Loss of Tension Increases Meniscus Degradation in a Degradative Microenvironment

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Introduction

The menisci are semi-lunar shaped fibrocartilaginous wedges located between the femur and the tibial plateau and support the structure and mechanical function of the knee joint. Menisci are comprised primarily of circumferentially aligned type 1 collagen bundles, which function to convert compressive forces into tensile hoop stresses[1]. In addition to circumferential fibers, the meniscus contains radial tie fibers that originate at the meniscus periphery and interdigitate amongst the circumferential fiber population[2]. Understanding meniscus structure and function is particularly important given the high incidence of meniscal pathology, and its association with progressive joint degeneration[3]. In particular, radial tears are clinically correlated with an increased incidence of chondral lesions[4] and are considered to be irreparable. These tears interrupt circumferential fibers and, as a result, reduce tensile strain transmission in the vicinity of a tear. Of note, several studies on tendon and other dense connective tissues have reported that when collagen fibrils are under a moderate amount of pre-strain, collagenase-mediated degradation is inhibited[5,6], as was evidenced by a reduction in the loss of mechanical properties in the presence of matrix degrading enzymes. Here, we evaluated whether a radial tear in the meniscus would predispose the tissue to collagenase-mediated digestion in the vicinity of the defect. For this, we used second harmonic generation (SHG) imaging, in which signal intensity is positively correlated with organized and aligned collagen[7–9]. Using this method, we visualized local changes in collagen organization as a function of pre-strain and location relative to a radial defect in the context of exogenous collagenase, which is upregulated in the joint in the context of meniscal injury[10].

Methods

Medial menisci (n = 4) were harvested from adult (skeletally mature) cows. The top third of each sample was removed, as were both the anterior and posterior horns. Menisci were then sectioned to 350 mm thickness in the transverse plane. Four samples (25.4 mm width) from each meniscus were sutured to rubber backing using 3-0 TiCron sutures placed at the apex of the outer body and the anterior and posterior inner edges. After suturing, the tissue was placed in a tensioning device (Figure 1A) with a gauge length of 25.4 mm. Type I Bacterial Collagenase (Worthington) was reconstituted in Phosphate Buffered Saline supplemented with magnesium and calcium (PBS+) at a concentration of 60U/mL. Each sample was randomly assigned to one of four conditions: 0% strain in PBS+ (CTL), 0% strain in collagenase (no strain, NS), 4.5% strain in collagenase (S), and 4.5% strain in collagenase with an added half-width radial defect in the body of the tissue (SD). After 8 hours of collagenase digestion at 37^{O}C, samples were washed thoroughly in 4^{O}C PBS and imaged whole-mount in the transverse plane in eight regions of interest (ROIs) relative to the defect (Figure 2A) at 25X magnification using SHG (840 nm excitation). Maximum intensity projections spanning 142 ± 7.1 microns of the tissue depth were generated, and the mean signal intensity was quantified in FIJI. Signal intensity was normalized to the PBS control for each sample. Data were compared using a 2-way ANOVA across treatment groups and ROIs with Tukey's post-hoc tests.

Results

Visual inspection post digestion showed that PBS+ (CTL) incubated tissues maintained their pre-incubation structural characteristics and opacity. Conversely, in collagenase-incubated tissues, areas of degradation were readily apparent via changes in tissue opacity (Figure 1B). Quantification of mean SHG signal intensity indicated that strained (S) tissues had no significant differences from CTL tissues ($p = 0.064$), whereas non-strained, collagenase treated tissues (NS) had a lower intensity than CTL tissues ($p < 0.0001$). Interestingly, radially defected (SD) tissues revealed no differences in intensity compared to NS tissues ($p = 0.999$), but showed significant differences compared to intact, strained tissues (S) that were also incubated with collagenase ($p = 0.0002$) (Figure 2B). There was no clear pattern of SHG signal change as a function of location ($p = 0.870$) though within just the SD group, the inner center ROIs were shown to have reduced signal compared to outer edge ROIs ($p = 0.0524$).

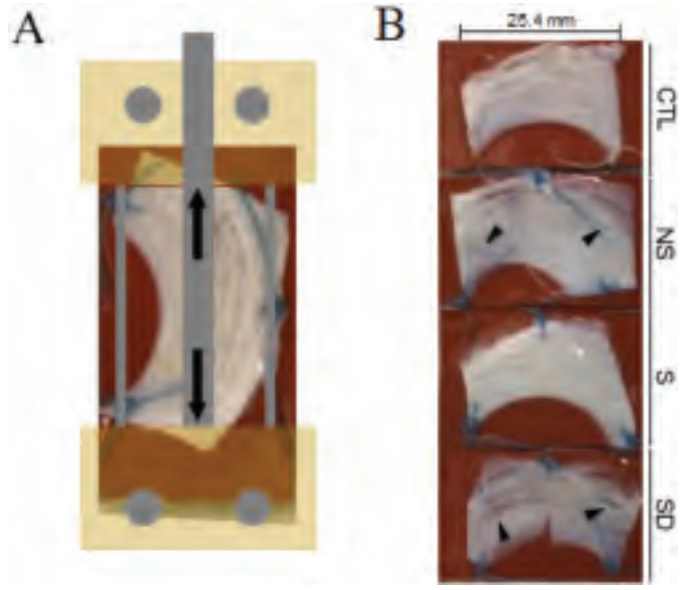

Figure 1. (A) Meniscus specimen sutured to backing with a schematic of the tension clamp device. Tension shown with black arrows. **(B)** Specimens from collagenase digestion groups and PBS control shown after digestion for 8 hours. Black arrowheads indicate regions of apparent digestion.

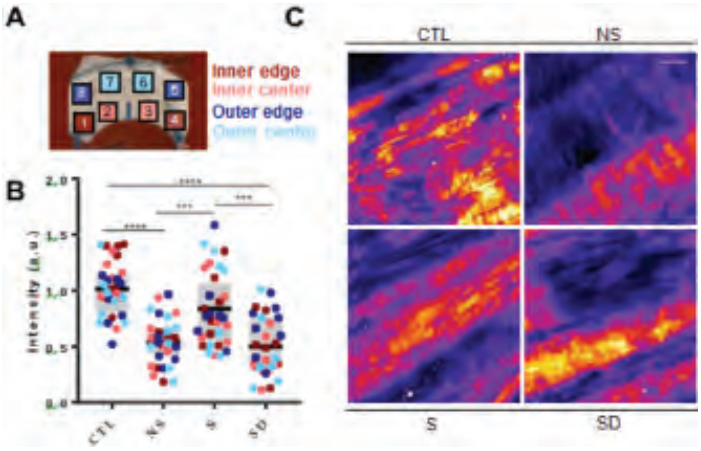

Figure 2. (A) Black boxes indicate regions of interest for imaging. **(B)** Quantification of SHG signal intensity normalized to CTL. Colors indicate ROI. **(C)** Representative images of the maximum projection of digested tissue from each treatment group. Images are from the same sample and ROI (inner edge). SB = 200 microns.

Discussion

This study establishes a platform to investigate structural reorganization and remodeling in the context of radial meniscus defects. The clamp system can be used to transmit variable amounts of strain to explant tissues that are either intact or contain a 'radial' defect to generate regions of reduced strain. The results of this study show that using this system, application of collagenase leads to a loss of SHG signal in the absence of pre-strain. When strain was applied in the context of added collagenase, greater SHG signal was retained compared to similarly collagenase treated unstrained controls. When the sample was defected, altering strain patterns in the tissue, collagenase treatment resulted in increased loss of SHG signal, indicating that a radial tear made the tissue susceptible to aberrant remodeling. These findings suggest that, like in other dense connective tissues, the application of strain can protect collagen from the action of local matrix degrading enzymes. The observation of increased local remodeling with loss of local strain may lead to aberrant cell mechanosensing in the vicinity of focal defects in the meniscus[11], which may exacerbate altered signaling and lead to a cascade of degeneration, ultimately compromising tissue function.

Significance

This study develops a model platform in which to investigate structural reorganization of meniscal explants in the context of loss of pre- strain in a degradative environment. Future studies using this platform will inform large animal studies of meniscal remodeling and degeneration after injury and provides a controlled setting in which to study how mechanical loading regulates the development of pathology after injury.

Acknowledgements

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