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Chondrocyte deformation under extreme tissue strain in two regions of the rabbit knee joint

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ABSTRACT

Articular cartilage and its native cells—chondrocytes—are exposed to a wide range of mechanical loading. Chondrocytes are responsible for maintaining the cartilage matrix, yet relatively little is known regarding their behavior under a complete range of mechanical loads or how cell mechanics are affected by region within the joint. The purpose of this study was to investigate chondrocyte deformations *in situ* under tissue loads ranging from physiological to extreme (0–80% nominal strain) in two regions of the rabbit knee joint (femoral condyles and patellae). Local matrix strains and cell compressive strains increased with increasing loads. At low loads the extracellular matrix (ECM) strains in the superficial zone were greater than the applied tissue strains, while at extreme loads, the local ECM strains were smaller than the applied strains. Cell compressive strains were always smaller than the applied tissue strains and, in our intact, *in situ* preparation, were substantially smaller than those previously found in hemi-cylindrical explants. This resulted in markedly different steady-state cell volume changes in the current study compared to those working with cartilage explants. Additionally, cells from different regions in the knee exhibited striking differences in deformation behavior under load. The current results suggest: (i) that the local extracellular and pericellular matrix environment is intimately linked to chondrocyte mechanobiology, protecting chondrocytes from potentially damaging strains at high tissue loads; and (ii) that cell mechanics are a function of applied load and local cartilage tissue structure.

1. Introduction

Articular cartilage is a fibrous connective tissue that lines the bony surfaces of articulating joints. It is essential to the health and integrity of a joint, providing load transmission and a relatively frictionless surface for gliding of joint surfaces. Structurally, cartilage is composed of an extracellular matrix (ECM), cells called chondrocytes, and their associated pericellular matrix (PCM). The PCM is a thin region of tissue surrounding chondrocytes and is thought to play a significant role in modulating the mechanical environment of the cartilage cells (Choi et al., 2007; Guilak et al., 2006; Poole, 1997). Chondrocytes are responsible for maintaining the tissue ECM through the synthesis of structural macromolecules and contribute lubricating proteins found on the cartilage surface and in the synovial fluid (Mow, Ratcliffe 1992; Stockwell, 1979). Osteoarthritis (OA) is a common joint disease characterized by a breakdown of the cartilage ECM. Mechanical loading has been shown to stimulate the metabolic activity of chondrocytes and has been linked to the adaptive/degenerative changes associated with OA (Sah et al., 1989; Wieland et al., 2005). Previous studies have shown that chondrocytes deform under load in many different preparations, including: isolated chondrocytes (Nguyen et al., 2010; Ofek et al., 2009), chondrocytes embedded in agarose gel constructs (Freeman et al., 1994; Knight et al., 1998; Lee et al., 2000), and chondrocytes in tissue explants (Guilak, 1995; Guilak et al., 1995; Wong et al., 1997). These previous works have greatly improved our understanding of cartilage and chondrocyte mechanobiology and serve as an important foundation for current research. However, these studies may not reflect the intrinsic behavior of chondrocytes in their physiological environment, surrounded by an intact ECM and PCM. Recently, techniques have been devised which allow for the analysis of chondrocytes in the intact cartilage attached to its native bone (Abusara et al., 2011; Han et al., 2009). It has been shown that cells are greatly influenced by their matrix environment, specifically by whether it is intact or has been compromised such as in tissue explant experiments (Nugent et al., 2011;

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Turunen et al., 2011). Therefore, studying chondrocytes in their intact environment has the potential to provide new insight towards the complex mechanobiological processes associated with joint homeostasis and remodeling. Additionally, cartilage and chondrocyte behavior under high strains has received little attention in previous work. Extreme tissue strains can occur in cartilage under certain loading conditions, such as impact loading, and thus are possible *in vivo* (Flachsmann et al., 2001; Krueger et al., 2003).

The composition and mechanical properties of articular cartilage are known to differ between joint regions (Arokoski et al., 1999; Jurvelin et al., 2000; Korhonen et al., 2002; Little and Ghosh, 1997, Treppo et al., 2000). Furthermore, local cartilage deformation and chondrocyte gene expression have been shown to vary topographically within a joint (Bevill et al., 2009). Therefore, the purpose of this study was twofold: to quantify chondrocyte deformations in the intact tissue for applied strains ranging from physiological to extreme; and secondly, to determine whether chondrocyte deformations vary with respect to location in a joint. It was hypothesized that (i) chondrocyte deformation increases with increasing tissue load up to a certain threshold value, but then, for increasing applied tissue loads, would not deform to the same extent because of limits imposed by the ECM and PCM; and that (ii) chondrocytes from different joint regions deform differently for a given applied tissue load.

2. Methods

2.1. Sample preparation

Cartilage samples were obtained from the knees of six to eight month-old New Zealand white rabbits. All experiments were approved by the Animal Ethics Committee of the University of Calgary. Patellae (PAT) and medial and lateral femoral condyles (COND) were extracted from the knee joints ($n=4$ per joint region) and stripped of all non-cartilaginous connective tissue while maintaining the underlying bone. Specimens were tested within 60 h of sacrifice and the order of testing randomized to mitigate the effect of time. All tissue samples not tested immediately were stored in Dulbecco's Modified Eagle's Medium (DMEM, Gibco, OR, USA) at 4 °C until the time of testing. Incubation in DMEM maintains the cartilage mechanical properties and improves cell viability beyond the maximum 60 h test point in this study (Bonassar et al., 1995; Schachar et al., 1994). Calcein-AM (excitation: 488 nm, emission: 515 nm, Invitrogen, USA) was suspended in DMEM at a concentration of 5 μM. Specimens were incubated in the Calcein-AM solution for one hour at 21 °C prior to testing. After staining, samples were rinsed in phosphate-buffered saline (PBS) for 15 minutes, then fixed in a specimen holder using dental cement for thickness measurement. Care was exercised to ensure samples were mounted such that loading occurred in the target area: a flat region of physiological relevance. For patellar cartilage, loading was applied slightly proximally and approximately midway between medial and lateral aspects; for the medial and lateral femoral condyles, loading was applied to the summit of the condyles (Han et al., 2012). Tissue thickness was determined by needle indentation conducted at two locations close to the loaded region.

2.2. Mechanical testing and confocal imaging

After determination of tissue thickness, cartilage samples were immersed in a PBS solution and placed into the chamber of a custom-designed *in situ* indentation system mounted to the stage of a confocal laser scanning microscope (LSM 510, Zeiss Inc., Germany) (Fig. 1A,B). The system consists of a light-transmissible indenter, piezoactuator, load cell, and displacement transducer (Han et al., 2009). The specimen position was adjusted to the point of initial contact between indenter and cartilage surface and characteristic groupings of chondrocytes were identified such that the same cells (typically $n=4$–8 cells per sample) were observed throughout the experiment. A series of static loads were then applied to the samples in the following order: 10%, 20%, 30%, 40%, 60%, and 80% compressive strain (Fig. 2). Tissue strains $\leq 20\%$ were considered physiologically relevant (Armstrong et al., 1980; Herberhold et al., 1999), whereas strains $\geq 60\%$ were considered extreme. Loading was applied using a light-transmissible, cylindrical sapphire indenter (diameter=2 mm) at an average rate of $0.7 \pm 0.2\%$/s (3.5 ± 1 μm/s) and controlled by a custom-written program (LabVIEW, National Instruments, USA). After the loading ramp was completed, displacements were held for 15 minutes to allow the tissue to reach near steady-state conditions (Fig. 2) (Clark et al., 2003; Morel et al., 2005). Confocal image sections were obtained using a 40×0.8 NA 0.17 mm cover glass-corrected water immersion objective (Zeiss Inc., Germany). Spatial resolution in the x–y plane (parallel to the cartilage surface) was 0.41 μm × 0.41 μm and images along the depth (z direction) were obtained every 0.5 μm. Images were taken in the superficial zone of the cartilage samples, from the surface to a depth of 50 μm.

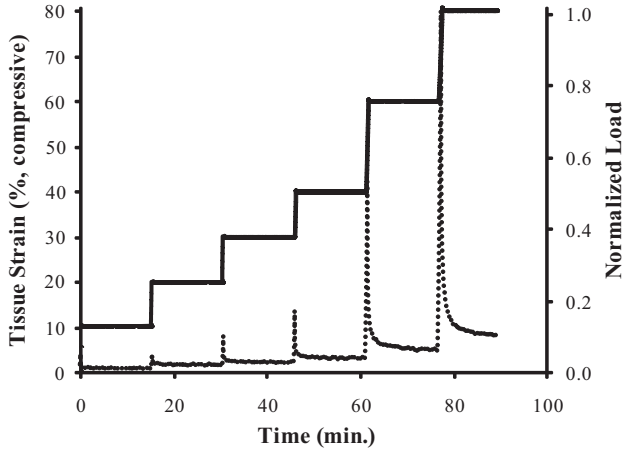

Fig. 2. Compressive loading protocol applied to the cartilage samples (black line) and corresponding force-time response of an exemplar condylar cartilage specimen (red line) showing the near steady-state conditions at the time of imaging (15 min after load application).

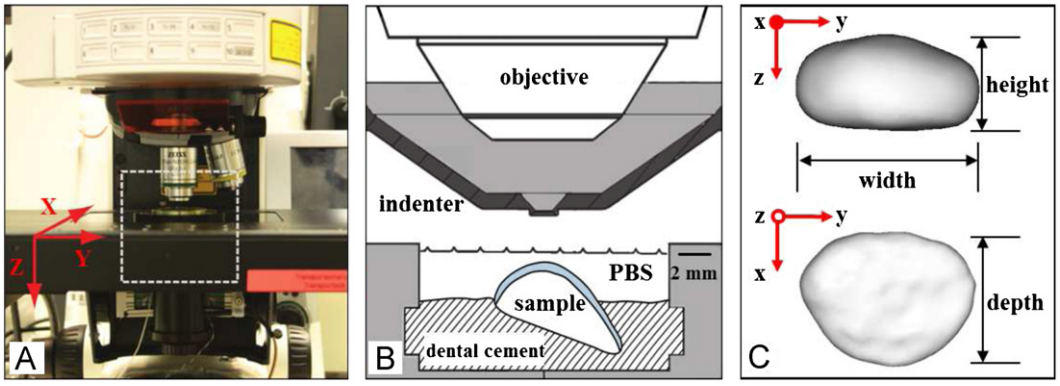

Fig. 1. (A) In situ chondrocyte loading system assembled and mounted to the stage of a confocal laser scanning microscope. (B) Schematic illustration of a femoral condyle fixed in the tissue holder component showing the indenter and objective configurations (scale bar=2 mm). (C) Definition of chondrocyte morphology used for cell reconstruction and data analysis.