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Moderate dynamic compression inhibits pro-catabolic response of cartilage to mechanical injury, tumor necrosis factor-α and interleukin-6, but accentuates degradation above a strain threshold

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SUMMARY

Objective: Traumatic joint injury can initiate early cartilage degeneration in the presence of elevated inflammatory cytokines (*e.g.*, tumor necrosis factor (TNF)-α and interleukin (IL)-6). The positive/negative effects of post-injury dynamic loading on cartilage degradation and repair *in vivo* are not well-understood. This study examined the effects of dynamic strain on immature bovine cartilage *in vitro* challenged with TNF-α + IL-6 and its soluble receptor (sIL-6R) with/without initial mechanical injury.
Methods: Groups of mechanically injured or non-injured explants were cultured in TNF-α + IL-6/sIL-6R for 8 days. Intermittent dynamic compression was applied concurrently at 10%, 20%, or 30% strain amplitude. Outcome measures included sulfated glycosaminoglycan (sGAG) loss (dimethylmethylene blue (DMMB)), aggrecan biosynthesis (^{35}S-incorporation), aggrecanase activity (Western blot), chondrocyte viability (fluorescence staining) and apoptosis (nuclear blebbing *via* light microscopy), and gene expression (qPCR).
Results: In bovine explants, cytokine alone and injury-plus-cytokine treatments markedly increased sGAG loss and aggrecanase activity, and induced chondrocyte apoptosis. These effects were abolished by moderate 10% and 20% strains. However, 30% strain amplitude greatly increased apoptosis and had no inhibitory effect on aggrecanase activity. TNF + IL-6/sIL-6R downregulated matrix gene expression and upregulated expression of inflammatory genes, effects that were rescued by moderate dynamic strains but not by 30% strain.
Conclusions: Moderate dynamic compression inhibits the pro-catabolic response of cartilage to mechanical injury and cytokine challenge, but there is a threshold strain amplitude above which loading becomes detrimental to cartilage. Our findings support the concept of appropriate loading for post-injury rehabilitation.
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Introduction

Joint injuries such as the anterior cruciate ligament (ACL) tear are a major risk factor for osteoarthritis (OA) later in life. The initial joint trauma can be a single disruption of the ligament, or accompanied by damage to cartilage, meniscus, synovium, and subchondral bone. Post-injury evaluation of the synovial fluid from ACL-deficient patients has revealed inflammation-associated biochemical changes including increased levels of pro-inflammatory cytokines (*e.g.*, tumor necrosis factor (TNF)-α, interleukin (IL)-1, and IL-6) as well as matrix protein degradation products generated by matrix metalloproteinases (MMPs) and ADAMTS aggrecanases (A Disintegrin and Metalloproteinase with Thrombospondin Motifs)[1–4]. This inflammatory response, which can be prolonged after the initial injury, is believed to act in conjunction with abnormal mechanical loading to accelerate cartilage degeneration that eventually leads to OA. Indeed, studies comparing OA patients with or without prior joint injury provided strong evidence that ACL tears can significantly increase the risk for early OA[5–7].

In vivo, articular cartilage is subjected to a complex combination of shear, compressive, and tensile stress under normal loading conditions. After joint injury, in addition to the inflammatory response, trauma-induced joint instability also alters the contact mechanics between articular surfaces[8]. In particular, Van de

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Velde[9,10] used dual fluoroscopic and MR imaging techniques to quantify tibiofemoral joint kinematics in both normal and ACL-deficient human patients. Their results showed that cartilage contact deformation increased significantly to ~20–30% in the ACL-deficient knee from ~15–20% in the contralateral healthy knee during lunge motion with 0–30° flexion[10]; while surgical reconstruction restored some of the *in vivo* contact biomechanics, the increased cartilage deformation was not ameliorated[11]. These studies raise the question of whether post-injury joint loading can cause additional damage to cartilage, and whether there exists a range of motion within which rehabilitative loading can be beneficial in maintaining cartilage structure and function.

Over the last two decades, *in vitro* injury models have been developed to facilitate understanding of cartilage mechanical injury on the onset and progression of OA[12–14]. Consistently, injurious loading has been shown to result in loss of proteoglycans[15], tissue swelling[14], collagen network damage[12], and reduced tissue stiffness[13]. In addition, significantly increased chondrocyte apoptosis was observed[16,17], especially in the superficial zone[18], and the degree of cell damage was age-dependent[19]. Matrix biosynthesis by remaining live cells was also suppressed by injury[13]. Furthermore, injury potentiates proteoglycan catabolism induced by exogenous cytokines TNF-α and IL-6[20], which were introduced to simulate the inflammatory environment seen *in vivo* after joint injury. These studies have furthered our understanding of the immediate effects of mechanical injury; however, the interplay between cytokines and post-injury mechanical signals is less understood.

Dynamic compression can induce anabolic responses in normal cartilage which promote matrix biosynthesis with a strong dependence on compression frequency, amplitude, and loading duty cycle[21–24]. The spatial profiles of cell-mediated matrix biosynthesis have been correlated with compression-induced interstitial fluid flow[25–27], and the mechano-transduction pathways involve MAPK activation, intracellular calcium and cyclic AMP[28,29]. Additionally, dynamic compression can mitigate the catabolic responses of chondrocytes to cytokines in tissue-engineered cultures[30]. However, little is known about the effects of follow-on dynamic compression after injury/cytokine-challenge in intact cartilage.

In the present study, we implemented a previously-characterized *in vitro* injury model involving cytokines TNF-α and IL-6/sIL-6R with or without initial mechanical injury, and investigated the effects of intermittent unconfined dynamic compression (10–30% strain amplitude) on immature bovine cartilage. We hypothesized that (1) dynamic compression can maintain anabolic effects in an inflammatory environment by rescuing matrix biosynthesis suppressed by cytokines[31]; (2) dynamic compression has an additional anti-catabolic role in reducing cytokine-mediated cartilage degradation; (3) there is a range of strain amplitudes within which dynamic compression is beneficial, while overload strain amplitudes above a threshold can be deleterious.

Materials and methods

Bovine articular cartilage harvest and culture

Articular cartilage disks were harvested from the femoropatellar grooves of 1-2-week-old calves, obtained on the day of slaughter (Research '87, Boylston, MA). A total of 19 joints from 15 different animals were used. Full-thickness cartilage cylinders were cored using a 3-mm dermal punch, and the top 1-mm disk containing intact superficial zone was harvested with a blade. Disks were incubated in serum-free medium (low-glucose Dulbecco's Modified Eagle's Medium [DMEM; 1 g/L]) supplemented with 1% insulin–transferrin–selenium (ITS, 10 g/ml, 5.5 g/ml, and 5 g/ml, respectively, Sigma, St. Louis, MO), 10 mM HEPES buffer, 0.1 mM nonessential amino acids, 0.4 mM proline, 20 g/ml ascorbic acid, 100 units/ml penicillin G, 100 g/ml streptomycin, and 0.25 g/ml amphotericin B for 2–3 days (5% CO_2; 37°C). Disks for each test were match for anatomic location on the joint surface, and the thickness variation for those receiving dynamic compression was limited to <5%.

Injurious compression and exogenous cytokines

After equilibration, groups of cartilage disks were injuriously compressed in a custom-designed, incubator-housed loading apparatus [Fig. 1(A)][32]. As described previously[33], each bovine disk was placed in a polysulfone chamber and subjected to radially unconfined compression to 50% final strain at a strain rate of 100%/s, followed by immediate release at the same rate [Fig. 1(B)]. After injury, disks were immediately placed in treatment medium in the presence or absence of rhTNF-α (25 ng/ml), rhIL-6 (50 ng/ml), and sIL-6R (250 ng/ml) (R&D Systems, Minneapolis, MN). Previous studies showed that this combination of cytokines caused significantly greater sGAG loss than either cytokine alone[20,34].

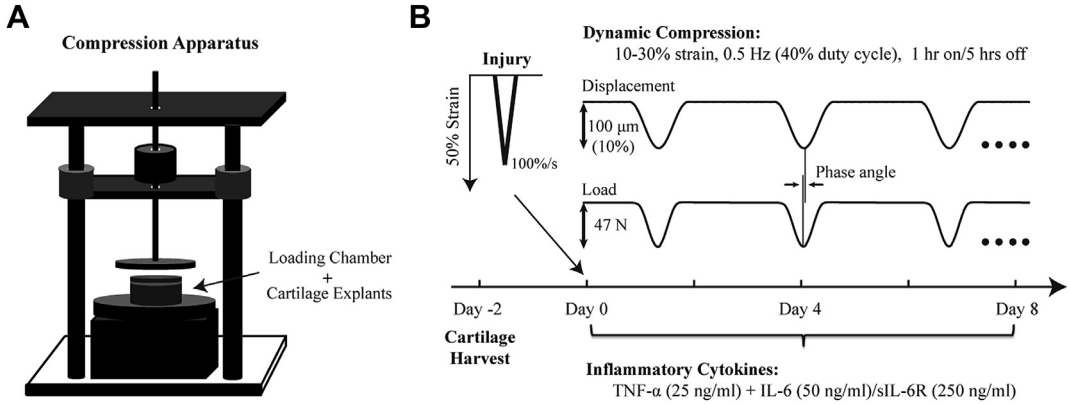

Fig. 1. **A**, Schematic of custom-designed, incubator-housed loading apparatus[32] used to perform injurious and dynamic compression. **B**, Experimental design: Injurious compression was applied to cartilage explants on Day 0, followed by immediate incubation in TNF-α + IL-6/sIL-6R. Intermittent dynamic compression started on Day 0 (10%, 20% or 30% applied strain amplitude) and continued up through Day 8. Representative waveforms shown for a 10% dynamic strain amplitude applied to a group of 12 disks within the loading chamber, and the corresponding measured total compressive load.

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