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Local and global measurements show that damage initiation in articular cartilage is inhibited by the surface layer and has significant rate dependence

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ABSTRACT

Cracks in articular cartilage are a common sign of joint damage, but failure properties of cartilage are poorly understood, especially for damage initiation. Cartilage failure may be further complicated by rate-dependent and depth-dependent properties, including the compliant surface layer. Existing blunt impact methods do not resolve local cartilage inhomogeneities and traditional fracture mechanics tests induce crack blunting and may violate underlying assumptions of linear elasticity. To address this knowledge gap, we developed and applied a method to indent cartilage explants with a sharp blade and initiate damage across a range of loading rates (strain rates 0.5%/s–500%/s), while recording local sample deformation and strain energy fields using confocal elastography. To investigate the importance of cartilage's compliant surface, we repeated the experiment for samples with the surface removed. Bulk data suggest a critical force at which the tissue cuts, but local strains reveals that the deformation the sample can sustain before reaching this force is significantly higher in the surface layer. Bulk and local results also showed significant rate dependence, such that samples were easier to cut at faster speeds. This result highlights the importance of rate for understanding cracks in cartilage and parallels recent studies of rate-dependent failure in hydrogels. Notably, local sample deformation fields were well fit by classical Hookean elasticity. Overall, this study illustrates how local and global measurements surrounding the initiation of damage in articular cartilage can be combined to reveal the importance of cartilage's zonal structure in protecting against failure across physiologically relevant loading rates.

1. Introduction

Cracks in articular cartilage are a common sign of joint damage. In clinical settings, fissures are often observed during arthroscopic inspection of an injured joint (Bauer and Jackson, 1988; Curl et al., 1997). Such injuries predispose patients to chronic joint damage and disease, including osteoarthritis (Brown et al., 2006). In orthopedics, clinicians and researchers acknowledge the importance of cracks by including them in various arthroscopy and histopathology grading schemes (Outerbridge, 1961; Pritzker et al., 2006). Basic science and engineering studies have also associated cartilage cracks with increased cell death and matrix degradation (Anderson et al., 2011; Lewis et al., 2003), suggesting they can disrupt the homeostasis that is essential for joint health. As such, cracks in cartilage have the potential to be an important early marker of cartilage damage and disease. However, beyond these basic observations, cartilage cracks are poorly understood and many questions must be answered before cracks can guide clinical decision-making.

One complication for studying cracks in cartilage is that cartilage is highly anisotropic and heterogeneous, with mechanical properties and composition that vary with depth. The superficial 100–300 μm of tissue, known as the surface layer, has lower compressive and shear moduli than the bulk (Buckley et al., 2010; Schinagl et al., 1997), which may be explained by variations in composition (Silverberg et al., 2014). Additionally, the collagen alignment varies with depth, where fibers near the surface are predominantly parallel to that surface with an additional in-plane alignment known as the split-line pattern (Below et al., 2002;

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Benninghoff, 1925; Roth and Mow, 1980). Recent results have further demonstrated that the surface layer may serve a mechanically protective role (Bartell et al., 2015; Buckley et al., 2013). Beyond variations in composition and alignment, cartilage has a complex rate-dependence from both viscoelastic and poroelastic effects (Mow et al., 1980). All of these factors may influence cartilage fracture and failure, but are difficult to disentangle without studying cartilage at spatial resolutions of around ten microns.

Experimentally, cartilage cracks are generally studied in two contexts: blunt overload or traditional fracture mechanics geometries, such as the notch test. In overload experiments, such as a drop-tower test, a blunt object rapidly and forcefully impacts cartilage (Argatov and Mishuris, 2015; Henak et al., 2016; Jeffrey et al., 1995, 1995; Repo and Finlay, 1977; Scott and Athanasiou, 2006; Waters et al., 2014). When this loading is faster than the poroelastic time scale, fluid is trapped and pressurizes, thus stressing the surrounding solid matrix, which ultimately ruptures (Morel and Quinn, 2004). This loading is analogous to physiologic injuries, but the geometry of the sample and loading both influence fluid pressurization and so the material properties are difficult to disentangle. Moreover, the exact location of crack initiation is unknown prior to loading, making it experimentally difficult to study local material behavior. In contrast, traditional fracture mechanics experiments apply standardized sample and loading geometries to articular cartilage that are designed to concentrate stress at a particular point, leading to material failure (Ahsan and Sah, 1999; Chin-Purcell and Lewis, 1996; Oyen-Tiesma and Cook, 2001; Taylor et al., 2012). By linking a specific geometry to linear elasticity, such data can be used to calculate material properties, such as toughness, which describes the ability to absorb energy without cracking. In soft tissues, however, finite strains may violate the assumptions of linear elasticity and it is unclear to what degree this affects the understanding of cartilage failure. Studies applying the well-known notch test to cartilage show the tissue failing by crack-blunting and plastic yielding rather than traditional brittle crack propagation, indicating that tissue microstructure inhibits the stress concentration necessary to propagate brittle-like cracks (Hui et al., 2003; Stok and Oloyede, 2007). Additionally, such methods study steady-state crack growth, rather than damage initiation, though the latter may be equally important physiologically. Thus, neither blunt overload nor traditional fracture tests are adequate to fully understand cracks in articular cartilage, especially damage initiation.

To address this knowledge gap, this study aimed to develop an indentation-based method to study damage initiation in articular cartilage. By indenting samples with a sharp blade, we created a crack at a known location, in a well-defined geometry, and with more stress-concentration than notch tests (Johnson, 1987). Moreover, because the crack location was known, we could utilize recently developed confocal elastography techniques to study both global and local material behavior and investigate the importance of material inhomogeneity and finite strains when interpreting damage initiation in cartilage. We further investigated how rate modulates the observed damage initiation by indenting over a wide range of loading rates. Combined, this method simultaneously observed the local, global and time-dependent processes that are potentially important to understanding damage initiation in articular cartilage.

2. Methods

2.1. Sample preparation

Chondral explants were harvested from condyles of 13 neonatal calves (sex unknown; Gold Medal Packing, Oriskany, NY) (Fig. 1A). Explants were immersed in PBS and stored at 4 °C for up to 48 h. For testing, explants were trimmed to 3 mm deep and bisected perpendicular or parallel to the known split-line direction (Silverberg et al., 2013), creating 125 hemi-cylindrical samples. In some samples, a sledge microtome was used to remove 500 μm from the articular surface, creating a surface-removed group. Information about each sample was recorded, including source animal, condyle (medial or lateral), orientation (parallel or perpendicular to split-line), time between dissection and testing, and surface condition (intact or removed).

2.2. Indentation device

To test cartilage failure properties, a razor blade (#27-251, Razor Blade Company, Van Nuys, CA; ~150 nm tip diameter, Appendix A) was mounted to the piezoelectric-driven plate of a Tissue Deformation Imaging Stage (TDIS; Harrick Scientific, Ithaca, NY) and used to indent cartilage, thus creating cracks in a known location (Fig. 1B and C). Samples were glued to the fixed plate of the TDIS, as described previously (Buckley et al., 2010). During indentation, force was measured using a 2 kg (19.6 N) load cell (S300, Strain Measurement Devices, Wallingford, CT) and blade displacement was recorded from the piezoelectric monitor. The blade was driven to 500 μm displacement and retracted at fixed speeds of 2.5–1000 μm/s. Each sample was immersed in PBS throughout testing and indented only once with a fresh blade.

The TDIS was mounted onto an inverted confocal microscope (LSM 5 LIVE, Carl Zeiss Inc., Oberkochen, Germany), to observe local sample deformation. For imaging contrast, samples were stained for 50 min in 14 μM 5-DTAF (Buckley et al., 2010). Videos of deformation during indentation were recorded at 15 to 60 frames per second, depending on blade speed, with a 512 pixel (666 μm) field of view (Fig. 1D).

2.3. Data analysis

For each experiment, force (F) verses blade depth (d) plots were characterized. Force was smoothed using a moving-average filter with a window size of 1 μm blade displacement (scaled in time based on the blade speed). The blade depth was calculated as the blade displacement minus the slight displacement of back plate resulting from the strain-based load cell. The critical force, F_C, and depth, d_C, at first-cut were extracted, and the data were integrated up to this critical point to extract strain energy, W_C. For these three responses (critical force, depth, and energy), mixed-effects linear regression models were implemented to test which parameters significantly affected each response (Appendix B).

Confocal videos were processed to extract local deformation and energy fields. Videos were analyzed from zero blade depth to just beyond the point of first cut using Ncorr (2D image correlation; widow size 19.7 μm, grid spacing 3.9 μm, smoothing radius 27.5 um; Blaber et al., 2015). Images were generally well tracked, except the area closest to the blade tip. When the first cut in the bulk response did not agree with that observed in the confocal video, likely due to misalignment, samples were excluded from local deformation analysis.

Deformation fields were used to compute local strain energy density. Strain energy was calculated by assuming a 2D neo-Hookean constitutive model with depth-dependent Lamé parameters taken from the literature (Schinagl et al., 1997; Silverberg et al., 2013). Local deformations were compared to the 2D functional form predicted by contact mechanics. According to Johnson (1987), line loading of a Hookean elastic half-space yields the radial displacement field:

$$u_r(r,\theta) = -\frac{(1-\upsilon^2)}{\pi E} 2P\cos\theta \ln\frac{r}{r_0} - \frac{(1-2\upsilon)(1+\upsilon)}{\pi E} P\theta\sin\theta \qquad (1)$$

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