

# Evaluation of the biomechanical properties in native and engineered cartilage with a non-destructive imaging technique

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**INTRODUCTION:** Current clinical approaches to cartilage repair and regeneration often result in mechanically inferior scar tissue (fibrocartilage) rather than functional hyaline cartilage of the articular surface. Traditional tissue engineering approaches for cartilage regeneration using chondrocytes or stem cells with natural or synthetic hydrogel scaffolds have not resulted in a clinical solution. We have pioneered a means to generate tissue-engineered articular cartilage matrix from chondrocytes, cells specific to cartilage, called dynamic Self-Regenerating Cartilage (dSRC). dSRC is produced by allowing the chondrocytes themselves to generate a natural matrix rather than constraining seeded cells to a specific hydrogel scaffold. Assessment of the biomechanical properties is essential to evaluate the development of engineered cartilage. We present here a new imaging methodology, Laser Speckle rheology (SHEAR), to evaluate the shear biomechanical properties of native and engineered cartilage. Current methods for mechanical testing of articular cartilage include compression, lubrication, tension, nanoindentation, and integration. Rheometry is the established conventional method for measuring shear mechanical properties, but it has limitations. SHEAR circumvents these limitations and is the only method that can measure shear viscoelastic modulus in whole tissue/tissue-engineered scaffold non-invasively without direct mechanical deformation. In contrast, shear rheometry applies only to bulk properties and requires direct mechanical deformation of the specimen; thus, it cannot assess the shear properties of engineered constructs in a non-destructive manner. SHEAR evaluates the shear modulus of native and engineered cartilage (dSRC) in a passive, non-contact, and non-destructive manner and additionally can also map microscopic variations in shear modulus within a tissue sample. The aim of this study was two-fold: (a) to demonstrate the feasibility of using SHEAR to evaluate the biomechanical properties in native and engineered cartilage and (2) to determine the biochemical properties of dSRC as a function of maturation time compared to native hyaline cartilage.

**METHODS:** dSRC were formed by placing freshly harvested swine knee chondrocytes in sealed 15-mL polypropylene tubes followed by culture on an oscillating platform at 40 cycles per minute for 14 days at 37°C. During this time, the chondrocytes generate a new extracellular matrix, forming a sheet or pellet of dSRC. SHEAR biomechanical analysis was performed on dSRC samples after 4, 8, and 12 weeks of in vitro culture and compared to native swine articular cartilage. **SHEAR:** A 632-nm HeNe laser beam was linearly polarized, expanded, and focused onto the back focal plane of a 10X 0.25 NA air objective for collimated illumination of the sample. Backscattered light was collected through the same objective lens via a beam splitter and acquired by a high-speed CMOS camera. A time series consisting of 1000 speckle frames over a 280 x 560  $\mu\text{m}$  region of interest was acquired at a frame rate of 430 Hz (dSRC) or 590 Hz (native cartilage). Speckle intensity fluctuation was analyzed to reconstruct the shear viscoelastic modulus,  $G^*$ , via the Generalized Stokes-Einstein Relation. The optical resolution of the SHEAR microscope was 1.5  $\mu\text{m}$ , supporting the spatial resolution of the reconstructed shear modulus map of approximately 15  $\mu\text{m}$ . dSRC sheets were placed flat in a petri dish for imaging. Native cartilage was imaged with the articular surface facing illumination. Three specimens were measured for each of the 4, 8, and 12-week dSRC and native cartilage groups. Values of shear modulus magnitude,  $|G^*|$ , measured in each group at 15 Hz, were combined for multiple pairwise comparisons (one-way ANOVA). **Shear rheometry of native cartilage:** Native swine articular cartilage was extracted with a biopsy punch sliced into a disk with a diameter of 8 mm and thickness of 1 mm. The bulk shear modulus of the cartilage disk was measured with an oscillatory shear rheometer. A frequency sweep test over the 0.5-40 Hz range was performed at 23°C with an oscillatory torque of 10  $\mu\text{N}\cdot\text{m}$  (resulting in <0.2% strain) and a normal force control at 3 N. Four cartilage disks from 1 knee were measured.

**RESULTS:** We have demonstrated the consistent formation of dSRC matrix in vitro, in the form of sheets or pellets, after 4, 8, and 12 weeks. The contiguous matrix is solid and robust. A 2D SHEAR map of the superficial shear modulus for each specimen was obtained over a 1.9 x 2.1 mm region by laterally translating the specimen on a 2-axis motorized stage. An increasing trend in shear moduli can be observed in dSRC pellets from 4 to 12 weeks, indicating that the engineered cartilage gradually gains mechanical integrity over the course of culture under dynamic reciprocating motion. dSRC pellets attain approximately 15–30% of the native shear moduli of native cartilage after 12 weeks under these conditions.

**DISCUSSION:** Micromechanical properties of the engineered cartilage (dSRC) and native swine articular cartilage were evaluated with SHEAR for the first time. SHEAR is a non-destructive and non-invasive tool that enables microscale spatial mapping of shear modulus in a wide range of biological samples. The biomechanical properties of the engineered cartilage (dSRC) increase over time. The engineered cartilage (dSRC) shear moduli are about a third of the native cartilage. Due to its non-invasive and non-contact capabilities, SHEAR is particularly suitable for the evaluation of fragile and arbitrarily shaped engineered cartilage such as dSRC.

**SIGNIFICANCE/CLINICAL RELEVANCE:** SHEAR is suitable for evaluating fragile and arbitrarily shaped engineered cartilage such as dSRC due to its non-invasive and non-contact capabilities. SHEAR technology can be used to evaluate the shear modulus of cartilage in vivo (current studies in our laboratory). SHEAR has the potential to assess osteoarthritis disease progression and the efficacy of regenerative treatments in vivo.

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## IMAGES AND TABLES:

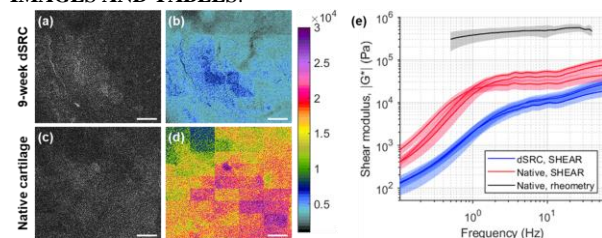


Figure 1. Non-invasive measurement of shear modulus in dSRC and ex vivo porcine articular cartilage. (a, c) Representative snapshots of laser speckle images recorded in dSRC and native cartilage. (b, d) Shear stiffness maps of dSRC and native cartilage obtained from speckle movies in (a, c). Color bar represents  $|G^*|$  at 3 Hz in Pascals. Scale bar: 400  $\mu\text{m}$ . (e) Frequency-dependent  $|G^*|$  of dSRC and native cartilage measured by SHEAR (blue, red) and parallel-plate shear rheometry (black). Results from 3 dSRC pellets and 3 native cartilage disks are shown in separate curves for SHEAR. The solid line and shaded region represent the mean and standard deviation of  $|G^*|$  map (2.2 mm x 2.5 mm area) from each sample. For rheometry, the solid line and shaded region represent the mean and standard deviation of bulk measurements in n=4 native cartilage disks.