Microscale Compositional Mapping Predicts Local Strains Across Interface of Cartilage Repair from an Eight Month Equine Model

Rebecca M. Irwin[1], Alexander J. Boys[1], Tianyu Gao[1], Itai Cohen[1], Lara A. Estroff[1], Lawrence J. Bonassar[1]
[1]Cornell University, Ithaca, NY
rmi8@cornell.edu

Disclosures: Rebecca M. Irwin (N), Alexander J. Boys (N) Tianyu Gao (N), Itai Cohen (N) , Lara A. Estroff (N), Lawrence J. Bonassar (N)

INTRODUCTION: Several surgical repair techniques exist for treating focal articular cartilage defects, yet achieving lateral integration of the repair tissue with surrounding native tissue remains a challenge[1]. Previous experimental studies have used macroscale failure tests to assess integration strength, but these tests do not provide information on tissue deformations under physiologic loads or on variations in integration strength between cartilage zones. Additionally, finite element analyses have predicted deformation gradients across the cartilage repair interface which could lead to detachment of the repair[2]. Deformations or high strains across the interface that show tissue inhomogeneity would potentially identify areas more prone to failure. While the failure adhesive strength of cartilage repair has been correlated with collagen deposition *in vitro*[3], no such data exist from an *in vivo* model. The objectives of this study were to establish a method to spatially correlate microscale collagen composition and tissue strains at cartilage repair interfaces under compressive loading and determine if collagen composition was predictive of interface strains in an *in vivo* long-term equine model of cartilage repair.

METHODS: Equine samples of cartilage repair interfaces were obtained from 9 horses evaluated in a previous study of cartilage repair[4]. Full thickness, 15 mm diameter osteochondral defects were created bilaterally in the lateral trochlear ridge of the femur and randomly treated with one of 3 treatments (1. fibrin glue alone, 2. naïve chondrocytes, or 3. IGF-I transfected chondrocytes; n=3/group). Horses were sacrificed at 8 months. Samples were sectioned across the repair interface, submerged in PBS, and imaged using Raman spectroscopy (785 nm laser, 25 µm resolution). After imaging, the same sample was fluorescently stained with 5-DTAF for 45 minutes, rinsed in PBS, and mounted to a microscale test frame on an inverted confocal microscope. Samples were axially compressed 8% with images collected during compression (Figures 1AB). Collagen composition was assessed using the Pro/Hypro peak area (830-890 cm^{-1}) normalized to the native deep zone peak area for each sample (Figure 1C). Tissue strains were calculated from tracked displacements in MATLAB using the open source software Ncorr (Figure 1D). The interface was optically identified from confocal images for all samples. As a control, native equine articular cartilage with no repair tissue was also tested (n=2).

RESULTS: Under compressive loading (i.e. E_{yy}), intact cartilage control samples experienced low shear strains <1% (Fig 2A, purple). One naïve chondrocyte treated sample did not show elevated shear strains across the interface similar to the control samples, indicating a well-integrated repair (Fig 2A, blue). Collagen concentration did not predict shear strains for the controls or the well-integrated naïve chondrocyte samples as there was little change in shear strain ($\Delta|E_{xy}|$ <1%, Fig 2B). In contrast, the remaining 8 repair samples experienced shear strain magnitudes ($|E_{xy}|$) exceeding those in control samples. The three samples from the fibrin treated group experienced changes in shear strain magnitude between 1-2% (Fig 2C), and repair tissue strains decreased with increasing collagen concentration (R^2=0.78, Fig 2D). The naïve chondrocyte and IGF-I treated samples experienced $\Delta|E_{xy}|$ greater than 2% (Fig 2EG). A subset of these samples had a peak in shear strain ~500 µm from the interface (Fig 2E), and shear strains in the repair tissue decreased with increasing collagen (R^2=0.36, Fig 2F). The remaining samples that experienced $\Delta|E_{xy}|$ greater than 2% had no discernable shear strain pattern (Fig 2G), and there was no relationship between collagen and repair shear strains for these samples (R^2=0.1, Fig 2H).

DISCUSSION: We developed a technique that allows for microscale spatial correlation of composition and mechanical behavior at a soft tissue repair interface. One repair sample was found to have superior integration as it achieved low shear strains consistent with intact control samples, but no repair treatment resulted in superior integration. Well-integrated samples showed no relationship between collagen concentration and shear strain as there was little variation in shear strain ($\Delta|E_{xy}|$ <1%). However, repair samples with larger shear strain variation (1%< $\Delta|E_{xy}|$) experienced higher shear strains in areas with lower collagen, and are therefore more prone to failure in interface regions with lower collagen content. Additionally, this technique allowed for the identification of a characteristic peak shear strain response 500 µm from the repair interface for 3 samples (Fig 2E).

SIGNIFICANCE: Microscale composition and strain mapping identified collagen as a key compositional feature for reducing strains at the repair interface in a clinically relevant *in vivo* cartilage repair model, and therefore increasing collagen deposition at the repair interface could be a potential target for improving cartilage repair integration.

REFERENCES: [1] Ahsan & Sah *Osteoarthritis Cartilage* 1999 [2] Wayne+ *J Biomech Engng* 1991 [3] DiMicco+ *Osteoarthritis Cartilage* 2002 [4] Ortved+ *Mol Ther* 2015

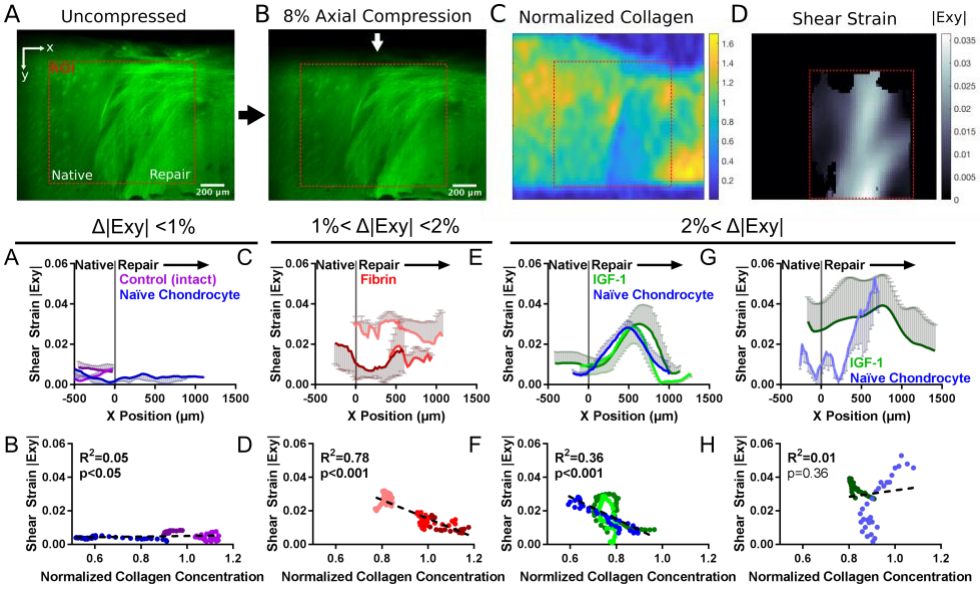

Figure 1: Articular cartilage repair interface from naïve chondrocyte treated group before (A) and after compression (B). Normalized collagen concentration from Raman spectroscopy identified lower collagen levels at the interface (C), and shear strains tracked from compression were elevated at the interface (D).

Figure 2: Control samples and a naïve chondrocyte treated sample had little variation in shear strains (A) and no change with collagen composition (B). Fibrin repair samples experienced 1%< $\Delta|E_{xy}|$ <2% (C) and strains decreased with increased collagen (D). A shear strain peak at 500 µm from the interface was observed for samples with $\Delta|E_{xy}|$ >2% (E), and shear strains also decreased with collagen concentration (F). Remaining samples with $\Delta|E_{xy}|$ >2% had no strain pattern (G) and no relationship with collagen concentration (H).