SUBCLINICAL MECHANICAL IMPACT OF ARTICULAR CARTILAGE LEADS TO A DEGENERATION CASCADE IN AN EXPLANT MODEL

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ABSTRACT INTRODUCTION:
Pathologic impact loading of articular cartilage can lead to post-traumatic osteoarthritis years after an initial injury. To further understand, and eventually prevent post-traumatic OA, better characterization of the pathophysiological behavior of articular cartilage post-impact loading is needed. In particular, information in regard to impact loading regimes that do not cause gross identifiable damage would be helpful in understanding the possible causes of 'idiopathic' or primary OA. To date, there is little work investigating gene expression following impact loading, which may aid in discovering clinical tools to prevent or manage post-traumatic OA. The goal of this study is to broadly characterize the temporal effects of two different levels of impact loading on articular cartilage (Low, 1.1 J at 3.7 MPa and High, 2.8 J at 6.5 MPa) in order to better understand how clinically silent impact injuries can lead to degeneration of the tissue. In this study we investigate the changes that take place in articular cartilage at 24 h and 1 and 4 weeks following impact loading at two levels. Our approach involves quantifying cell death, changes in gene expression, alterations in the biochemical composition of the ECM, and changes to the tissue’s biomechanical properties. We hypothesize that low levels of impact that do not result in immediate tissue changes will begin a degeneration cascade that will appear and worsen in culture. In contrast, we expect that high levels of impact will result in immediate changes that will persist and become exacerbated.

METHODS:
After mechanical impact, bovine articular cartilage explants were cultured acutely and temporally up to 4 weeks. The levels were chosen such that the Low impact level did not visibly damage the cartilage whereas the High impact level caused grossly identifiable damage. Cell viability was performed with a Live/Dead® assay (Molecular Probes) and quantified using ImageJ (NIH). Tissue morphology was scored on a standardized scale. Quantitative real-time polymerase chain reaction (qRT-PCR) was used to obtain the gene expression profiles for seven genes. The amounts of collagen, glycosaminoglycans (GAGs), and DNA in the extracellular matrix (ECM) was assayed using a hydroxyproline assay, dimethyl-methylene blue (DMMB) assay, and PicoGreen® assay, respectively. The DMMB assay was also used to quantify GAG released into the media. The creep indentation properties were obtained using an automated creep indentation instrument, which allowed calculation of the aggregate modulus, Poisson’s ratio, and permeability. A sample size of n = 5 was used for viability data and for gene expression. For GAG, collagen, DNA, and creep indentation measurements, a sample size of n = 6 was used. A single factor ANOVA with repeated measures (StatView) was performed on all data. If significance (p < 0.05) was found, a Student-Newman-Keuls post-hoc test was performed.

RESULTS:
Morphology scores after impact showed no difference between Low (1.6 ± 1.0) and baseline (2.1 ± 1.7), but were significantly increased for High (8.5 ± 1.0) impact (Fig. 1). Control, Low, and High had 1.8% ± 0.8%, 9.9% ± 1.0%, and 18.8% ± 3.7% dead cells at 24 h, respectively. Cell death increased over 1 and 4 weeks for the Low and High explants. qRT-PCR showed significantly suppressed abundance of gene expression for TIMP-1 by ten-fold and aggrecan expression by four-fold inside the impact zone at week 1.

GAG release into the media was significantly increased over controls for Low and High impact explants at 24 h and during week 1. GAG content of Low and High impact explants significantly decreased at week 4. Culture controls did not illustrate increased GAG release during any week or a decreased GAG content at week 4. The DNA content of the Low and High impact zones were significantly decreased compared to baseline and culture controls.

In terms of the aggregate modulus at 24 h, Low and control explants were equivalent. Within week 1, the aggregate modulus of the High impact group was significantly lower than the Low impact group (Fig. 2). Further, at weeks 1 and 4, the Low and High impact groups’ aggregate modulus was significantly lower than culture control. At week 1, Low was 69% of culture control and High was 53% of Low. At week 4, Low and High were not significantly different. The permeability of the Low impact group was increased significantly at week 1.

DISCUSSION:
The results presented here describe the acute and temporal effects of mechanical impact on articular cartilage. They also, for the first time, investigate gene expression profiles and creep indentation biomechanics and compare them to the ECM biochemistry and morphology. Our results support our hypothesis, with data from the various assays combining to demonstrate that High and Low impact injuries to articular cartilage both produce similar degenerative changes over a period of 4 weeks, even though Low impact produces no initial or acute gross damage, but only subtle changes in gene expression and GAG release. Though both Low impact and baseline tissue were morphologically equivalent initially, Low impact explants exhibited temporal behavior that was comparable to those of High impact. Overall, the High impact level caused initial tissue damage at 24 h and week 1, which worsened by week 4. While at week 1 the Low impact tissue was not observed to be as damaged as High group, at week 4 the degenerative cascade of Low impact explants had reached that of the High impact explants in terms of biomechanics, GAG release, and gene expression profile. Further, Low impact samples displayed the same tableau of temporal cell death pattern and cell viability as the High. From the results presented in this investigation, it follows that interventions to slow or prevent the early changes associated with Low impact may be able to rescue the tissue from the ensuing degeneration cascade. These interventions may translate into clinical tools to decrease the prevalence of post-traumatic osteoarthritis.

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