INTRODUCTION:
Articular cartilage is a heterogeneous tissue that serves as a protective layer at the surface of every long bone. Although high shear stress is known to predispose the tissue to failure and disease [1], the mechanisms responsible for shear energy dissipation in articular cartilage are poorly understood. We previously reported that a narrow region of the tissue 100-200μm below the articular surface is responsible for the vast majority of shear energy absorption by cartilage. This surface region is also damaged or degraded early in the process of arthritis. However the consequences of damage to this region on energy absorption are not well understood. To better understand the effects of such surface degeneration the goal of this study was to characterize the effect of collagenase or trypsin exposure on the shear energy absorption of articular cartilage.

METHODS:
Sample Preparation: A total of ten cylindrical explants 3-3.5 mm thick and 6 mm in diameter of articular cartilage were harvested from the patellofemoral groove of 1-3 day old calves. They were then cut along their long axes into 2 hemi-cylinders, yielding a total of twenty hemi-cylinders and placed into PBS and 7 μg/mL 5-dichlorotriazinylaminofluorescein (5-DTAF) for 2 hours to stain extracellular matrix proteins [2]

Enzymatic Digestion: Cartilage samples designated for enzymatic digestion and CR imaging of collagen [3] were coated with epoxy resin to ensure that enzyme exposure occurred only at the articular surface [3]. Samples were imaged on an CR microscope in real-time at a frame rate of 4 frames/min. Samples were digested for 15, 30, and 90 minutes using two different enzymes, collagenase (2mg/mL) and trypsin (50µg/mL), to remove collagen and proteoglycans, respectively. Enzymatic solutions were removed by serial washing with protease inhibitors and were fixed, embedded, sectioned, and stained with Safranin-O to observe proteoglycan distribution.

Confocal Strain Mapping: The local shear modulus of samples was measured using grid resolution automated tissue elastography (GRATE) as described previously [4]. The undigested hemi-cylinders were loaded between two parallel shearing plates of a tissue deformation imaging stage that was mounted on an inverted confocal microscope [5]. Two lines were photobleached onto the hemi-cylinder along the long axis, perpendicular to the articular surface [5]. Each sample was subjected to a 10% axial strain and sinusoidal shear displacement with amplitude of 16µm at a rate of 0.1 Hz. After completion of the shear testing on the undigested tissue, each sample was removed from the TDIS, enzymatically digested as described above, reloaded into the TDIS, and tested again.

Data Analysis: Custom software written in Matlab (The Mathworks, Inc., Natick, MA) was used to track the motion of the photobleached lines as a function of depth [6]. Local shear strains (γs) were calculated from the local slope of the photobleached lines and used to calculate the local viscoelastic properties of the tissue including the shear modulus (G*) and phase angle (δ). The rate of energy dissipation was calculated by the following equation

\[ \Delta \varepsilon = \pi (G^* \sin \delta / \gamma_s) ^ 2. \]

RESULTS:
Safranin-O histology revealed a decrease in proteoglycans near the articular surface after collagenase and trypsin digestions, with proteoglycan depletion increasing with time. Similarly confocal reflectance micrographs showed increasing collagen degradation in collagenase treated samples, although the collagen network remained intact after trypsin treatment. (Fig. 1A) As previously reported (G) changed in narrow band near surface (~400μm); changes were significant up to 50-fold decrease in local (G). Collagenase and trypsin exposure increased the phase angle (δ) at articular surface with collagenase exposure increased (δ) deep into the tissue, as far as 1000μm. (Fig. 1B) In addition, both enzymes increased ΔE by cartilage up to 4-fold locally progressing higher at longer exposure times; similar to (δ), this penetrated deeper into tissue, up to 1000μm. (Fig. 1C)

DISCUSSION:
This study evaluated the local viscoelastic shear properties of articular cartilage after degradation by both enzymes. Since the tissue is more compliant and more viscous at the articular surface. Surface digestion by trypsin or collagenase at the articular surface led to increased local energy dissipation. However, proteoglycan removal alone increased viscous behavior counter to the idea that friction between proteoglycan and collagen causes viscous behavior. Combination of proteoglycan removal and collagen removal increased viscous behavior slightly more than proteoglycan removal alone. For depth of penetration (trypsin at 90 mins and collagenase at 15 mins -200μm) peak of (δ) and ΔE are similar, but mechanical effects of collagen degradation are seen deeper in tissue (~1000μm). These results suggest removal of tension on the collagen network contributes to viscous behavior.

SIGNIFICANCE: Loss of collagen and proteoglycans are hallmarks of early-stage osteoarthritis. This study shows that proteoglycan removal increases viscous behavior and suggests that tension in the collagen network is an important contributor to the viscoelastic properties

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Figure 1: (A) Histology and CRM Intensities as a function of vertical position during collagenase and trypsin digestion (B) Phase angle (δ) vs. depth z (C) Energy dissipated (ΔE) vs. depth z (n=9)