Validation and Screening in a High Throughput Mechanical Injury Model of Engineered Cartilage

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Introduction

Joint injuries increase the likelihood of developing post-traumatic osteoarthritis (PTOA) due to the initiation of catabolic processes that lead to progressive degeneration of the joint cartilage. Several in vitro models have been developed (using chondral explants) to investigate the pathologic mechanisms of PTOA and to evaluate the efficacy of putative small molecule therapeutics to stop or reverse the degenerative process. These agents include anti-apoptotic agents that inhibit caspase activity,[1,2] free-radical scavengers,[2] and polymers that aid in the repair of cell membranes.[3,4] Unfortunately explant models are limited in their throughput and in the homogeneity of explant response to injury, making larger scale drug screening studies impractical. To address this limitation, we recently developed a high throughput mechanical testing platform capable of applying injurious compression to engineered cartilage constructs, showing increased matrix degradation and cell death comparable to that reported for cartilage injury models.[5] In the current study, we further validated this high throughput mechanical injury (HiTMI) model of PTOA by: 1) directly comparing the response of native chondral explants and engineered cartilage analogs subjected to identical injuries, and 2) using the HiTMI system to evaluate bioactive molecules previously reported to reduce cell death and proteoglycan loss post-injury.

Methods

Engineered Cartilage

Engineered cartilage tissue analogs (CTAs) were fabricated as previously described;[6] briefly, cartilage was harvested from juvenile bovine knees, minced, digested overnight in Collagenase II, washed (PBS w/ 2X PSF) and centrifuged (1750 rpm, 3X) to collect chondrocytes. Chondrocytes were seeded into polyhydroxyethylmethacrylate coated 96-well plates at $1x10^6$ cells/construct and cultured in high glucose DMEM with 10% FBS and vitamin C (50 ng/mL). CTAs were matured for ≥14 weeks and their height was measured prior to placement in 48 well plates for high throughput impact (75% strain at 50% strain/s; total impact time: 10s). CTAs were harvested 24, 48, and/or 120 hours post-injury.

Cartilage Explants

Chondral plugs were harvested from the trochlear groove of juvenile bovine knees (Ø4mm) and trimmed to similar heights, removing the subchondral bone (H: 3-4mm). Explants were injured as above, but were processed one at a time as the high peak stresses generated during injury exceeded the load threshold of the high throughput sensor.

Chemical Compounds

Immediately following injury, CTAs were treated with one of three agents: (1) N-Acetyl-Cysteine (NAC, 2mM) a nitric oxide scavenger, (2) Z-VAD-FMK (ZVF, 100μM), a pan-caspase inhibitor, or (3) Polaxamer 188 (P188, 8mg/mL), an amphiphilic polymer capable of inserting into and closing the ruptured cell membrane. All were included in culture for the initial 48 hours post-injury. As a positive control, some CTAs were treated with IL-1 (10 ng/mL) for 120 hours.

Analysis

For all time points, constructs were harvested to measure matrix content (GAG by DMMB assay and collagen by OHP assay; N=4). Medium was collected at each time point to assess release of matrix (GAG) and cell viability (LDH assay; N=2-4). Values were normalized to wet weight and to the control, un-injured group. Comparisons between treated and non-treated impacted and control groups were made using a two-way ANOVA with Bonferroni's post-hoc ($p<0.05$).

Results

Although injury of native cartilage generated peak stresses approximately 20-fold higher than engineered cartilage (~20 vs. 1 MPa; Figure 1, inset), the response to injury was similar as measured by enzyme and matrix release. Both cartilage and CTAs released similar amounts of GAG (0.18+/−0.024 vs. 0.22+/−0.003 %ww) to the medium, while LDH release was slightly higher from native tissue (650+/−123 vs. 280+/−32.9 %ww, Figure 1). Subsequent

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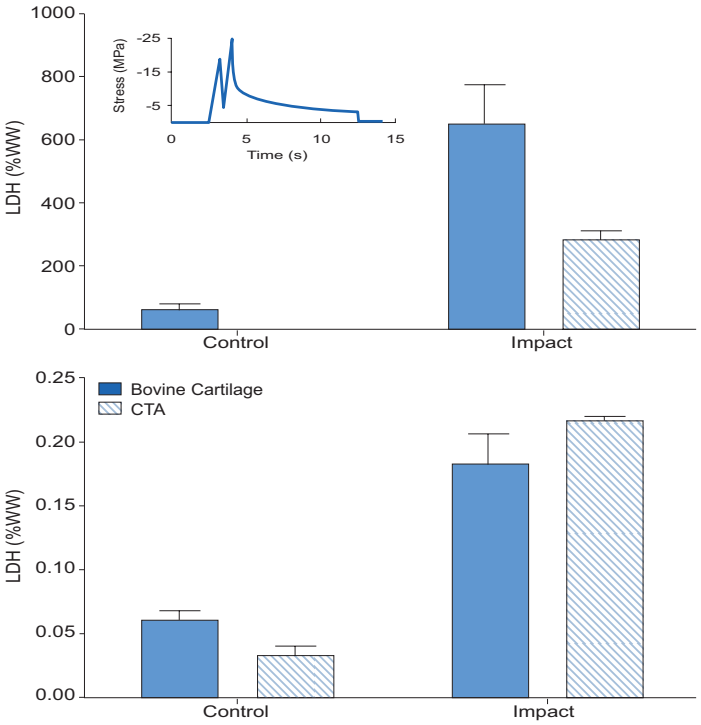

Figure 1. Comparison of cartilage and CTA response to injury shows similar trends in (A) LDH and (B) GAG release 24 hours post-injury (CTA: N=2 with two constructs/well; cartilage: N=4).

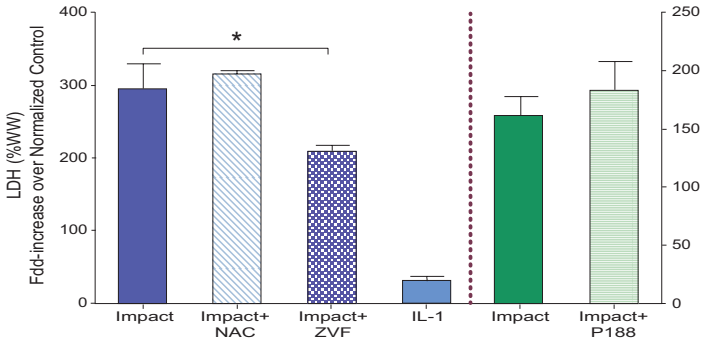

Figure 2. LDH release 24 hours post-injury. (Left) ZVF significantly reduces enzyme release by ~28%, while NAC and (Right) P188 have no effect compared to injury alone (N=2 with two constructs/well).

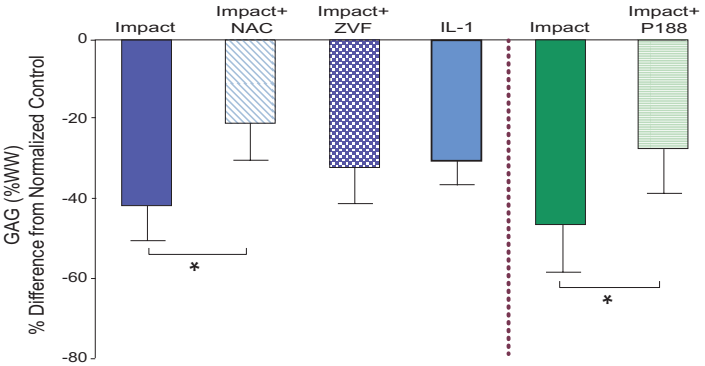

Figure 3. GAG in constructs 120 hours post-injury. While injury alone reduces GAG by ~40% compared to control, (Left) NAC and (Right) P188 treatment are able to retain ~20% more matrix as compared to injury alone.

screening of several putative PTOA therapeutics showed reduced cell death and matrix loss in the short-term following injury in this model system. In the initial 24 hours, ZVF treatment significantly reduced LDH by ~28% compared to injury alone (211+/−6 vs. 295+/−34.6; $p<0.05$), however NAC and P188 did not (Figure 2). By 120 hours post-injury, however, both NAC and P188 decreased GAG loss (by 18 and 20% respectively; $p<0.05$) compared to injury alone, although neither agent restored GAG to control levels (Figure 3). Despite increasing initial cell viability after injury, ZVF treatment did not alter GAG content in injured samples at 120 hours.

Discussion

In this study, we validated our in vitro high throughput mechanical injury (HiTMI) model by directly comparing the response of native cartilage to that of engineered cartilage under identical injurious compression conditions. Although peak stresses differed significantly with 75% strain applied at 50% strain/sec (due to differences in mechanical properties), release of GAG and LDH were comparable over the first 24 hours post-injury. Following this validation step with native tissue, we next carried out a small screen of putative PTOA therapeutics that target the first events to occur following injury. Consistent with the literature, application of these compounds to engineered cartilage injured in the HiTMI system resulted in similar protection against loss of cell viability and matrix changes. For example, ZVF is reported to increase cell viability by 15-20% with 48 hours of treatment of explants compressed to 30% strain in 500ms[1] or impacted at 7J/cm^2;[2] in this study, ZVF similarly decreased LDH release from CTAs post impact. NAC has likewise been reported to increase viability by ~30% following injury.[2] Here we found that NAC had no effect on reducing membrane damage. However, our results with NAC do match those reporting a reduction of GAG loss from cartilage by ~20% in chondral explants post-impact at 7J/cm^2.[2] Findings for P188 have been more variable, with some reports noting a ~20% increase in cell viability[3] with smaller changes in GAG loss;[4] our findings support the notion that GAG retention is improved with P188 treatment. Together, these results suggest that CTAs (and engineered cartilage in general) may serve as an appropriate surrogate for studying the mechanisms underlying the progression of PTOA. We additionally demonstrated the feasibility of using the CTA to screen even larger small molecule libraries for PTOA therapeutics using this novel HiTMI system.

Significance

We established that engineered cartilage can serve as a surrogate for the study of PTOA. Given the ability to form many such analogs in a micro-scale format, this finding sets the stage for the high throughput screening of large chemical libraries to identify novel compounds that may attenuate PTOA pathology and offset progressive degenerative joint changes.

Acknowledgments

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References

1. D'Lima DD, Hashimoto S, Chen PC, et al. Impact of mechanical trauma on matrix and cells. *Clin. Orthop.* S90–99 (2001).

2. Martin JA, McCabe D, Walter M, et al. T. O. N-acetylcysteine inhibits post-impact chondrocyte death in osteochondral explants. *J. Bone Joint Surg. Am.* 91, 1890–1897 (2009).

3. Phillips DM, Haut RC. The use of a non-ionic surfactant (P188) to save chondrocytes from necrosis following impact loading of chondral explants. *J. Orthop. Res.*22, 1135–1142 (2004).

4. Natoli RM, Athanasiou KA. P188 reduces cell death and IGF-I reduces GAG release following single-impact loading of articular cartilage. *J. Biomech. Eng.* 130, 041012 (2008).

5. Mohanraj et al. ORS 2013.

6. Estrada LE, Dodge GR, Richardson DW, et al. Characterization of a biomaterial with cartilage-like properties expressing type X collagen generated in vitro using neonatal porcine articular and growth plate chondrocytes. *Osteoarthr. Cartil.* 9, 169–177 (2001).