Glycosaminoglycan Stabilization Reduces Tissue Buckling in Bioprosthetic Heart Valves

Sagar R. Shah and Naren R. Vyavahare

Department of Bioengineering, Clemson University, Clemson, SC 29634-0905

Introduction: Currently, bioprosthetic heart valves are crosslinked with glutaraldehyde to prevent tissue degradation and to reduce tissue antigenicity. Glutaraldehyde forms stable crosslinks with collagen via a schiff base reaction of the aldehyde with an amine group of the hydroxylysine/lysine in collagen. However, within a decade of implantation, 20-30% of these bioprostheses will become dysfunctional and over 50% will fail due to degeneration within 12-15 years post-operatively. One of the disadvantages of glutaraldehyde crosslinking is its incomplete stabilization of valvular extracellular matrix components such as glycosaminoglycans (GAGs)[1,2], which are important in maintaining a hydrated environment necessary for absorbing compressive loads, dissipating shear stresses, and resisting buckling. Previous studies have reported a greater depth of buckling, one of the major causes of failure in these bioprostheses, in glutaraldehyde crosslinked aortic valves[3,4]. Buckling occurs at sites of sharp bending, producing large stresses that can eventually lead to mechanical fatigue and consequent valvular degeneration. Thus, we hypothesized that the retention of valvular GAGs may reduce the extent of buckling in bioprosthetic heart valves and subsequently improve the durability of these bioprostheses. To evaluate the potential role of GAGs in reduction of buckling in bioprosthetic heart valves, we used two 1-ethyl-3-(3-dimethyl aminopropyl) carbodiimide (EDC) based crosslinking chemistries that would link GAG carboxyl groups to the amine groups of proteins.

Materials and Methods: Fresh porcine aortic heart valves were obtained from a local abattoir and thoroughly rinsed in ice-cold saline. Within 3-hours of harvesting, intact aortic valves were stuffed with cotton to maintain diastolic morphology and chemically crosslinked in three fixation groups:

Group I: Glut (0.6% for 24 hrs followed by 0.2%Glut in HEPES buffer, pH 7.4 for 6 days)

Group II: 30 mM EDC/ 6 mM N-Hydroxysuccinamide (NHS) followed by Glut (EDC/NHS+Glut),

Group III: GAG stabilizing agent followed by EDC/NHS and Glut as shown in Group II.

Fresh porcine aortic valves that were not chemically fixed were used as controls to observe buckling in native valve tissue. Following the above-mentioned preparations, cusps were excised from the aortic root, cut into strips, and bent to desired curvatures by bending them against natural curvature. To maintain a bent configuration, stainless steel pins were pierced through either ends of the strips; the ends were separated to a desired radius of curvature; and held in place by using cork stoppers at either ends of the pin for 24 hours in 0.2 % Glut solution. The radius of curvature was varied by changing the length of the tissue. Histological preparations of the samples were performed; stained with Alcian Blue to identify GAGs; and the extent of buckling was quantified. The actual curvature of the bending, tissue thickness, and depth of buckling were measured following histological evaluation.

Results and Discussion: Per histological observations, it was evident that as the radius of curvature decreased or as the curvature of bending increased, the extent of buckling increased. To demonstrate this relationship, fractional depth of buckling versus the product of tissue thickness and curvature of bending were plotted (as described previously by Vesely, I. *et al*[3,4]). Glutaraldehyde-crosslinked tissue buckled to a greater extent than fresh tissue (data not shown). Following enzymatic digestion of GAGs, the tissue buckled to an even greater extent, suggesting that the loss of GAGs may play a role in buckling of cusps.

Valvular tissue treated with GAG-targeted fixation chemistries (Group II and III), showed lesser degree of buckling as compared to glutaraldehyde-crosslinked tissue (Group I). The Group III treated tissue showed the least amount of buckling compared to other chemically fixed tissues, mimicking fresh tissue buckling pattern (Fig 1).

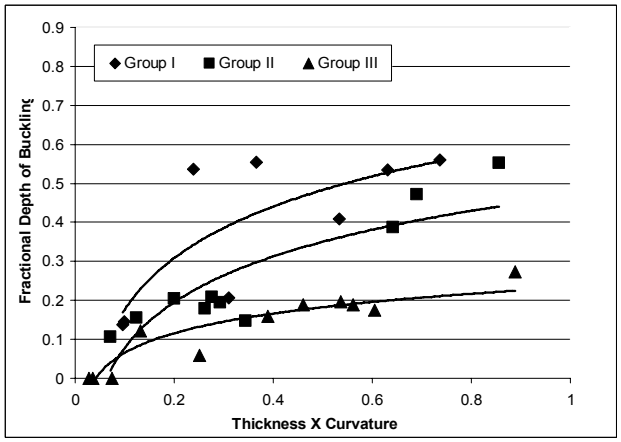

Fig 1. Linear plot of fractional buckling depth versus product of tissue thickness and curvature.

Further studies are being performed to examine buckling in GAG-stabilized valvular tissue after enzyme digestion.

Conclusion: Retention of valvular GAGs using GAG-targeted fixation chemistries may in fact reduce the extent of buckling in bioprosthetic heart valves.

Acknowledgement: This study was supported by a grant from NIH (HL070969).

References:

1. Lovekamp, J. *Biomaterials.* 2006; **27** (8): 1507-1518

2. Vyavahare, N. *J Biomed Mater Res.* 1999; **46** (1):44-503**.** Vesely, I. *Ann Thorac Surg.* 1988; **46** (3): 302-308

4**.** Vesely, I. *J. Heart Valve Dis.* 1998; **7** (1): 34-39