Biomimetic Proteoglycans Increase the Indentation Modulus of the Porcine Aortic Valve Leaflet Spongiosa Without Compromising Cross-Linking Stability

Mark Petrovic[1], Elizabeth R. Kahle[1,2], Lin Han[2], Michele S. Marcolongo[1]
[1]Department of Materials Science & Engineering, Drexel University, Philadelphia, PA
[2]School of Biomedical Engineering, Science, and Health Systems, Drexel University, Philadelphia, PA

Statement of Purpose: The utility of bioprosthetic heart valves (BHVs) is limited to certain patient populations because of their poor durability compared to mechanical valves[1], likely due to extracellular matrix (ECM) degradation[2]. Several strategies have been proposed to address this deterioration, including novel chemical fixatives to stabilize more of the ECM[3] and incorporation of small molecule inhibitors of catabolic enzymes implicated in the deterioration of the BHV ECM[4]. However, since the introduction of glutaraldehyde (GA) treatment for BHVs in the 1960s, few material changes have yielded significant improvements in long-term clinical performance[5]. We demonstrate a novel approach to this problem using biomimetic proteoglycans (BPGs) developed in our lab[6], which recently demonstrated to augment the mechanical properties of cartilage in animal models of stress urinary incontinence and osteoarthritis[7]. We hypothesized that diffusion of BPG10, a biomimetic proteoglycan shown to have 20% greater water uptake than hyaluronic acid[6], into the ECM of porcine aortic valve leaflet would increase the indentation modulus of the leaflet spongiosa. These findings suggest that a targeted molecular engineering approach of the valve leaflet ECM may be a viable way to improve the durability, and ultimately clinical performance of BHVs.

Methods: Fresh porcine aortic valve explants were randomly assigned to three groups (N=8 per group): fresh (as a control), glutaraldehyde-fixed (GA, as previously described[4]), with and without BPG10 (GA+BPG10, with 10 mg/mL BPG10 in the 0.2% glutaraldehyde solution). The effect of BPG10 on tissue hexosamine content was assessed using a modified hexosamine acid assay[8,9]. Differential scanning calorimetry (DSC) was performed on hydrated leaflet tissue in hermetically sealed pans to evaluate collagen crosslink stability. 12 μm thick sections of the non-coronary leaflets were taken in the plane approximately perpendicular to the leaflet-aortic wall junction using Kawamoto's film assisted cryosectioning technique[10]. Three pairs of consecutive sections, ≥ 500 μm apart, were taken from each valve. In each pair, one section was stained using Movat Pentachrome to guide atomic force microscopy (AFM) experiments on the paired section. AFM was conducted using microspherical AFM tips (R ≈ 2.25 μm, k ≈ 0.6 N/m) on a Bruker Icon. For each tissue section, indentation was performed on ≥8 locations of the leaflet spongiosa up to a ~1.5 μm maximum indentation depth at an indentation rate of 10.3 μm/s. The indentation modulus, E_{ind}, was calculated by fitting the entire loading portion of the force-displacement curve to the Hertz model with finite thickness correction[12]. Statistical significance was evaluated using separate Mann-Whitney *U*-tests for hexosamine acid assay and DSC, and Kruskal-Wallis test followed by Tukey Kramer multiple comparison for E_{ind}.

Results: The E_{ind} of untreated aortic valve leaflet (fresh) spongiosa was 2.455 ± 1.346 kPa. Glutaraldehyde fixation (GA) significantly increased the compressive modulus of the tissue to 16.57 ± 4.182 kPa. Incorporation of BPG10 into the fixation protocol (GA + BPG10) further increased the indentation modulus to 35.72 ± 2.648 kPa (Figure 1a). BPG10 increased offset the loss of tissue hexosamines during the GA fixation process (Figure 1b) and did not compromise glutaraldehyde-mediated tissue crosslinking (Figure 1c and 1d).

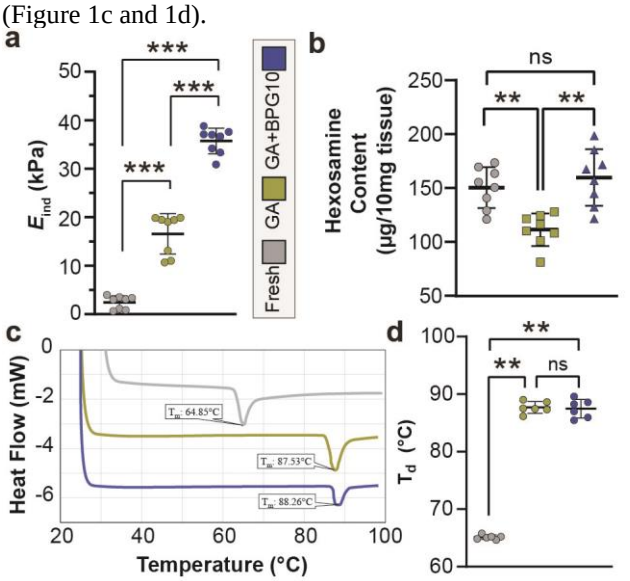

Figure 1. a) BPG10 increased E_{ind} beyond the increase imparted by glutaraldehyde-mediated crosslinking. **b)** Incorporation of BPG10 into the fixation solution resulted in a hexosamine content after seven days similar to fresh tissue (N=8). **c)** Representative thermograms for each experimental group, offset for clarity **d)** BPG10 does not compromise GA cross-linking efficacy (N=6). Data are presented as mean ± SD. ($p^{**} < 0.01$, $p^{***} < 0.001$)

Conclusions: BPG10 offset hexosamine loss during the fixation process, and significantly increased the indentation modulus of the leaflet spongiosa without interfering with GA fixation – a key step in the manufacturing of modern BHVs. Further investigation is needed to assess the biocompatibility of BHVs. These findings suggest that a targeted molecular engineering approach of the valve leaflet ECM may be a viable way to improve the durability of BHVs clinically.

References: [1] Arsalan M. Nat. Rev. Cardiol 2016; 13, 360–367. [2] Pomar J.L. Ann. Thorac. Surg 1984; 37, 78–83. [3] Mercuri J. Biomaterials 2007; 28, 496–503. [4] Raghavan D. Biomaterials 2007; 28, 2861–2868. [5] Zilla P. Biomaterials 2008; 29, 385–406. [6] Prudnikova K. Biomacromolecules 2017; 18, 1713–1723. [7] Phillips E. R. J. Orthop. Res 2019; 37, 403–411. [8] van de Loo H. M. Anal. Biochem 1976; 76, 555–560. [9] Leong J. J. Biomater. Appl 2013; 948–960. [10] Kawamoto T. Arch. Histol. Cytol 2003; 66, 123–143. [11] Li Q. J. Biomech 2015; 48, 1364–1370. [12] Dimitriadis E. Biophys. J 2002; 82, 2798–2810