Osmotic Loading of in Situ Chondrocytes in Their Physiological Environment

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**Introduction:** One of the signs of osteoarthritis is swelling of articular cartilage, leading to decreased, hypotonic extracellular osmolarity and increased cell volumes [1, 2]. Results from earlier studies on isolated chondrocytes or chondrocytes near the cutting edge of explant tissues suggested that cell volume changes following hypotonic osmotic challenge are complete within minutes of exposure, [3, 4], while it takes hours for the extracellular matrix to reach its steady-state. However, none of the earlier studies were made on chondrocytes of fully intact cartilage samples attached to their native bone. The mechanical environment of chondrocytes in fully intact tissue differs conceptually from that of isolated cells and cells near the cutting surface of explant tissues, thus, the results from these earlier studies may have little, if any, physiological meaning.

The aim of this study was to analyze cell volume changes and swelling of the extracellular matrix of chondrocytes in intact rabbit patellae. Volume changes were evaluated as a function of time post hypotonic osmotic challenge using confocal laser scanning microscopy. We hypothesized that cell volume changes might be significantly modulated by the extracellular matrix, and that they might be substantially different from those presented for isolated cells or cells of explant tissues.

**Materials and Methods:** Twelve knee joints of mature New Zealand white rabbits were prepared for osmotic loading experiments within 30 minutes post mortem. Intact patellae were harvested and incubated in a physiological phosphate buffered saline solution (300 mOsm) with 50 μM Calcein-AM (Molecular Probes, Eugene, OR, USA) for 30 minutes. After staining, samples were attached to a Petri dish with dental cement, and then the dish was filled with the phosphate buffered saline solution.

A standard confocal laser scanning microscope (Zeiss LSM 510, Carl Zeiss, Germany) was used for cell imaging. Images of the chondrocytes were obtained through the articular surface. A series of planar optical sections were obtained at a 0.5 μm spacing (z-axis) using a x40, 0.8 N.A. water immersion objective (Zeiss, Carl Zeiss, Germany). Cells from the top 50 μm of the articular cartilage were analyzed. The pixel size in the x-y plane was 0.4x0.4 μm. After imaging the samples in a physiological saline solution, the saline concentration was changed to hypotonic (10 mOsm) in ~30 seconds. Confocal images were taken at 5 - 240 minutes following solution exchange.

The Visualization Toolkit 5.0.1 (Kitware Inc.) was used to reconstruct 3D cell images, and a Python programming code with a threshold of 40% of the maximum fluorescence intensity [4] was used for cell volume and area calculations. Local tissue strain as a function of time was estimated from the changes in the distances between cell centroids. Swelling of the entire tissue was estimated from the displacements of the cell centroids. Wilcoxon signed rank tests were used for statistical analysis (SPSS Inc., Chicago, Illinois, USA).

**Results:** Cell volumes changed rapidly initially (0 - 5 min, p < 0.05), followed by constant cell volumes for an hour (5 → 15, 60 min; 15 → 60 min, p > 0.05) (Fig. 1). However, two and four hours following the hypotonic challenge, cell volumes were significantly larger than those at all earlier time points (5, 15, 60 → 120 min; 5, 15, 60, 120 → 240 min, p < 0.05) (Figs. 1 and 2). Time-dependent changes in cell surface areas were similar to those in cell volumes.

The rate of tissue swelling was fast immediately following the osmotic challenge, and then slowed down but was still substantial between two and four hours (Fig. 2). Local tissue strains caused by tissue swelling were small but positive (data not shown).

**Discussion:** Confocal laser scanning microscopy was used to study chondrocyte volume changes of intact rabbit patellae following hypotonic osmotic loading. The time-dependent volume changes of chondrocytes were substantially different than those found earlier for isolated cells or in situ cells of explant tissues [1, 3, 4]. In the short term (0 - 5 min), we observed ~25% increase in cell volumes, while others found over 50% and several 100% increases in volumes for in situ cells of explants and isolated chondrocytes, respectively [1], suggesting that the peri- and extracellular matrix may have a remarkable capability to protect chondrocytes in their native environment. In the long term (up to 4 hours) chondrocytes were significantly larger than in the short term, a phenomenon which has not been observed before, suggesting that the peri- and extracellular matrix with long-lasting swelling properties may have modulated cell volumes as a function of time. These differences are likely caused by the different mechanical boundary conditions of chondrocytes in our study and those of previous studies where isolated cells were used or cell mechanics were measured within 40 μm from the cutting edge of the explants. Thus, our results add novel and potentially relevant information to the mechanical behaviour of chondrocytes in the intact joint. They further suggest that cartilage degeneration, such as occurs in osteoarthritis disease, should be studied in the intact tissue with fully intact mechanical and biological boundary conditions, as results from isolated chondrocytes and cells observed in explant tissues might vastly differ from those observed here in the intact tissue, and may lead to inappropriate evaluation and treatment of osteoarthritis disease.


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