Strain Effects on Collagen Proteolysis in Heart Valve Tissues

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Introduction: Compositionally, morphologically, and mechanically, heart valve leaflet tissues are characterized by their complex heterogeneity, which confer valves their mechanical integrity and unique functional characteristics. Healthy leaflets imply homeostasis, with valvular interstitial cells catabolizing damaged collagen alongside collagen synthesis. By contrast, severe collagen depletion caused by matrix metalloproteinases (MMPs) can induce matrix destruction and change the viscoelastic property of the heart valve tissues. Strain subjected on collagen fibers may effectively enhance or block proteolytic sites and could potentially accelerate or resist degradation of fibers, leaving them selectively susceptible to collagenases and MMPs. With application of collagenase to the tissues to simulate collagen degradation by MMPs, this project focused on characterizing stress relaxation behaviors of fresh porcine aortic (AV) and pulmonary valve (PV) tissues and collagenase-treated ones under different stretching conditions. We hypothesize that a decrease in stress on the leaflet is associated with the increased collagenase concentration induced on the heart valve tissue. We also hypothesize that strain levels consistent with normal heart conditions will aid in resisting degradation of collagen fibers.

Materials and Methods: Aortic and Pulmonary valves were dissected from a fresh porcine heart, 10mm x 10mm samples were cut in the middle of the leaflet, and biaxial mechanical testing was performed. Stress relaxation tests were run at 20%:70% (circumferential:radial) and 25%:87.5% for AV and 20%:90% or 25%:112.5% for PV. The sample was held for 10,000 seconds at the prescribed strain levels. At 3,000 seconds, the HBSS solution the sample was immersed in was drained and replaced with a Type II collagenase solution of 0.2 or 0.5 mg collagenase/ ml HBSS to simulate endogenous MMPs for collagen degradation. A collagen assay was performed, extracting collagen for 72hr, to measure collagen concentration of the leaflets after mechanical testing [1]. Samples were also histologically prepared and stained with Masson's Trichrome, Hematoxylin & Eosin (H&E), and Verhoff stains and digitized as photomicrographs using a Zeiss Axiophot upright microscope.

Results and Discussion: The normalized stress of AV and PV decreased as the collagenase concentration was increased, indicating the degradation of collagen fibers, visible from histological images, where there was a significant loss of collagen fibers as the collagenase concentration increased. The collagen assay results, however, did not show a statistically significant decrease for collagen remaining in the samples after testing. In comparison to previous tests using the same equipment and protocols testing equibiaxially [2] (**Figure 1**), the current tests displayed a smaller stress-relaxation at the end of the 10,000-second testing period. The samples tested at physiologically accurate strain levels displayed a higher stress at the end of testing, indicating that at these levels, the leaflets can actually resist degradation of the collagen fibers. It is suggested that at the strain levels of 20%:70% or 25%:87.5% (AV) and 20%:90% or 25%:112.5% (PV), the leaflets could resist degradation of the collagen fibers and stay strong and durable, with a longer lifespan.

Conclusion: The AV and PV showed an increase in stress at the completion of mechanical testing when subjected to physiological strain rates as compared to equibiaxial strains, indicating a mechanism present that aids in resisting degradation of collagen when strained physiologically. This suggests that at these strain levels, collagen fibers are able to effectively block proteolytic sites to resist degradation of fibers and selectively choose which fibers are subjected to collagenases or MMPs.

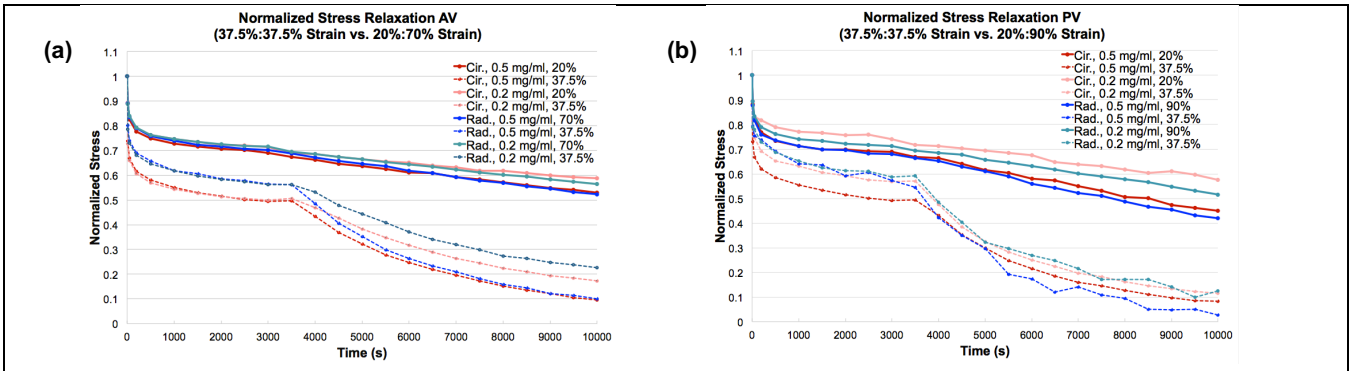

Figure 1: Comparison of equibiaxial testing (dashed) vs. **(a)** 20% to 70% non-equibiaxial testing (solid) for AV and **(b)** 20% to 90% non-equibiaxial testing (solid) for PV at 0.2 and 0.5 mg/ml collagenase [2].

References: [1] Huang H.S, et al. J Eng Med, 226(11), 2012. [2] Huang S, et al. J Eng Med, 229(10), 2015.