Journal of Biomechanics ∎ (∎∎∎∎) ∎∎∎–∎∎∎

Contents lists available at ScienceDirect

Journal of Biomechanics

journal homepage: www.elsevier.com/locate/jbiomech
www.JBiomech.com

Measuring microscale strain fields in articular cartilage during rapid impact reveals thresholds for chondrocyte death and a protective role for the superficial layer

Lena R. Bartell [a,*], Lisa A. Fortier [b], Lawrence J. Bonassar [c,d], Itai Cohen [e]

[a] School of Applied and Engineering Physics, C7 Clark Hall, Cornell University, Ithaca, NY 14853, USA
[b] Department of Clinical Sciences, Cornell University, Ithaca, NY, USA
[c] Department of Biomedical Engineering, Cornell University, Ithaca, NY, USA
[d] Sibley School of Mechanical and Aerospace Engineering, Cornell University, Ithaca, NY, USA
[e] Department of Physics, Cornell University, Ithaca, NY, USA

ARTICLE INFO

Article history:
Accepted 26 May 2015

Keywords:
PTOA
Indentation
Elastography
Arthritis initiation
Injury

ABSTRACT

Articular cartilage is a heterogeneous soft tissue that dissipates and distributes loads in mammalian joints. Though robust, cartilage is susceptible to damage from loading at high rates or magnitudes. Such injurious loads have been implicated in degenerative changes, including chronic osteoarthritis (OA), which remains a leading cause of disability in developed nations. Despite decades of research, mechanisms of OA initiation after trauma remain poorly understood. Indeed, although bulk cartilage mechanics are measurable during impact, current techniques cannot access microscale mechanics at those rapid time scales. We aimed to address this knowledge gap by imaging the microscale mechanics and corresponding acute biological changes of cartilage in response to rapid loading. In this study, we utilized fast-camera and confocal microscopy to achieve roughly 85 μm spatial resolution of both the cartilage deformation during a rapid (~3 ms), localized impact and the chondrocyte death following impact. Our results showed that, at these high rates, strain and chondrocyte death were highly correlated ($p < 0.001$) with a threshold of 8% microscale strain norm before any cell death occurred. Additionally, chondrocyte death had developed by two hours after impact, suggesting a time frame for clinical therapeutics. Moreover, when the superficial layer was removed, strain – and subsequently chondrocyte death – penetrated deeper into the samples ($p < 0.001$), suggesting a protective role for the superficial layer of articular cartilage. Combined, these results provide insight regarding the detailed biomechanics that drive early chondrocyte damage after trauma and emphasize the importance of understanding cartilage and its mechanics on the microscale.

1. Introduction

Osteoarthritis involves the degradation of articular cartilage in joints and is a leading cause of disability (Birchfield, 2001; Jackson et al., 2001; Murphy et al., 2008). Clinically, 12% of osteoarthritis is post-traumatic (PTOA), wherein initiation stems from a distinct mechanical insult (Brown et al., 2006) and trauma is known to initiate progressive cartilage degradation (Anderson et al., 2011; Brown et al., 2006; Felson et al., 2000; Fischenich et al., 2015; Goldring and Goldring, 2010; Radin et al., 1972; Vellet et al., 1991). After decades of research, PTOA initiation is poorly understood and a cure remains elusive (Anderson et al., 2011; Goldring and Goldring, 2010; Krasnokutsky et al., 2008; Scott and Athanasiou, 2006).

Understanding PTOA has proven difficult, due in part to the complexities of cartilage material properties and the scope of the disease. For example, cartilage has a distinct superficial layer that is more compliant and dissipates more shear energy than the bulk (Buckley et al., 2013; Schinagl et al., 1996; Silverberg et al., 2014; Wang et al., 2002; Wong et al., 2008). Additionally, a traumatic, pathologic event can deliver forces over a fraction of a second (10^{-3} s) (Aspden et al., 2002, p. 200), while a patient may not present with symptoms for years (10^{8} s) (Scott and Athanasiou, 2006). This represents ten orders of magnitude in time that are important to the problem. Currently available animal and explant models can monitor bulk cartilage mechanics on injury time scales (10^{-3} s) and investigate biomechanical effects of trauma over hours

* Corresponding author. Tel.: +1 607 255 8853; fax: +1 607 255 6428.
E-mail address: lrb89@cornell.edu (L.R. Bartell).

http://dx.doi.org/10.1016/j.jbiomech.2015.05.035
0021-9290/

to months (10^1 – 10^6 s) (Backus et al., 2011; Jeffrey et al., 1995; Newberry et al., 1998; Repo and Finlay, 1977; Scott and Athanasiou, 2006; Waters et al., 2014). Studies have also investigated mechanics at the cellular level (Abusara et al., 2011; Guilak et al., 1995; Upton et al., 2008). Although loading rate affects cellular response (Moo et al., 2013), methods to measure microscale mechanics (e.g. 10–100 μm) at physiological time scales (e.g. 10^{-1}–10^0 s) have only recently been developed (Buckley et al., 2010). This leaves an important, unexplored gap in understanding microscale cartilage changes during and immediately following rapid traumatic injury.

The purpose of this study was to design and implement techniques to investigate the microscale mechanics of articular cartilage during rapid impact and to statistically describe the acute chondrocyte response. In particular, our method correlated microscale tissue strains during rapid impact (85 μm and 1 ms resolutions) with the microscale, time-dependent decrease in cell viability following that impact (85 μm and 10 min resolutions). This correlation enabled identification of microscale thresholds and sensitivities of chondrocytes to microscale deformation. Additionally, we tested for mechanical and biological changes in response with the superficial layer removed. This directly linked the mechanics of rapid cartilage impact to acute biological changes, giving new insight on the mechanisms of PTOA initiation.

2. Methods

A custom method enabled the correlation of chondrocyte death and microscale mechanics: chondral explants were impacted while a fast-camera recorded their rapid deformation; subsequent cell death was captured via confocal microscopy.

2.1. Impact device

The setup consisted of a custom impact device on an inverted confocal microscope (LSM 5 LIVE, Carl Zeiss Inc., Jena, Germany) (Fig. 1) with a 10 × objective. The confocal housing interfaced with a high-speed camera (v7.1, Vision Research, Wayne, NJ) and a mercury arc lamp (HBO 100, Carl Zeiss Inc., Germany) to enable epi-fluorescence microscopy at 1000 frames per second.

The custom impact device (Fig. 1A) includes a spring (2.96 kN/m, McMaster-Carr, Elmhurst, IL) of adjustable compression which, upon triggering, drives an aluminum piston and the attached 0.8 mm diameter steel rod (McMaster-Carr, Elmhurst, IL) into the sample (design inspired by Alexander et al., 2013). All impacts were energy-controlled at ~0.12 J (8.9 mm spring compression) to consistently cause cell death without micro-cracking and thus can be considered moderately pathologic (Aspden et al., 2002). Each sample was glued, as described previously (Buckley et al., 2010), to a cantilevered aluminum backplate with the articular surface facing the impact tip. Impacts were observed from below through a glass slide, showing the rod's circular cross section approaching the sample (Fig. 1B). Samples were mounted 2 mm above the glass slide, leaving a fluid layer in between. Using known weights, the backplate was calibrated as a cantilevered spring (152 kN/m). Backplate motion during impact was tracked using intensity-based image correlation in MATLAB (The MathWorks Inc., Natick, MA) and used to measure impact force. Peak force was combined with area of impact indentation to estimate peak bulk stress.

2.2. Sample preparation

Eighteen full-thickness, 6 mm-diameter chondral explants (without bone) were harvested sterilely from the outer rim of the tibial plateau of 6 neonatal calves (sex unknown, assumed random; Gold Medal Packing, Oriskany, NY). Explants were immersed in phenol red-free DMEM with 10% FBS (Invitrogen, Waltham, MA) and 1% antibiotics (100 × penicillin–streptomycin, Mediatech, Manassas, VA) and stored at 4 °C for up to 48 h. Using tissue slicer blades (Stadie-Riggs, Thomas Scientific, Swedesboro, NJ), explants were cut in half to create two hemi-cylinders, and trimmed to approximately 2.5 mm deep. Paired hemi-cylinders were used as control and impacted samples. For eight explants, 1 mm was removed from the articular surface, maintaining the 2.5 mm thickness, creating two sample populations: surface-intact and surface-removed.

2.3. High-speed deformation imaging

A fluorescent stain with a photobleached grid was used to visualize tissue motion during impact. All samples were stained in 28 μM 5-DTAF (ex/em 492/516 nm; Invitrogen, Waltham, MA) for 45 min followed by a 10-min PBS rinse to provide general cartilage staining (Buckley et al., 2010; Silverberg et al., 2014). Using a precision wire mesh (TWP Inc., Berkley, CA) a 120 μm grid was photobleached on the samples. This grid size was chosen to be resolvable over motion blur, to ensure adequate cell counting statistics (> 50 cells/grid box), and to capture tissue mechanical inhomogeneities. Paired control and impact samples were mounted side-by-side to the backplate and surrounded by PBS. Upon impact triggering, cartilage deformation was recorded using the fast camera.

2.4. Cell viability imaging

Chondrocyte death after impact was imaged. 2 μM ethidium homodimer (EthD) (Invitrogen, Waltham, MA) was added to the cavity, staining for 30 min before impact. Dead cells were imaged every 10 min for 3 h following impact. Preliminary, 12-h studies demonstrated that nearly all cell death occurred within 3 h. To

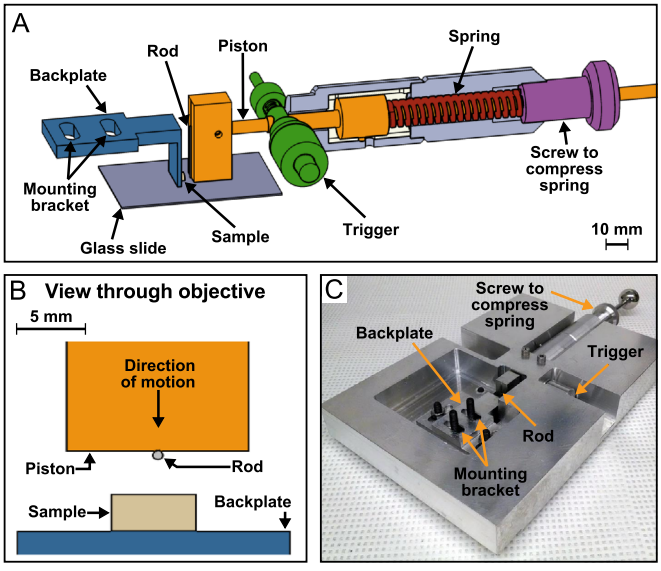

Fig. 1. A technique for rapid impact microscopy was developed. A custom impact device (A and B) included a compressed spring which was released by a trigger to drive the piston and attached rod into the sample. The sample, which was mounted on the backplate, was immersed in PBS to maintain tissue hydration during testing. The cantilevered backplate was mounted to the stable body of the impact device via a mounting bracket. A glass slide allowed the sample to be viewed from below. The impact device was designed to mount on an inverted confocal microscope such that the objective looks up through the glass slide, as depicted (B). The microscope also interfaced with a mercury arc lamp and high-speed camera (not shown) to allow epifluorescence imaging at 1000 frames per second. (C) A photograph of the impact device.