Changes in flexural mechanical behavior of Neomycin treated GLUT fixed aortic valve tissue

Michael D. McCall[1], Chad E. Eckert[1], Vincent M. Friebe[2], Naren R. Vyavahare[2], and Michael S. Sacks[1]
[1]University of Pittsburgh and [2]Clemson University

Introduction: Current bioprosthetic heart valve technology is centered around the use of glutaraldehyde as the universal crosslinker in order to prevent degradation. Although glutaraldehyde is widely used, it does have a major shortcoming – its failure to crosslink the entire extracellular matrix. Glutaraldehyde treated bioprosthetic heart valves are more resistant to collagen degradation, but glycosaminoglycans (GAGs) are not stabilized. Raghavan et al were able to show that a novel chemical treatment using neomycin was able to inhibit enzyme mediated GAG degradation[1]. In addition, our lab has previously shown a correlation between GAG loss and flexural rigidity[2]. Therefore, in theory, a neomycin treatment combined with glutaraldehyde would allow for the entire extracellular matrix to be stabilized, preventing degradation of the valve.

Methods: Porcine heart valves were obtained from a local slaughterhouse and the leaflets (right coronary, left coronary, and non-coronary) were divided randomly into four treatment groups: glutaraldehyde fixed (GLUT), glutaraldehyde fixed and subjected to GAG specific degrading enzyme (GLUT+ENZ), neomycin and glutaraldehyde fixed (NEO), and neomycin and glutaraldehyde fixed and subjected to GAG specific degrading enzyme (NEO+ENZ). The leaflets exposed to the GAG degrading enzyme were incubated with the mixture for 24 h at 37 C. The GAG specific degrading enzyme was composed of 1.2 mL of 5.0 U/mL hyaluronidase and 0.1 U/mL chondroitinase in 100 mM ammonium acetate buffer, pH 7.0. The leaflets that were not exposed to the GAG degrading enzyme were left attached to the root for as long as possible to prevent GAG leaching.

Flexure specimens were excised from the leaflet as per Fig. 1a with approximate dimensions of 15 mm circumferentially and 3 mm radially. Once obtained, the specimens were markered and loaded into the bending apparatus as seen in Fig. 1b.

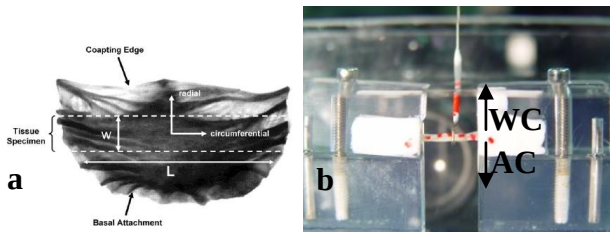

Figure 1. (a) Representative location of testing specimen for flexural testing. (b) Specimen placed in bending apparatus in preparation for against-curvature (AC) and with-curvature (WC) flexure.

GLUT and GLUT+ENZ specimens were subjected to changes in curvature (ΔK) ranging from 0.2 (WC) to -0.05 (AC) while NEO and NEO+ENZ specimens were subjected to ΔK limits of 0.1 (WC) to -0.01 (AC).

Results: As seen in Fig. 2, hysteresis decreased in the GLUT samples with the introduction of GAG degradation enzyme. In the NEO treated groups, hysteresis remained at similar levels after exposure to the GAG degradation enzyme. Also, interestingly, the neomycin treatment resulted in a small, but measurable increase in flexural rigidity.

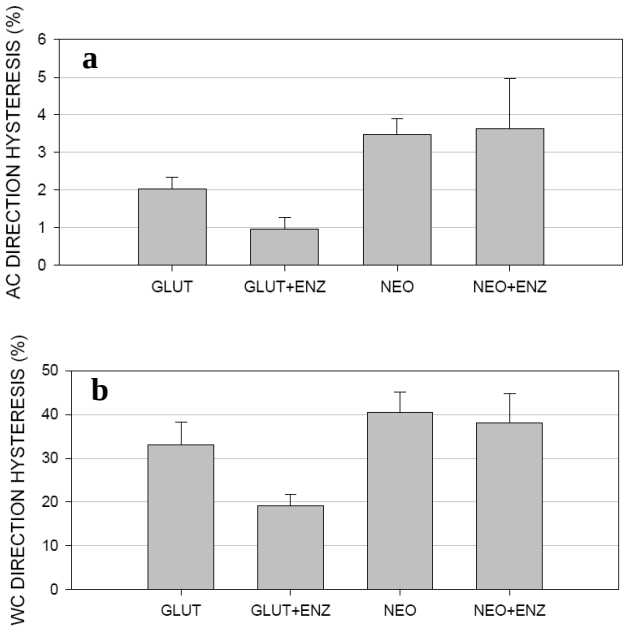

Figure 2. AC (a) and WC (b) hysteresis (%) values with standard errors, n=7.

Conclusions: This preliminary work was able to support both the claims of Raghavan et al and Sacks et al in unison. We were able to show that introducing neomycin into the fixation treatment stabilizes GAGs in porcine leaflets during flexural loading. This finding will help to play a role in the ever evolving development of bioprosthetic heart valves.

References:

[1]Raghavan et al. *Biomat*; **28**; 2861-68, 2007.
[2]Sacks et al. *Expert Rev Med Devices*; **3**; 817-34, 2006.

Acknowledgments:

NIH/NHLBI R01 HL070969