A High Throughput Mechanical Model to Study Injurious Compression of Engineered Cartilage

Introduction

Post Traumatic Osteoarthritis (PTOA) is caused by a traumatic injury to the joint, where cartilage damage initiates events that progress to OA later on in life. In vitro explant models have been developed to evaluate the mechanisms that govern cartilage degeneration following injury. Several variables (peak stress, final strain level, strain rate) have been correlated with the degree of tissue damage. These models have also been used to evaluate the effect of various small molecules on cell viability and matrix degradation caused by injury. Although these studies provide insight into the mechanisms of injury, rapid screening of libraries of small molecules, with reproducible samples, is not possible. Alternatively, engineered cartilage tissues analogs (CTA) can be produced in reproducible formats and impacted using a high throughput mechanical screening (HTMS) device. This system may provide a valuable platform to study the timeline of events following traumatic injury, and to discover new molecules that block injury progression into PTOA. Here we show that the degree of impact induced injury to CTAs is dependent on loading parameters, and that high throughput impact initiates a degenerative response.

Methods

Chondrocytes isolated from juvenile bovine cartilage were seeded into a self-aggregating suspension culture model to create CTAs using poly-HEMA coated 96 well plates (1X10⁶ cells/well). Constructs were pre-cultured for 4-6 months in DMEM with 10% FBS and vitamin C. Single Impact: Four protocols were evaluated using an Instron (5848): 75% and 50% strain at strain rates of 50%/s and 10%/s for a total compression time of 10s. CTAs were harvested at 12, 24 and 120 hrs post-injury and analyzed for live/dead (N=1) and histology (N=2), biochemical content (GAG, DNA, HYP) (N=3), or frozen in LN₂ for gene expression (N=3). Media was collected to measure soluble GAG, nitric oxide (NO), and MMP (N=3). HTMS: Based on induced injury with single impact, CTAs were impacted to 60% strain at 50%/s in a 48 well format. CTAs were harvested at 24 and 120 hrs post-injury for biochemical (N=3) and gene expression (N=3-4) analysis. Media was collected at 24 hrs (N=12-14) and 120 hrs (N=5-7) for assays above. The real-time load response was monitored during impact, and peak stress calculated based on measured CTA geometry.

Statistics: Significance was calculated using one way ANOVA with Tukey’s post hoc test, with p<0.05.

Results

The load response of CTAs during single impact testing and the apparatus used are shown in Figure 1A. CTAs in these tests had an equilibrium modulus of 90.1 ±/− 29.0 kPa. Peak stresses at 75% strain were significantly higher than the 50% strain group for both strain rates. Histological sections showed that 50% strain caused internal fissure formation with reduced GAG staining at the fissure edges (Fig 1B). In contrast, 75% strain resulted in significant edge matrix disruption and less diffuse GAG staining throughout. Aggrecan expression decreased compared to control (24 hrs; p<0.05) at 75% strain, but by 120 hrs expression had recovered (p<0.05). With impact to 75% strain, MMP-13 expression increased compared to previous time points, control, and 50% strain at 120 hrs. Analysis of NO showed a strain dependent increase from 12 to 24 hrs; from this peak level at 75% strain, NO decreased back to 12 hr levels over the remaining time course. Following single tests, CTAs were impacted using the HTMS device (Fig 3A, B) to 60% strain at 50%/s. CTAs had an equilibrium modulus of 97.5 ±/− 65.1 kPa. To ensure accurate displacement of the platen at the appropriate strain rate, and to test analysis methods for determining peak stresses and sensor-to-sensor variation, 10% agarose constructs were first impacted using the device. Peak stresses calculated for engineered cartilage were similar to that of the single tests to 50 and 75% strain. NO released from impacted CTAs was 7-fold higher than controls at 24 hrs, but fell 2-fold by 120 hrs, similar to single impact tests (p<0.05) (Fig 3C). Histological analysis and live/dead staining showed cell death in regions...
adjacent to fissures and internal fissure formation, similar to single tests at 50% strain (Fig 3D).

**Discussion**

Impact of engineered cartilage analogs resulted in catabolic events that caused chondrocyte cell death and altered biosynthesis. Previous studies have shown similar changes following impact of osteochondral explants with significant cell death and loss of matrix associated with surface fractures due to high strain rates and peak stresses. In this study, injury to engineered cartilage was dependent on the final strain, and the injury response was modulated through gene expression.

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**Figure 1A-B.** (A) Representative load profile and peak stresses of CTAs for single impact tests. (B) Histological sections of impacted CTAs 120 hrs post-injury show structural disruption and loss of GAG due to impact.

**Figure 2A-B.** (A) AGG expression is initially decreased (24 hrs), but recovers by 120 hrs; MMP-13 is only increased at 120 hrs for the highest strain. p<0.05: * vs 12hr; $ vs 24hr; # vs 75% strain; @ vs control (same time point). (B) NO levels show a strain dependent increase up to 24 hrs after impact. p<0.05 * vs 50 and 75% strain and $ vs 75% strain at the same time point; @ vs other time points.
and production of inflammatory molecules. Both single and HTMS impact tests showed similar trends of increased NO during the first 24 hrs after injury, followed by recovery towards baseline by 120 hrs. As nitric oxide is an acute response to injury and inflammation, increased NO may activate cell stress signaling pathways and result in the up-regulation of catabolic molecules and induce matrix degradation. Attenuation of this signal and the return of aggregan expression to that of control, may indicate a late reparative response after injury. This data describes an impact protocol that causes reproducible, acute injury in engineered cartilage. Following further validation of the impact HTMS device using additional injury-related, cellular responses, it will be possible to begin screening chemical libraries for drugs that might limit progression to PTOA.
Significance
This HTMS device allows for the study of PTOA-related cellular responses using an engineered cartilage impact platform and allows for screening of chemical libraries for modulators of cell injury and repair towards the discovery of new drugs for the treatment of OA.

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References