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Tissue-engineered articular cartilage exhibits tension–compression nonlinearity reminiscent of the native cartilage

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ABSTRACT

The tensile modulus of articular cartilage is much larger than its compressive modulus. This tension–compression nonlinearity enhances interstitial fluid pressurization and decreases the frictional coefficient. The current set of studies examines the tensile and compressive properties of cylindrical chondrocyte-seeded agarose constructs over different developmental stages through a novel method that combines osmotic loading, video microscopy, and uniaxial unconfined compression testing. This method was previously used to examine tension–compression nonlinearity in native cartilage. Engineered cartilage, cultured under free-swelling (FS) or dynamically loaded (DL) conditions, was tested in unconfined compression in hypertonic and hypotonic salt solutions. The apparent equilibrium modulus decreased with increasing salt concentration, indicating that increasing the bath solution osmolarity shielded the fixed charges within the tissue, shifting the measured moduli along the tension–compression curve and revealing the intrinsic properties of the tissue. With this method, we were able to measure the tensile (401 ± 83 kPa for FS and 678 ± 473 kPa for DL) and compressive (161 ± 33 kPa for FS and 348 ± 203 kPa for DL) moduli of the same engineered cartilage specimens. These moduli are comparable to values obtained from traditional methods, validating this technique for measuring the tensile and compressive properties of hydrogel-based constructs. This study shows that engineered cartilage exhibits tension–compression nonlinearity reminiscent of the native tissue, and that dynamic deformational loading can yield significantly higher tensile properties.

1. Introduction

Native articular cartilage exhibits low compressive Young's modulus (E_{-Y}; 0.1–1 MPa) (Wang et al., 2002a, 200b; Park et al., 2004) relative to its high tensile Young's modulus (E_{+Y}; 3–6 MPa) (Kempson et al., 1968; Williamson et al., 2003a, 2003b), which regulates the mechanical response of cartilage in unconfined compression (Cohen et al., 1998; Soulhat et al., 1999; Soltz and Ateshian, 2000; Huang et al., 2001, 2003Chahine et al., 2004) and in contact configurations (Krishnan et al., 2004). Previous studies indicate that a high E_{+Y} combines with interstitial fluid pressurization to produce an elevated dynamic compressive modulus (G^*) at least 6 × greater than E_{-Y} (Soltz and Ateshian, 2000; Park et al., 2003; Park and Ateshian, 2006). This mechanism arises because the high E_{+Y} of cartilage restricts lateral expansion of the tissue upon axial compression. Since the interstitial fluid cannot exude rapidly, the initial deformation must be nearly isochoric, occurring only if the interstitial fluid pressurizes considerably to help resist the compressive load. In order to distribute and absorb loads similar to native cartilage, engineered cartilage should exhibit similar tension–compression nonlinearity (TCN). Previous studies have demonstrated that chondrocyte seeded agarose constructs are capable of achieving native values for E_{-Y} and glycosaminoglycan (GAG) content (Kelly et al., 2006; Bian et al., 2009a, 2009b, 2010; Natoli et al., 2009a, 2009b). While E_{-Y} and E_{+Y} have been independently analyzed previously, the role of TCN in developing engineered cartilage has not been addressed. Therefore, we adapted a method used previously to examine TCN in native cartilage (Chahine et al., 2004), which permits determination of

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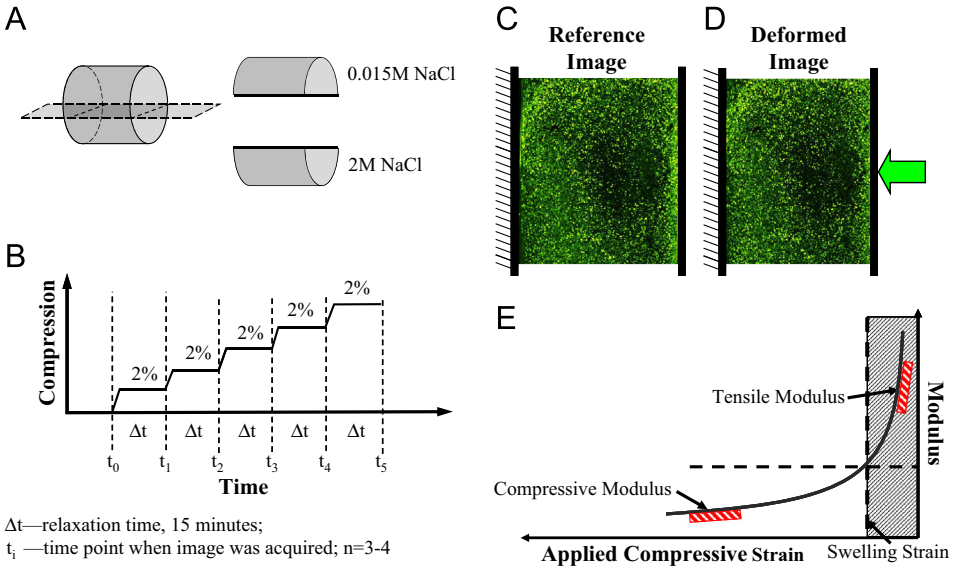

Fig. 1. (A) Prior to osmotic testing the semi-cylindrical samples were equilibrated in the appropriate saline solution (solution A and solution B). The order of testing was rotated to prevent bias from multiple testing of the same semi-cylindrical sample. (B) For each test, the semi-cylinder was loading into the microscope testing device and compressed at 2% increment to a final compression of 10–12% and allowed to equilibrate for 15 min between each compression. Images were acquired prior to each compression. (C) and (D) Images of typical construct before (C) and after (D) compression. (E) Schematic tension–compression curve: By controlling the osmolarity of the bathing fluid we can swell or shrink a test sample along its tension–compression curve. By then applying a compressive load to a swollen construct and comparing its properties to an unswollen construct we can extract its tensile modulus under compression without special grips. Hatched region represents region where specimen is under tension.

E_{+Y} and E_{-Y} from a single specimen. Using this technique, E_{+Y} and E_{-Y} were found to be similar to values obtained from direct measurements using more conventional methods (Huang et al., 2001, 2003, 2005; Wang et al., 2002a, 200b; Williamson et al., 2003a, 2003b; Park et al., 2004). This technique uses osmotic swelling to place the sample in an initial state of tension. Small compressive displacement increments are then applied and the resultant loads are measured during the tissue's transition from tensile to compressive strains.

The underlying principle behind this technique stems from the fact that hydrated tissues possessing a fixed charge density swell and stiffen under hypotonic loading. Conversely, these tissues shrink and become softer under hypertonic loading via concomitant changes in Donnan osmotic pressure (Maroudas, 1976; Lai et al., 1991; Wang et al., 2002a, 2002b). Therefore, compressing swollen tissues allows for measurement of E_{+Y} when the *applied* compressive strain is smaller than the *true* tensile swelling strain of the solid matrix (Fig. 1E). As the strain increases, the measured response yields E_{-Y}.

The objective of this study is to determine the TCN in engineered cartilage grown under free swelling (FS) or dynamically loaded (DL) cultures. We hypothesize that DL will improve both E_{+Y} and E_{-Y} of engineered cartilage compared to FS controls. Therefore, we acquired a spectrum of engineered cartilage moduli from tension to compression using compressive loading in the presence of osmotic swelling and report for the first time E_{+Y} and E_{-Y} of the same engineered cartilage specimen.

2. Materials and methods

2.1. Sample preparation and tissue culturing

Articular cartilage was harvested from adult canine knee joints. Three to five joints were used and cells were pooled from all joints, as previously described (Lima et al., 2007; Bian et al., 2010). Cartilage chunks were digested with 390 U/mL collagenase type VI (Sigma) for 11 h with slight agitation. Isolated chondrocytes were passaged in DMEM containing 10%FBS, 10 ng/mL PDGF, 1 ng/mL TGF-β1, 5 ng/mL FGF-2 and 1% antibiotics/antimycotics. Chondrocytes were seeded in 2% (w/v) agarose at 30×10^6 cells/mL and cast between parallel plates.

Cylindrical constructs ($\varnothing4.0\times2.3$ mm) were cored and cultured in DMEM containing 50 μg/mL L-proline, 100 μg/mL sodium pyruvate, 1% ITS+ premix (BD Biosciences), 100 nM dexamethasone, 1% antibiotics/antimycotics, 50 μg/mL ascorbic acid, and 10 ng/mL TGF-β3 (R&D Systems). Constructs were maintained in FS culture for 14 days. After day 14, constructs were either cultured under DL conditions or maintained under FS conditions until day 42. For DL, a sinusoidal deformation with a magnitude of 10% surface-to-surface strain at a frequency of 1 Hz (5 days/week, 3 h/day continuous) was applied, with an initial 2% tare strain.

2.2. Average mechanical properties

A custom unconfined compression device (Mauck et al., 2000) with rigid-impermeable glass loading platens and a 250 g load cell (Honeywell Sensotec) was used to assess the E_{-Y} of the whole construct at days 0, 14, 28 and 42 (n=4–11). Before each test, the construct thickness and diameter were measured, specimens were equilibrated under a 0.02 N tare load, and a 10% strain was applied at 0.05% strain/sec. E_{-Y} was calculated from the equilibrium stress and initial cross-sectional area. The average unconfined dynamic modulus (G^*) was subsequently measured by superimposing a 2% sinusoidal strain at 1 Hz.

To establish whether E_{-Y} of engineered tissue was dependent on strain, immature bovine articular chondrocytes were harvested and used to create constructs, as described above. On days 0 and 42, these constructs were used in a series of stress-relaxation tests at 5%, 10%, 15%, and 20% strains (n=5). For comparison, freshly harvested explants were also tested.

2.3. Direct tensile testing of agarose constructs

Acellular constructs were cast as described above to test the tensile mechanical properties of 2% (w/v) agarose. Rectangular samples were cut from the slab (length=12 mm, width=3 mm, thickness=2.34 mm, n=7). Sandpaper grips were glued to the top and bottom edge of the sample and secured in metal grips that attached to the mechanical testing device (Instron). A quasi-static ramp was applied at a rate of 0.01%/s, and load and displacement data were recorded until failure. The tensile modulus was calculated as the slope of the stress-strain curve.

2.4. Tension–compression analysis

A custom glass-bottom device was mounted on the stage of an inverted microscope and used to mechanically test semi-cylindrical specimens (Wang et al., 2002a, 2002b, 2003; Chahine et al., 2004). Prior to testing, the thickness of each construct was measured, the constructs were halved, and each half was maintained in isotonic saline (Fig. 1A). At days 0 (n=4) and 42 (n=4–6), the semi-