Introduction

In diarthrotic joints, articular cartilage cells, the chondrocytes, experience relatively high levels of intermittent hydrostatic pressure (5 to 10 MPa) as a normal physiological stimulus. Clinical observations in humans and experimental studies in animal models confirm that mechanical loads provide an essential stimulus for maintenance of normal articular cartilage extracellular matrix homeostasis. Alterations in joint loading due to immobilization, ligamentous laxity, traumatic impact and increased subchondral bone stiffness result in pathological changes in cartilage characteristic of osteoarthritis. Previous in vitro studies confirm that extracellular matrix homeostasis. Alternations in joint loading due to immobilization, ligamentous laxity, traumatic impact and increased subchondral bone stiffness result in pathological changes in cartilage characteristic of osteoarthritis. Previous in vitro studies confirm that extracellular matrix homeostasis.

Methods

Normal adult human articular cartilage samples (N=5) were collected steriley from patients undergoing knee reconstruction (3), amputation (1) or total joint arthroplasty for avascular necrosis (1). Chondrocytes were released from matrix following overnight enzymatic digestion at 57 °C in 5% CO2 in collagenase (2 mg/ml) in 15 ml of Dulbecco’s Modified Eagle’s Medium/Ham’s F12 (DMEM/F12) containing 25 µg gentamicin. The chondrocytes were collected by centrifugation, washed three times with PBS and plated on 60mm tissue plates at high density (1 x 10^6 cells/cm^2) in DMEM/F12 supplemented with 10% of fetal bovine serum (FBS) and 25 µg/ml gentamicin. Serum-containing medium was removed 24 hours prior to loading by washing in PBS (3x) and serum free medium (DMEM/F12 supplemented with HEPES, sodium, ascorbate, liposomes and gentamicin) was added for an overnight incubation prior to loading. The serum free culture medium was then replaced and the cells were treated with intermittent hydrostatic pressure (IHP) with different levels (1 MPa, 5 MPa and 10 MPa) and different durations of loading. Each culture plates were exposed to IHP in individual heat-sealed bags that were completely filled with serum free DMEM/F12 medium (42 ml). The bags were then submerged in a water-filled high-pressure vessel connected to a Mitsui servo-hydraulic loading frame. Control cultures were maintained under identical culture conditions with the exception that load was not applied. For the experimental protocol, cells and medium samples from each culture plate were harvested either after a loading period of 4 hours per day for 1 day (4x1) or 4 hours per day for 4 days (4x4). Total cellular RNA was extracted and analyzed by the reverse transcription polymerase chain reaction (RT-PCR) using specific primers for aggrecan, type II collagen and beta-actin. The beta-actin signal was used for as a nested reference gene, controlling for differences in amplification conditions and initial concentration or loading the cDNA. PCR products were separated on 1.2% agarose gel and visualized by ethidium bromide staining. The amount of expression relative to beta-actin was analyzed by image quantification of software (QMac v 1.2).

Results

With a loading period of 4 hours per day for 1 day of IHP (4x1), the expression of aggrecan mRNA relative to beta-actin mRNA was not significantly stimulated at a level of 1 MPa (Figure 1). However, aggrecan mRNA signal was increased relative to beta-actin following exposure to IHP at a level of 5 MPa and 10 MPa (p<0.05). The increase in aggrecan mRNA signal level for 5 MPa and 10 MPa was 1.3 fold and 1.5 fold, respectively, when compared to unloaded control cultures. With a loading regimen of 4 hours per day for a duration of 4 days (4x4), the difference between unloaded control culture and the IHP treated cultures was more extensive. Application of IHP increase aggrecan mRNA signal levels by 1.4 fold (1 MPa), 1.8 fold (5 MPa) and 1.9 fold (10 MPa) relative to beta-actin when compared to unloaded cultures. Expression patterns for type II collagen mRNA was different from the pattern of observed for aggrecan. Following IHP treatment for 4x1, the mean level of expression of type II collagen was not significantly stimulated by IHP at any load level. In contrast, expression of type II collagen mRNA signal was upregulated at loading levels of 5 MPa and 10 MPa following a loading regimen of 4 x 4. The mean relative increases of type II collagen signal were 1.6 fold (5MPa) and 1.7 fold (10 MPa), which were statistically different from control cultures (p<0.05).

Discussion

The results presented here show that IHP is an effective stimulus for modulation of articular chondrocyte extracellular matrix macromolecule expression. The modulation of chondrocyte metabolism was subject to both level of load and duration of load. Furthermore, individual matrix molecule expression was differentially regulated by mechanical loading. With respect to type II collagen, a level of 1 MPa of IHP did not influence the mRNA signal at either loading period tested. In addition, short term exposure of cell to IHP (4 x 1) at levels of 5 MPa and 10 MPa did not influence the expression of type II collagen mRNA; however, 5 and 10 MPa of IHP increased type II collagen mRNA if applied for using the 4 x 4 loading regiment. In contrast to collagen, expression of aggrecan mRNA continued to increase with the duration and level of load.

A number of experimental approaches demonstrate that cartilage cells react to confined and unconfined compressive loading under in vitro testing conditions depending on time, magnitude and frequency of loading. The data presented here suggest that repair and regeneration of articular cartilage extracellular matrix will be facilitated by defined loading conditions. Our previous studies with hydrostatic pressure and shear stress demonstrate a plasticity of the articular chondrocyte with respect to modulation of gene expression. The expected outcome of this study is that mechanical stimulation may serve as an adjunctive therapy to facilitate cartilage repair and regeneration in diarthrotic joints.

![Figure 1](image)

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