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Technical note

A compression system for studying depth-dependent mechanical properties of articular cartilage under dynamic loading conditions

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ABSTRACT

The biological activities of chondrocytes are influenced by the mechanical characteristics of their environment. The overall real-time mechanical response of cartilage has been investigated earlier. However, the instantaneous local mechano-biology of cartilage has not been investigated in detail under dynamic loading conditions. In order to address this gap in the literature, we designed a compression testing device and implemented a dual photon microscopy technique with the goal of measuring local mechanical and biological responses of articular cartilage under dynamic loading conditions. The details of the compression system and results of a pilot study are presented here. A 15% ramp compression at a rate of 0.003/s with a subsequent stress relaxation phase was applied to the cartilage explant samples. The extra cellular matrix was imaged throughout the entire thickness of the cartilage sample, and local tissue strains were measured during the compression and relaxation phase. The axial compressive strains in the middle and superficial zones of cartilage were observed to increase during the relaxation phase: this was a new finding, suggesting the importance of further investigations on the real-time local behavior of cartilage. The compression system showed promising results for investigating the dynamic, real-time mechanical response of articular cartilage, and can now be used to reveal the instantaneous mechanical and biological responses of chondrocytes in response to dynamic loading conditions.

1. Introduction

Articular cartilage is composed of a solid phase, a fluid phase, chondrocytes and a small amount of charged anions (carboxyl or sulfate groups) and cations (e.g., $Na+$, $K+$, $Ca++$) [1], which are dissolved in the interstitial fluid. Using advanced microscopy techniques, it has been possible to elucidate some of the biological processes inside chondrocytes and selected mechanical interaction of chondrocytes with their environment. For instance, the deformation of the extracellular matrix (ECM) was found to be a stimulus regulating chondrocyte function [2–4]. The superficial zone in articular cartilage has been the region of primary interest for researchers because of the importance of surface-to-surface contact and the high density of biologically active cells [5]. Moreover, imaging superficial zone chondrocytes is less challenging because of their proximity to the surface of the tissue. Cartilage has depth-dependent structural properties [6–9] that provide protection against shear, compressive, and tensile stresses. The compressive modulus in the deep zone of cartilage is as much as 27 times greater than that of the superficial layer in bovine cartilage [10,11]. Therefore, merely studying the superficial zone of cartilage does not represent well the mechanical behavior and biological processes of the entire tissue.

In order to better understand the natural environment of chondrocytes and ECM, a local investigation across the entire cartilage depth is required. Although bulk cartilage mechanics have been investigated under dynamic loading conditions [12–18], the local mechanical behavior of cartilage under dynamic conditions has not been studied systematically. Custom-designed compression testing devices have been used to determine the depth-dependent properties of cartilage and chondrocytes [8–11,18–25]. Bartell et al. [24] used a custom-designed testing setup to study the relationship between chondrocyte death and microscale mechanics under impact loading conditions. Their epi-fluorescence microscopy technique allowed for imaging at a frequency of 1000 frames per second, but the spatial resolution of 85 µm was not sufficient to study local cell strains. Guilak et al. [9,22] developed

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an imaging technique for measuring chondrocyte deformations across the entire cartilage depth but only for steady-state static conditions.

Cell nuclei have frequently been used as fiducial markers to measure full thickness cartilage strains with video microscopy [9–11,26]. Measurement of the positions of cells nuclei is subject to errors, and strains determined in this manner may not accurately represent tissue deformations because cell nuclei are not fixed in the cartilage ECM. Han et al. [23] designed a system for simultaneous superficial cell strain and loading measurements in explant cartilage samples. However, due to the limitations of the testing device [23], dynamic measurements could only be made for the most superficial 80 µm of the cartilage.

The biological response of chondrocytes is thought to be depth-dependent and to occur virtually exclusively when cells deform dynamically. Therefore, a proper understanding of the mechanobiology of articular cartilage depends crucially on detailed information of the depth-dependent dynamic properties and associated biological signaling of articular cartilage chondrocytes. Consequently, the purpose of this study was to design a device that allows for measuring tissue strain and chondrocyte deformation and signaling across the entire cartilage sample thickness dynamically at high temporal resolution. This device was then used for a pilot study aimed at measuring full-depth, local, dynamic and static deformations of the cartilage ECM for comparisons with previous studies.

2. Methods

2.1. Sample preparation

Cylindrical cartilage-bone explant samples of 4 – 6 mm diameter were extracted using a dental drill (KaVo MULTIflex® system INTRAmatic 20 ES handpiece with Hu-Friedy 6 mm trephine bur). The cylindrical samples were then cut into two identical semi-cylinders (see supplementary material).

2.2. Compression testing device components

A schematic of the compression system is shown in Fig. 1. Displacement, force and actuator signals are collected using a custom-written program (LabView software, National Instruments, TX, USA). The compression testing device is positioned on the *x-y* stage of a multi-photon microscope and the cartilage sample is imaged continuously during the mechanical testing. The system delivers up to 0.3 mm/s displacement rate with 2 mm stroke and load capacity of 300 N capable of applying strain rates as high as 50%/s on 2 mm thick cartilage samples. A variety of loading protocols at physiological rates can be applied, such as single/multiple ramp compressions and sinusoidal/triangular/square cyclic compressions. The main components of the compression testing device are an *xyz* linear stage, an actuator, a load cell, a DVRT, a connecting rod, a compression plate, a sample holder and a bath enclosure (Fig. 1). Detail of compression testing device is available in the supplementary material.

2.3. Pilot test: measuring depth-dependent tissue strain

2.3.1. Samples

Eighteen cylindrical core plugs of 6 mm diameter were prepared from the tibial plateau of skeletally immature pig knee joints (age 4 ± 1 month) according to the steps explained above. The average thickness of the samples was 648 µm with a standard deviation of SD = 35 µm. Following harvesting, the cartilage samples were rinsed with PBS and stored at +4 °C until testing the next day. Prior to testing, cartilage samples were immersed in PBS for one hour at room temperature. A 0.17 mm thick cover slip was used to cover the flat surface of the sample, which prevented fluid exchange from this face (see the supplementary material).

2.3.2. Loading protocol

The thickness of the cartilage samples was measured from the microscope images and was used as a reference to calculate the traveling distance of the compression plate. A 15% linear ramp compression was applied using an impermeable compression plate (unconfined compression) in 50 s and was maintained for another 300 s, during which stress relaxation occurred.

2.3.3. Microscopy

The compression testing device was secured on a multi-photon excitation microscope (Olympus FVMPE-RS, Olympus Corp., Japan) equipped with a 25x/1.05 NA water-immersion objective (XLPLN25XWMP2, Olympus Corp., Japan). Two filters (FF01 400/40 and FF01 520/60, Semrock Inc., USA) permitted the collection of second harmonic generation (SHG) and two-photon fluorescence (TPF) signals upon excitation by two multi-photon lasers tuned to 800 nm and 940 nm respectively. Prior to applying the compressive loading, selected areas of the cartilage were burned by narrowing the image frame into a 4×4 µm rectangular frame and using 100 – 110 mW of laser power. Squares obtained in this manner were used as markers and were tracked during compression testing to calculate local strains across the cartilage thickness (Fig. 2). The most superficial square was placed 10 to 30 µm below the cartilage surface, followed by 3 squares at 50 µm depth intervals. Further squares were placed at intervals of 80 – 120 µm. The spatial resolution, imaging frame size, and capturing rate were 0.995 µm/pixel, 512×512 µm and 2.2 frame/sec, respectively. The sensitivity of the detector was increased to obtain a clear contrast between the matrix and the markers (Fig. 2). The cartilage samples were imaged continuously during the compression phase and the following stress relaxation phase. Three time points were selected to present the local strains in the direction of compression through the thickness of the cartilage samples: (i) immediately after the 15% nominal strain was reached ($t = 50$ s, dynamic state), (ii) after two minutes of stress relaxation ($t = 170$ s), and (iii) after five minutes of stress relaxation ($t = 350$ s).

2.3.4. Statistical analysis

To compare the results of this study with those available in the literature, the axial compressive strains across the cartilage thickness were averaged for three sections; $0.0 < \zeta < 0.1$, $0.1 < \zeta < 0.3$, and $0.3 < \zeta < 1.0$, roughly representing the superficial, middle, and deep zones of the cartilage, respectively, where ζ is the normalized depth. The articular surface and tidemark are represented by $\zeta = 0$ and $\zeta = 1$, respectively. Wilcoxon Signed-Rank testing ($\alpha = 0.05$) was used to compare strains in the stress relaxation and dynamic states. The *t*-test ($\alpha = 0.01$) for two independent means was used to compare strains in superficial and middle zones.

3. Results

The cross-section of a cartilage sample along its thickness is shown in Fig. 2 before and after applying the 15% compression. The averaged ECM local axial strains in the superficial, middle, and deep zones in the dynamic and relaxation phases are shown in Fig. 3. As expected, the local strains were significantly greater in the superficial zone compared to the other regions for the dynamic and stress relaxation states, with average magnitudes of 41.2% and 47.7%, respectively. The ECM in the deep layers, i.e. $\zeta > 0.3$, had average compressive strains of 5.4% and 4.5% in the dynamic and stress relaxation states, respectively (see Fig. 3).

The dynamic strains in the middle zone (13.7%), i.e. $0.1 < \zeta < 0.3$, were close to the applied 15% nominal strain.

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