engineering of porcine aortic valve tissue resulting functional modulation of its nanobiomechanical and biochemical properties
- BPG10 increases the effective indentation modulus of the porcine aortic valve leaflet spongiosa
- BPG10 diffuses throughout the porcine aortic valve leaflet and offsets hexosamine losses typically seen during glutaraldehyde-mediated fixation without compromising collagen cross-link stability

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References: [1] Benjamin, E.J. et al. *Circ.* 2019. [2] Bloomfield, P. et al. *N. Engl. J. Med*. 1991. [3] Vesely, I. et al. *Ann. Thorac. Surg.* 1988. [4] Vyavahare, N. et al. *Ann. Thorac. Surg.* 1999. [5] Prudnikova, K. et al. *Biomacromolecules* 2017. [6] Prudnikova, K. et al. *Acta Biomater.* 2018. [7] Philips, E.R., et al. *J Orthop Res.* 2019 [8] Kriete, A.S. et al. *J. Biomed. Mater. Res.* 2019. [9] Tseng, H. et al. *Acta Biomater.* 2011 [10] Pomar, J.L et al. *Ann. Thorac. Surg.* 1984. [11] Walimbe, T. and Panitch, A. *Front. Pharmacol.* 2020. [12] Kawamoto *T. Arch. Histol. Cytol.* 2003. [13] Dimitriadis, E.K et al. *Biophys. J.* 2003.