Cartilage Mechanical Properties after Injury


Abstract: Cartilage injury often results in matrix degradation and in secondary osteoarthritis. This study was designed to correlate cell death and matrix degradation with biomechanical properties of cartilage. Full-thickness mature bovine femoral articular cartilage was harvested as 5 mm diameter cylindrical disk explants. Explants were divided into three groups: control, injury, and IL-1. The injury group was subjected to mechanical compression of 40% strain for five minutes. The IL-1 group was cultured in media containing 10 ng/mL of IL-1beta. The control group was not injured or exposed to IL-1beta. Chondrocyte viability, glycosaminoglycan release in media, and equilibrium creep were measured ten days after injury. A reduction in cell viability was seen after injury. A significant increase in glycosaminoglycan release and in equilibrium creep was detected in injured explants and in explants exposed to IL-1beta. A correlation was also found between the equilibrium creep and glycosaminoglycan content after injury and IL-1beta stimulation.

Keywords: Chondrocyte, cartilage, injury, viability, biomechanics, biomechanical properties, elastic modulus, glycosaminoglycan, trauma, cartilage repair, cartilage degeneration, cartilage lesion.

1Director, Research Associates, and Senior Bioengineer, respectively, Orthopaedic Research Laboratories, Scripps Clinic Center for Orthopaedic Research and Education, 11025 N. Torrey Pines Road, Suite 140, La Jolla, CA 92037.
2Director, Scripps Clinic Center for Orthopaedic Research and Education, 11025 N. Torrey Pines Road, Suite 140, La Jolla, CA 92037.
3Professor, Division of Arthritis Research, Scripps Research Institute, 10550 N. Torrey Pines Road, MEM161, La Jolla CA 92037.
Introduction

It is well recognized that impact loads can cause cartilage injury resulting in matrix degradation and in subsequent loss of cartilage tissue. The relationship between injury and matrix degeneration is readily apparent when the injury involves the mechanical disruption of the cartilage surface. However, cartilage degradation can also occur in the absence of gross matrix damage. Cell death has been reported after cartilage injury\cite{1-4} and can occur in the form of apoptosis\cite{5-9}. A correlation between chondrocyte apoptosis and osteoarthritis has been proposed\cite{10-12}. Chondrocyte apoptosis has also been detected at significantly higher levels in osteoarthritic cartilage relative to normal cartilage in human subjects\cite{12}. However, the link between injury, apoptosis, matrix loss, and loss of mechanical integrity has not been clearly established. The goal of this study was to demonstrate that injury-induced chondrocyte death and glycosaminoglycan release correlated with changes in the mechanical properties of cartilage over time.

Methods

Cartilage Explants

Full-thickness cartilage explants, 5 mm in diameter, were harvested from the weight-bearing region of the femoral condyles of mature fresh bovine joints using a dermal punch. The explants were cultured at 37°C, 5% CO$_2$, and 100% humidity in Dulbecco’s Modified Eagle’s Medium (DMEM) supplemented with 10% fetal calf serum. Explants were cultured in media for 48 hours before injury to allow glycosaminoglycan release levels to stabilize.

Experimental Design

Explants were divided into three groups: control, injury, and IL-1. The injury group was subjected to mechanical injury as described below. The IL-1 group was stimulated with 10 ng/mL of IL-1beta (interleukin-1 beta). IL-1beta is known to cause matrix degradation. The control group was not subjected to injury or to IL-1beta stimulation. A single experiment used cartilage (n = 4 to 6 explants in each group) harvested from two bovine joints. Each experiment was repeated separately four times.

Mechanical Injury

To simulate mechanical injury, bovine cartilage explants were subjected to 40% compressive strain in radially unconfined compression for five minutes. The injurious compression fell within the range of strain values previously reported to induce apoptosis\cite{6,8}. Explants from the control or IL-1 groups were not mechanically injured. After injury, explants were cultured for ten days before analysis.
**Cell Viability**

Cell viability was quantitated by staining with Calcein-AM[13]. Calcein-AM is an uncharged nonfluorescent esterase substrate which diffuses freely into cells. Viable cells containing esterase activity convert Calcein-AM to Calcein, which is a charged, fluorescent green stain, and only retained by cells with an intact plasma membrane. Propidium iodide was used as a counterstain as it reacts with nuclear DNA to produce a red fluorescence. Since propidium iodide is not cell membrane permeable, it cannot stain cells with an intact cell membrane. Cell viability was quantified as the percentage of fluorescent green cells in the explant section at 48 hours after injury.

**Glycosaminoglycan Assay**

The concentration of glycosaminoglycans released into the media was measured using 1,9-Dimethylene Blue (DMMB) to monitor spectrophotometric changes which occur during the formation of the sulfated GAG dye complex[14]. Explants were digested in papain and collagenase, and glycosaminoglycan concentration was determined at ten days after injury.

**Mechanical Properties**

The elastic modulus of the solid phase was measured on day ten after injury by allowing the explants to creep under a constant load. Explants were placed in a creep chamber and were preloaded with a small tare load. An additional 120 gm of constant load was added and the creep monitored until equilibrium (slope of creep against time was less than 1x10^{-6}). The equilibrium creep is directly related to the load and to the elastic modulus ($E_s$) of the cartilage matrix[15]. For the same constant load, the equilibrium creep is inversely related to $E_s$. The confined compression creep test is more widely accepted and was initially chosen to measure the aggregate modulus of the explants. However, injury often changed the shape and the size of the explant, making it difficult to consistently fit the explant in the confined compression chamber.

**Statistical Analysis**

Multifactor ANOVA (Analysis of Variance) was used to test differences in results between experimental groups. Neuman-Keuls pairwise contrasts was used to test for individual pairwise differences. Linear regression was used to test the association between glycosaminoglycan content and creep. A p value < 0.05 was used to denote statistical significance.

**Results**

**Cell Viability**

Figure 1 displays the mean chondrocyte viability (with standard deviation bars) for the different groups, ten days after injury. Cell viability was significantly reduced in the injury group (p < 0.05) compared with the control and IL-1 group.
Glycosaminoglycan Release and Content

The concentration of glycosaminoglycan in culture media from control explants was less than 10 microgm/mL per mg of explant (over ten days of culture). This more than doubled after injury (19.9 microgm/mL/mg, Figure 2, p < 0.01). An even higher release of glycosaminoglycan was detected in explants treated with IL-1beta (p < 0.001).

A corresponding decrease was noted in glycosaminoglycan content in injury and in IL-1beta explants after ten days in culture (Figure 3). This links the glycosaminoglycan released in media with the glycosaminoglycan content of the explant.

Mechanical Properties

Figure 4 shows the mean equilibrium creep after mechanical testing in unconfined compression with standard deviation bars. At ten days after injury mean equilibrium creep was significantly greater in the injured explants and in the explants cultured in IL-1, relative to control (p<0.01). Since the equilibrium creep inversely relates to $E_s$, this suggests a proportionate reduction in $E_s$.

A negative correlation was also found between glycosaminoglycan content and equilibrium creep in unconfined compression (Figure 5, $R^2 = 0.3$, p = 0.03). This finding links the biomechanical properties with the biochemical changes during the process of matrix degradation.
Figure 2 – *Mean Glycosaminoglycan Released in Media Over Ten Days Post Injury (n = 24, in 4 separate experiments).*

Figure 3 – *Mean Glycosaminoglycan Concentration of Explant Ten Days after Injury (n = 24, in 4 separate experiments).*
Figure 4 – Mean Equilibrium Creep at Ten Days after Injury (n = 16, in 4 separate experiments).

Figure 5 – Linear Regression of Creep with Glycosaminoglycan Content
Discussion

Cartilage injury is a significant contributor to secondary osteoarthritis. Because the inciting event can be easily identified, it is easier to document the progression of cartilage degeneration leading to arthritis compared with primary osteoarthritis or more insidious forms of secondary osteoarthritis. Cartilage injury models are therefore attractive in providing insights in the processes of cartilage degeneration and arthritis. This in vitro model of cartilage injury allows for carefully controlled evaluation of cellular, biochemical, and biomechanical events that may be a significant part of the posttraumatic degenerative process.

In this model, cell viability, matrix loss, and biomechanical strength were found to correlate with each other after injury. Analysis of matrix constituents can serve as valuable surrogate markers of cartilage damage. However, the primary function of articular cartilage is biomechanical support. Hence, the evaluation of biomechanical properties is more important in the context of cartilage degradation and repair. Previous reports have found significant correlations between the elastic modulus of cartilage and the biochemical composition such as glycosaminoglycan and collagen content\[16,17\]. Patwari et al. reported increased swelling of injured calf cartilage explants when placed in hypotonic sodium chloride solution\[18\]. Cartilage swelling is an estimate of damage to the collagen network. Loening et al. also reported a significant reduction in equilibrium modulus of injured calf cartilage explants when tested in unconfined compression immediately after injury\[6\]. The authors attributed this to damage to the collagen network. The present study was also able to correlate the equilibrium creep (which relates inversely to elastic modulus) with the glycosaminoglycan concentration of cartilage explants. This further served to validate surrogate markers of cartilage injury (such as glycosaminoglycan release in media).

This study demonstrated a pattern of cartilage degradation after injury similar to, although less severe than, that seen with IL-1 stimulation. This provides indirect evidence that the posttraumatic degradation may have a significant biochemical component. Other studies have also suggested this by reporting MMP-3 (stromelysin-1) release after similar injurious compression\[18\]. MMP-3 levels in synovial fluid have been reported to rise after clinical joint injury\[19\]. MMP-3 is known to cause degradation of glycosaminoglycan and collagen and therefore can affect both the aggregate modulus and the tensile strength of cartilage.

In early osteoarthritis, there may sometimes be increased synthesis of glycosaminoglycan\[20\]. However, this has been shown to be associated with decreased retention of newly synthesized glycosaminoglycan in matrix\[21\]. This may be attributed to decreased synthesis of link protein, which may have reduced the formation of large aggregates\[22\].

The consequences of cell death are also clinically relevant. There is a constant turnover of the macromolecules (predominantly glycosaminoglycan and type II collagen) that constitute cartilage matrix. Chondrocytes are the sole source of the components of these macromolecules and are therefore primarily responsible for the maintenance, repair, and remodeling of matrix. In mature articular cartilage, a limited pool of chondrocytes is available for this maintenance, and therefore any reduction in this finite pool of cells can result in reduction of net synthesis (leading to progressive degradation).
Coupled with the initial matrix damage, a significant loss in cell viability can lead to accelerated degradation. This opens a novel therapeutic approach through cytoprotection. Preliminary studies suggest that chondrocyte apoptosis can be prevented resulting in increased cell viability after injury[8,23]. Whether increased cell viability results in enhanced matrix synthesis, reduction in cartilage degeneration, or improved repair has yet to be demonstrated. A model of cartilage injury that permits accurate analysis of the cellular, biochemical, and biomechanical properties of cartilage can be extremely useful in evaluating such newer therapies.

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Reference List


