The Stress Relaxation Behaviors of Diseased Heart Valve Tissues

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INTRODUCTION

Compositionally, morphologically, and mechanically, heart valve leaflet tissues are characterized by their planar and transmural heterogeneity, with the complexity of the distinct free edge, belly, nodulus and commissure regions, and valvular excellular matrix (ECM) is largely comprised of collagens, elastin, and glycosaminoglycans, which in coordination confer valves their mechanical integrity and unique functional characteristics. Healthy leaflets imply homeostasis, with subpopulations of valvular interstitial cells (VICs) catabolizing damaged collagen and the same or other VICs mediating de novo collagen synthesis. By contrast, disruption, depletion or excess accretions of collagens are hallmarks of various valvular diseases, likely manifesting as both causes and effects of disrupted mechanotransduction across the macro to micro length scales. 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. Conceptually, this paradigm could potentially explain how molecular-level collagen damage elicits selective collagen fiber remodeling, and has broader implications for developmental tissue growth, physiological load adaption, and pathological degeneration. 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.

METHODS

Preparation of Samples

Aortic and Pulmonary valves were dissected from a fresh porcine heart and 10mm x 10mm samples were cut in the middle of the leaflet and mounted on the BioTester 5000 (CellScale Biomaterials Testing, Waterloo, ON, Canada) (Fig.1). Hanks' Balanced Salt Solution (HBSS) was heated to 37°C, filled in the tray, and the sample was immersed into the solution for testing.

Mechanical Testing

Two samples were tested at once under the assumption the elastic modulus is the same for both samples if they come from the same heart. A pre-stretch of 10 cycles (i.e., preconditioning) was conducted at 2% strain to ensure to generate repeatable results before mechanical testing. The stress relaxation test was run at 20% strain in the circumferential direction and 70% strain in the radial direction for AV and 20% and 90% for PV (n=6 for each collagenase concentration). The sample was held for 10,000 seconds at the prescribed strain levels. At 3,000 seconds, the HBSS 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.

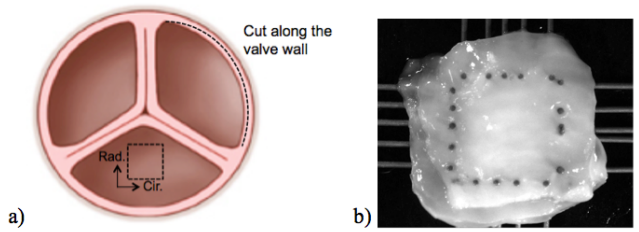

Fig. 1: a) Leaflets are cut along the heart valve wall and square sections are cut from the central area. b) Two samples are placed on the BioRakes.

Collagen Assay

A collagen assay was performed to measure collagen concentration of the leaflets after collagenase is applied. Collagen was extracted for 72hr for 6 samples for each collagenase concentration for AV and PV. The collagen masses of each sample were calculated by comparing absorbance values to that of the collagen standards [1]. The collagen concentration of each sample was then calculated by normalizing to the wet weight of the individual collagen sample.

Histology

Samples were fixed in 10% buffered formalin immediately after stress relaxation testing. Each sample was paraffin embedded, sectioned, and stained with Masson's Trichrome and Hematoxylin & Eosin (H&E) stains. The histological slides were digitized as photomicrographs using a Zeiss Axiophot upright microscope at 400x magnification.

RESULTS AND DISCUSSION

The normalized stress of AV and PV are presented in Fig. 2-3. The normalized stress decreased as the collagenase concentration is increased, indicating the degradation of collagen fibers, it can been seen from histological images (Fig. 5), where there was a significant loss of collagen fibers (pink) 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 (Fig. 4).

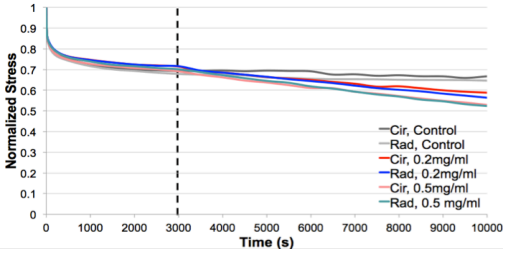

Fig. 2: Normalized Stress Relaxation for AV

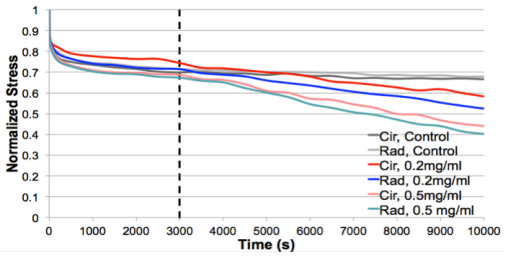

Fig. 3: Normalized Stress Relaxation for PV

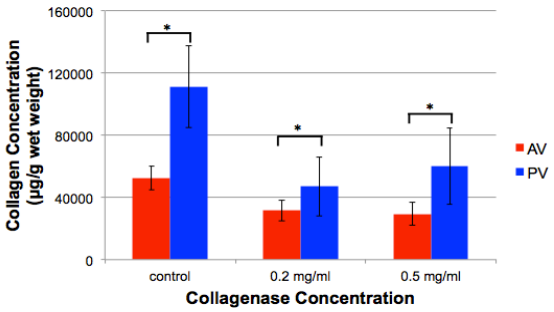

Fig. 4: Collagen Concentration for AV and PV. (* Statistical significance, $p<0.05$)

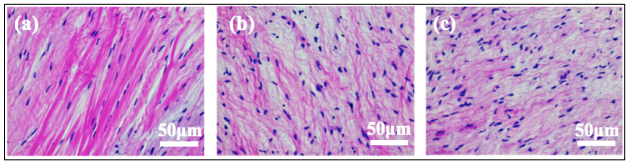

Fig. 5: Histological AV samples stained with H&E. (a) 0mg/ml, (b) 0.2mg/ml, (c) 0.5mg.ml collagenase concentrations

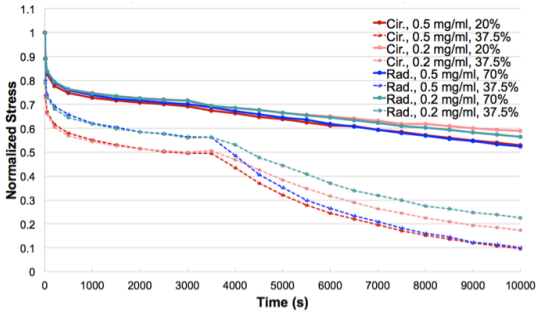

Fig. 6: Comparison of equibiaxial testing (dashed lines) vs. 20% to 70% non-equibiaxial testing (solid lines) for AV at 0.2 and 0.5 mg/ml collagenase [2].

In comparison to previous tests using the same equipment and protocols testing equibiaxially [2] (Fig. 6), 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% (AV) and 20%:90% (PV), the heart valve leaflets are able to resist degradation of the collagen fibers and stay strong and durable, resisting disease and having a longer lifespan.

REFERENCES

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