STRESS-RELAXATION BEHAVIORS OF DISEASED HEART VALVE TISSUES

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INTRODUCTION

Heart valve tissue with collagenous ECM is regarded as a load-adapting biomaterial. Mechanical forces have significant influence for stimulating biomechanical regulation of heart valve cells that modulate tissue remodeling through collagen fibers, such as homeostasis, synthesis, or degradation of collagen. Generally, coordinated collagen synthesis and degradation are processes of matrix remodeling under physiological conditions. However, severe collagen depletion caused by matrix metalloproteinases (MMPs) pathologically induces matrix destruction, changes viscoelastic properties of the heart valve, further affects cellular regulations mediated by heart valve cells, and even leads to heart valve diseases. Moreover, during disease, it has been observed that the mechanical force enables the heart valve leaflet to retain structural strength via accumulation of collagen fibers and prevents deterioration or tissue destruction from collagen cleavage mediated by cellular activities.

The impact of collagen degradation on the biochemical regulation of heart valves in matrix and at cellular levels has been demonstrated comprehensively in previous studies. In this study, therefore, to understand the mechanical behavior in stress and the viscoelastic properties of the heart valve as they vary with collagen degradation, a tissue-level investigation of collagen-deficient heart valve tissue responding to its mechanical environment was conducted via testing of stress relaxation. Collagenase was applied to simulate collagen degradation by MMPs in matrix remodeling. A series of stress relaxation tests under different strain levels and with application of collagenase solution were performed to test the sensitivity of collagen fibers to proteolytic degradation. Further, the quantity of collagen released from the heart valve tissue was also measured to verify the relationship among collagenase concentrations, strain levels, and amounts of collagen remaining in heart valve tissues, corresponding with stress drops under different conditions.

METHODS

Preparation of specimens and collagenase solutions: Eight porcine hearts (sows, eight months to one year old) were dissected in the Nahunta Pork Center (Pikeville, NC) and returned to the laboratory within 60 minutes. Twenty-four AVs and 24 PVs were dissected axially along the aorta and pulmonary artery in each heart, and then square specimens (10 mm × 10 mm) were cut from all AV and PV leaflets [1]. To better maintain the physiological functions of the tissues in a biomimic environment, all 48 leaflet specimens were immediately immersed in HBSS solution (Lonza, Walkersville, MD) at 37°C. Meanwhile, three concentrations (0.2 mg/ml, 0.5 mg/ml, and 1.0 mg/ml) of collagenase solution (Worthington, Lakewood Township, NJ) were prepared for collagen degradation and then stored in the refrigerator at 4°C.

Biaxial Tissue Tester: Our laboratory's biaxial testing system and protocols have been described in detail previously [1]. In brief, a biaxial tissue tester (BioTester 5000, CellScale, Canada) equipped with two load cells (500 mN) for two perpendicular axes of loading was used for measuring the force and displacement of the semilunar heart valve tissue leaflets. The measured values were used to further obtain stress–strain curves and to calculate the parameters of the material stiffness. Synchronized time lapse video for real-time monitoring and postprocess analysis was performed with the charged-couple device (CCD) camera, which acquired images with a pixel resolution of 1280×960 at an acquisition rate of 15 Hz, with a lens focal length of 75 mm. A temperature-controlled saline chamber with data logging capability provided a physiological environment for testing soft tissue specimens. BioRakes provided fast and accurate

sample mounting: each BioRake consisted of five tungsten tines used to anchor one edge of the specimen. Four rakes provided uniform attachment across the edges of the samples and evenly distributed load spanning 4 mm in length on each side of the sample.
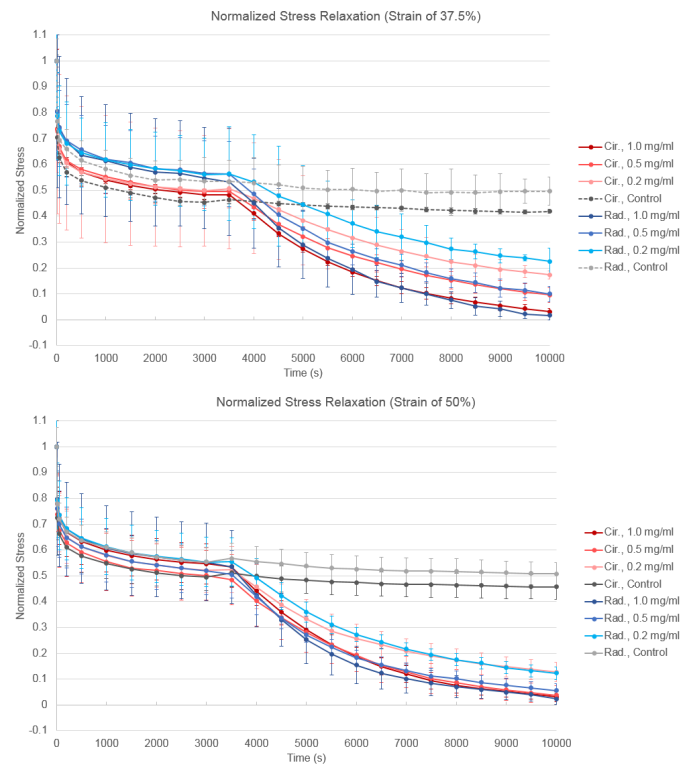
Stress Relaxation: Stress relaxation is a viscoelastic property of soft tissue which involves a decrease in stress under a constant strain. It is known that the magnitude of stress relaxation changes with variation of external mechanical stretching or the tissue structure. Here two groups were tested: 18 AV and 18 PV specimens were assigned to the collagenase-treated group (experimental group) and other fresh specimens were defined as the untreated group (control group). Six conditions where three different collagenase concentrations combined with two strain levels were applied to each AV or PV experimental group while two strain levels but no application of collagenase were set for each control group. Three AV or PV specimens were tested under each condition. Moreover, in order to avoid rapidly tearing the tissue sample during stretching before stress relaxation, 10 cycles of pre-conditioning stretching under 10% of strain and step increasing the strain rate at 2.5% per second were applied [2]. When the strain reached 37% or 50%, respectively, the specimen was held for 10,000 seconds to mimic comparable levels of relaxation [3-4]. For each AV or PV collagenase-treated group, during stress relaxation testing the specimens were immersed in the fluid chamber filled with 37°C HBSS for the first 3,000 seconds, and then HBSS was replaced immediately with collagenase solution. HBSS was gently removed from the chamber and collagenase solution was added using a Powerpette controller (VWR, Radnor, PA). However, solution replacement was not applied to each AV or PV untreated group. At the end of the testing, each specimen was gently removed from the biorakes and stored in individual centrifuge tubes filled with HBSS at 37°C for collagen assay.

Normalization of Data for Stress Relaxation: The stress relaxation responses at the various strain levels and collagenase solutions varied in magnitude. Higher strain level led to higher peak stress at the beginning of testing, and a lower state of stress relaxation resulted from smaller applied strain. To understand how applied strain and collagenase concentration affected stress relaxation, normalization of the stress relaxation that was exhibited by each stress drop relative to the peak stress was performed, which was beneficial for observing whether the patterns of all the curves were consistent. Therefore, normalization of stress relaxation was applied so as to more easily observe the mechanical behaviors of all specimens under different conditions.

RESULTS
The average AV responses to stress relaxation versus time at each condition are shown in **Figure 1**. Over a time period of 3,000 seconds (t = 0 ~ 3,000), the stress drops rapidly at the beginning of stress relaxation and then decreases more slowly to a constant value at each condition. The normalized stresses in the control group under all conditions remain between 0.4 and 0.5 after 3,000 seconds until the end of testing. However, after adding collagenase solution (t = 3,000 s), each stress-time curve for the collagenase-treated group starts to decline again until t = 10,000 s. At the end of testing, circumferential and radial normalized final stresses were below 0.1 for both conditions at 37.5% and 50% of strain when the 0.5 mg/ml or 1.0 mg/ml collagenase solution is applied. The smallest collagenase concentration (0.2 mg/ml) apparently has a smaller influence on stress drops, and the final normalized stresses in two directions are close to 0.2 and 0.15 under the strain conditions at 37.5% or 50%, respectively. Up to 98% of circumferential and radial normalized stresses drop under the condition of highest concentration of collagenase (1.0

mg/ml) for both strain levels. Further, it shows that only about 80% to 87% of the normalized stress relaxation in two directions occurs under the condition of lowest concentration of collagenase (0.2 mg/ml) for 37.5% or 50% of strain, respectively.

Figure 1: Average AV responses for stress relaxation versus time at two strain levels, 37.5% and 50%. Specimens immersed in HBSS are stretched under 37.5% or 50% of the strain control and holds for 10,000 seconds. For the collagenase-treated groups, HBSS is replaced with the 0.2 mg/ml, 0.5 mg/ml, or 1.0 mg/ml collagenase solution, respectively, after 3,000 seconds. For the control group, HBSS in the chamber is not changed. The circumferential direction of the specimen is denoted "Cir.," while the radial direction is "Rad."

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