Journal of Biomechanics 47 (2014) 1004–1013

Contents lists available at ScienceDirect

Journal of Biomechanics

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

Extracellular matrix integrity affects the mechanical behaviour of in-situ chondrocytes under compression

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ARTICLE INFO

Article history:
Accepted 3 January 2014

Keywords:
Chondrocyte mechanics
Tissue lesions
Tensile strain
Multi-scale finite element modelling
Transient response
Osteoarthritis

ABSTRACT

Cartilage lesions change the microenvironment of cells and may accelerate cartilage degradation through catabolic responses from chondrocytes. In this study, we investigated the effects of structural integrity of the extracellular matrix (ECM) on chondrocytes by comparing the mechanics of cells surrounded by an intact ECM with cells close to a cartilage lesion using experimental and numerical methods. Experimentally, 15% nominal compression was applied to bovine cartilage tissues using a light-transmissible compression system. Target cells in the intact ECM and near lesions were imaged by dual-photon microscopy. Changes in cell morphology ($N_{cell}=32$ for both ECM conditions) were quantified. A two-scale (tissue level and cell level) Finite Element (FE) model was also developed. A 15% nominal compression was applied to a non-linear, biphasic tissue model with the corresponding cell level models studied at different radial locations from the centre of the sample in the transient phase and at steady state. We studied the Green-Lagrange strains in the tissue and cells. Experimental and theoretical results indicated that cells near lesions deform less axially than chondrocytes in the intact ECM at steady state. However, cells near lesions experienced large tensile strains in the principal height direction, which are likely associated with non-uniform tissue radial bulging. Previous experiments showed that tensile strains of high magnitude cause an up-regulation of digestive enzyme gene expressions. Therefore, we propose that cartilage degradation near tissue lesions may be due to the large tensile strains in the principal height direction applied to cells, thus leading to an up-regulation of catabolic factors.

1. Introduction

Every day movements of synovial joints occur nearly without friction on a thin layer of articular cartilage covering the ends of bones. Articular cartilage consists of four major components: water (65–80%), collagen fibres (10–20%), proteoglycans (4–7%) and chondrocytes (1–10%) (Mow et al., 1984). The collagen fibres form a structural network that provides tensile strength and structural integrity to the tissue, while proteoglycans (PGs) give the tissue compressive strength through their water-retaining properties (Poole, 1997). Collagen and PGs form the extracellular matrix (ECM) that provides protection for the chondrocytes from excessive deformation during joint loading. Chondrocytes, in turn, maintain the integrity of the ECM by load-dependent release of matrix molecules (Bachrach et al., 1995; Honda et al., 2000; Poole, 1997; Sauerland et al., 2003).

Damage to articular cartilage often leads to joint degeneration and osteoarthritis (OA) (Link et al., 2003), a debilitating disease that causes joint pain and stiffness, and affects the quality of life of many people, especially the elderly (Wieland et al., 2005). Focal cartilage lesions may be found in otherwise healthy people with no symptoms of OA (Cicuttini et al., 2005). Once a lesion is formed, damage seems to spread from the lesion to the rest of the cartilage (Squires et al., 2003) resulting in cartilage erosion (Wang et al., 2006). There is also evidence for the presence of digestive enzymes in the vicinity of tissue lesions (Shlopov et al., 1997), suggesting a change in local chondrocyte behaviour, and an active release of proteolytic enzymes from cells near cartilage lesions (Wieland et al., 2005).

The mechanical microenvironment of chondrocytes is known to influence their biosynthetic activity (Grodzinsky et al., 2000; Guilak et al., 1997; Stockwell, 1987). Depending on the type (e.g., compression, tension) and nature (e.g., magnitude, frequency) of tissue loading, the biosynthetic response of chondrocytes is anabolic or catabolic

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0021-9290/$ - see front matter © 2014 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.jbiomech.2014.01.003

(Bachrach et al., 1995; Griffin and Guilak, 2005; Honda et al., 2000; Sauerland et al., 2003). An intact ECM has also been shown to be crucial in the proper regulation of cell volumes (Turunen et al., 2011). Since collagen fibres and proteoglycans are compromised at the site of tissue lesions, chondrocytes adjacent to lesions are thought to experience an altered mechanical milieu resulting in a catabolic response. However, the details as to which mechanical factors drive this detrimental change in chondrocyte behaviour remain unclear.

Therefore, the purpose of this study was to investigate the effects of cartilage tissue lesions on the mechanical behaviour of *in-situ* chondrocytes using experimental and numerical approaches. Differences in cell mechanics between intact and lesioned ECM conditions may provide insight into the mechanical factors that induce catabolic responses in chondrocytes near tissue lesions. Since the ECM near lesions is damaged, we hypothesised that cells near lesions experience excessive deformations during joint loading, thus producing different mechanical signals than cells surrounded by an intact matrix.

2. Methodology

2.1. Experiments

2.1.1. Specimen preparation

Metatarsal–phalangeal joints of adult cows ($N_{joint}=12$) were obtained from the local abattoir. 10 mm × 10 mm rectangular osteochondral blocks (experimental set $N_{joint}=6$; time lapse control set $N_{joint}=3$; loading order control set $N_{joint}=3$) of 5 mm-thickness were harvested from the medial load bearing surface of the joints and maintained in serum-free culture medium consisting of Dulbecco's Modified Eagle's Medium (DMEM) supplemented with HEPES, L-Glutamine, Penicillin/Streptomycin and L-ascorbate (Sigma Aldrich, Canada) (Moo et al., 2013). These explants were cultured in an incubator (5% CO_2) at 37 °C until the experiment (within 2 days) (Changoor et al., 2010; Moo et al., 2011).

On the day of experiments, cylindrical samples of 6 mm diameter were obtained from the rectangular blocks using a cylindrical punch. Cells in the specimens were stained with calcein AM (8 μM; excitation$_{max}$: 488 nm; emission$_{max}$: 515 nm; Molecular Probe, USA) and CFDA-SE (0.2 mM; excitation$_{max}$: 492 nm; emission$_{max}$: 517 nm; Molecular Probe, USA) for 30 min (Moo et al., 2013). Specimens were then washed three-times in a dye-free saline solution prior to attachment to a specimen holder using dental cement. Tissue thickness was determined by needle indentation at three locations close to the loaded region. Specimens were immersed in serum-free culture medium throughout the experiment to prevent dehydration.

2.1.2. Mechanical loading protocol and dual-photon excitation imaging

Osteochondral specimens were compressed in a custom-designed light-transmissible compression system (Fig. 1a) (Han et al., 2009) mounted onto the stage of a laser scanning microscope (LSM 510, Zeiss Inc. Germany) coupled to a mode-locked Chameleon XR infrared laser (Coherent Inc., USA).

The specimen was first subjected to a tare load of 0.3 N. A 15% nominal tissue strain was then applied in 30 s with a low-friction, impermeable compression platen allowing for lateral expansion and lateral fluid flow from the cartilage. The final displacement was held for 20 min. Image stacks were acquired at the centre of the tissue (intact ECM condition) at three time points: before compression (initial phase), 20 min into compression (compression phase), and 20 min after load removal (recovery phase). After that, the tissue was cut vertically into half (at the dashed line in Fig. 1a and b) and the same 15% tissue strain was re-applied. Cells located within 150 μm from the cut edge (lesioned ECM condition) were imaged using the same protocol as described for the intact tissue condition (Fig. 1b).

In a control set not involving tissue compression (time lapse control), cells in intact and lesioned ECMs were imaged at three time points (0 min, 20 min, and 40 min). In another control set (loading order control), two consecutive cycles (compression-recovery) of 15% tissue strain were applied to cartilage specimens in the intact ECM, and cells were imaged at the three time points for each loading cycle.

Chondrocyte images (experimental set $N_{cell}=32$ for both boundary conditions; time lapse control set $N_{cell}=23$ for intact ECM condition, and $N_{cell}=21$ for lesioned ECM condition; loading order control set $N_{cell}=20$) were obtained using dual-photon excitation (DPE) microscopy with a 40 × /0.8 NA water immersion objective (Zeiss Inc., Germany) at a wavelength of 780 nm. DPE microscopy was used to minimise photobleaching effects (Moo et al., 2013). A series of planar images (pixel size: 0.41 μm × 0.41 μm; bit-depth: 8) were acquired along the objective axis (z-axis) at 1 μm intervals. Images were taken within 40 μm of the articular surface.

2.1.3. Image analysis

The spatial coordinates of paired cells were tracked throughout the experiment (intact ECM conditions=28 pairs, lesioned ECM conditions=30 pairs). Changes in vertical separation of paired cells were measured for calculating local compressive tissue strains.

Image stacks were analysed using the '40% maximum intensity' method (Bush and Hall, 2001), with the highest threshold chosen for image segmentation. Distortions along the z-axis were corrected through microsphere calibration (Han et al., 2009).

Three-dimensional (3D) reconstruction of chondrocytes was performed using custom-written code (VTK, Kitware Inc., USA) for cell volume computation (Alyassin, 1999). 3D-reconstructed chondrocytes were best-fit to an ellipsoid (Feddema and Little, 1997). Cell widths and depths were defined along the major and minor axes of the cross section taken perpendicular to the cell height, respectively (Fig. 1c).

2.1.4. Statistical analysis

All data were expressed as means ± 1 standard error (SEM). Where applicable, means were compared using two-way repeated measures ANOVA, Student's independent t-test, or Student's paired t-test with $\alpha=0.05$.

2.2. Finite Element (FE) Modelling

An established two-scale (cartilage-chondrocyte) model (Han et al., 2007; Moo et al., 2012; Wu and Herzog, 2000) was used to study the *in-situ* mechanical behaviour of chondrocytes.

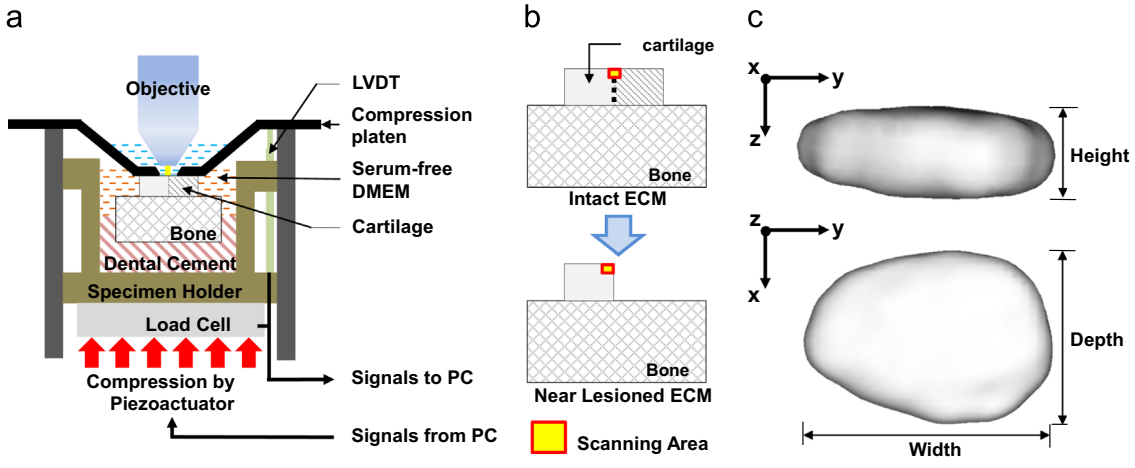

Fig. 1. (a) Schematic illustration of the *in-situ* compression system. The osteochondral block was compressed against a light-transmissible compression platen by a piezoactuator. The vertical displacement was monitored by a displacement transducer. The images of *in-situ* chondrocytes were scanned using dual photon laser scanning microscopy (b) Scanning protocol used for live cell imaging. The cells at the centre of the cartilage sample (intact ECM condition) were studied before the removal of tissue at the dashed line. Following the removal of that tissue, cell responses near the tissue lesion were studied (lesioned ECM condition). (c) Definition of chondrocyte morphology used for three-dimensional reconstruction. Images drawn are not to scale.