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A high-throughput model of post-traumatic osteoarthritis using engineered cartilage tissue analogs

B. Mohanraj † ‡, G.R. Meloni †, R.L. Mauck † ‡ § ||, G.R. Dodge † § || *

† *McKay Orthopaedic Research Laboratory, Department of Orthopaedic Surgery, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA 19104, USA*
‡ *Department of Bioengineering, School of Engineering and Applied Science, University of Pennsylvania, Philadelphia, PA 19104, USA*
§ *Collaborative Research Partner Acute Cartilage Injury Program of AO Foundation, Davos, Switzerland*
|| *Translational Musculoskeletal Research Center, Philadelphia Veterans Administration Medical Center, Philadelphia, PA 19104, USA*

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SUMMARY

Objective: A number of *in vitro* models of post-traumatic osteoarthritis (PTOA) have been developed to study the effect of mechanical overload on the processes that regulate cartilage degeneration. While such frameworks are critical for the identification therapeutic targets, existing technologies are limited in their throughput capacity. Here, we validate a test platform for high-throughput mechanical injury incorporating engineered cartilage.
Method: We utilized a high-throughput mechanical testing platform to apply injurious compression to engineered cartilage and determined their strain and strain rate dependent responses to injury. Next, we validated this response by applying the same injury conditions to cartilage explants. Finally, we conducted a pilot screen of putative PTOA therapeutic compounds.
Results: Engineered cartilage response to injury was strain dependent, with a 2-fold increase in glycosaminoglycan (GAG) loss at 75% compared to 50% strain. Extensive cell death was observed adjacent to fissures, with membrane rupture corroborated by marked increases in lactate dehydrogenase (LDH) release. Testing of established PTOA therapeutics showed that pan-caspase inhibitor [Z-VAD-FMK (ZVF)] was effective at reducing cell death, while the amphiphilic polymer [Poloxamer 188 (P188)] and the free-radical scavenger [*N*-Acetyl-L-cysteine (NAC)] reduced GAG loss as compared to injury alone.
Conclusions: The injury response in this engineered cartilage model replicated key features of the response of cartilage explants, validating this system for application of physiologically relevant injurious compression. This study establishes a novel tool for the discovery of mechanisms governing cartilage injury, as well as a screening platform for the identification of new molecules for the treatment of PTOA.

Introduction

The primary function of articular cartilage is as a load-bearing structure that supports and distributes the high stresses generated during normal physiological activities[1]. While cartilage generally functions well over a lifetime of use, acute instances of supra-physiologic loading (e.g., accident or other traumatic event), often result in tissue damage that initiates degenerative processes within the joint. Indeed, a subset of osteoarthritis (OA), termed post-traumatic osteoarthritis (PTOA) represents the significant fraction of patients who develop OA secondary to such joint trauma. Based on the incidence of knee, hip, and ankle OA for patients with a history of joint injuries[2], it is estimated that up to ~6 million individuals are burdened with PTOA in the US alone. Cartilage pathology and PTOA incidence generally correlate with the intensity of the original injury; patients with ligamentous or meniscal injuries are 10-fold more likely, and those with articular fractures are 20-fold more likely to develop knee OA compared to individuals without previous joint injuries[3,4]. Despite a growing understanding of the mechanical thresholds that instigate PTOA,

* Address correspondence and reprint requests to: G.R. Dodge, McKay Orthopaedic Research Laboratory, Department of Orthopaedic Surgery, Perelman School of Medicine, University of Pennsylvania, 424 Stemmler Hall, 36th Street and Hamilton Walk, Philadelphia, PA 19104, USA. Tel: 1-215-898-8653; Fax: 1-215-573-2133.
E-mail addresses: mbhavana@mail.med.upenn.edu (B. Mohanraj), gmeloni@mail.med.upenn.edu (G.R. Meloni), lemauck@mail.med.upenn.edu (R.L. Mauck), gdodge@mail.med.upenn.edu (G.R. Dodge).

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the molecular pathogenesis and mechanisms of disease progression are not yet well understood.

To that end, a number of *in vitro*, *ex vivo*, and *in vivo* models of cartilage injury have been developed to explore the temporal patterns of anabolic and catabolic events that culminate in cartilage degeneration. These models serve as useful platforms in which to explore variables that regulate the extent of damage, including the impact energy, peak stress/strain, and stress/strain rate. Common markers of load-induced injury include tissue swelling and fibrillation[5], cell death at or near the injury site[6,7], and increased expression of proteases and inflammatory cytokines[7,8]. Biologic mediators of PTOA act collectively to decrease chondrocyte matrix biosynthesis[5] and instigate a loss of proteoglycans and other matrix elements[5,6,9,10]. Together, these molecular and compositional changes culminate in a loss of tissue mechanical integrity[5,7].

The timeline of activation of these degenerative processes (and the controlling signaling mechanisms) is particularly important, as the different stages of response post-injury may represent opportunities for therapeutic intervention[11]. Indeed, previous studies have focused on small molecules targeting the early events, including mechanisms that lead to cell death, release of inflammatory mediators, and proteoglycan loss. Examples of such compounds include pan-caspase inhibitors[12,13] to decrease cell death, amphiphilic surfactants[14–16] to repair disrupted cell membranes, oxidative free-radical scavengers[13] to limit early inflammatory processes, as well as growth factors[17,18] and glucocorticoids[19] to increase anabolic response post-injury. In the above studies, these factors have shown varied success in reducing cell death and matrix degradation in *in vitro* and *in vivo* models of PTOA, indicating that such early pathologic changes are appropriate targets for therapeutic intervention.

To date, the selection of agents that might abrogate PTOA initiation has been based on their roles in canonical pathways involved in cell physiology and/or OA progression. Since the mechanisms of PTOA have not yet been fully elucidated, there may be other agents not previously known to play a role in PTOA that could have chondro-protective effects. In recent work, Sampson *et al.* showed that parathyroid hormone (clinically used to improve bone mass) administered to mice after meniscus destabilization surgery was chondro-protective (or regenerative) in that it limited hypertrophic changes after onset of instability[20]. Wang *et al.* also showed that the inflammatory complement system regulated cartilage degradation in mouse models of joint instability[21]. These studies illustrate the significant role that such non-canonical pathways may play in mediating the degenerative response in situations of chronic overload; the acute injury response may similarly initiate heretofore unexplored signaling pathways.

High-throughput (HT) screening enables the rapid evaluation of small molecule libraries for the discovery of novel compounds relevant to tissue development and healing without prior knowledge of the mechanism of action. Recently, Johnson *et al.* developed an image-based high-throughput screening system to identify molecules that promoted chondrogenic differentiation of mesenchymal stem cells (MSCs)[22]. From the 1000s of molecules screened in that study, several “hits” were identified, with the small molecule kartogenin emerging as the most promising. Follow-up secondary *in vitro* assays (e.g., Real-Time Polymerase Chain Reaction (RT-PCR)) and tertiary *in vivo* investigations (rodent joint instability models) illustrated that kartogenin also had a chondro-protective effect and acted by disrupting the binding of a specific transcription factor subunit to an actin associated protein. Given the non-intuitive mechanism of action, this study highlights the need for unbiased screening tools to guide molecular discovery specific to a particular disease process.

To enable such screens in the context of PTOA, we developed a high-throughput *in vitro* mechanical injury platform that is compatible with drug screening. While *in vitro* models of injury using explants have been valuable in elucidating regional changes in cell viability and matrix loss, explants are not ideal for high-throughput screening due to the large number of samples required and variation in the cellular and molecular stratifications found throughout the joint. Cartilage tissue engineering, which aims to mimic the biochemical and mechanical properties of native cartilage for joint repair, can generate cartilage-like analogs with which to study the pathogenesis of PTOA. Engineered cartilage can also be fabricated in a uniform manner and in large quantity, and as such are ideal for high-throughput screening applications. In particular, we have studied a scaffold-less method to generate cartilage tissue analogs (CTAs) that closely mimic native cartilage both in terms of extracellular matrix composition and biomechanical properties[23–25].

Here, we adapted our high-throughput mechanical testing system[26] to apply compressive injury to CTAs in a rapid and reproducible manner. The primary goals of this study were to determine the strain and rate dependent response of engineered cartilage to compressive injury, to evaluate the progression of degeneration, and to validate this response with respect to native articular cartilage explants treated similarly. Our findings validate the use of engineered cartilage as a surrogate for studying mechanisms of PTOA pathogenesis and introduce a new screening tool with which to identify novel compounds that can attenuate degeneration following cartilage injury.

Methods

Fabrication of cartilage tissue analogs

Engineered CTAs were produced as described previously[24,25]. Briefly, articular cartilage was harvested from juvenile bovine knees (2–6 months old, Research 87, MA), finely minced, and digested overnight (12–16 h) in Dulbecco's Modified Eagle Medium (DMEM) containing collagenase Type II (298 U/mL Worthington, NJ). Tissue digests were filtered (70 μm pore mesh), washed with PBS containing 200 U/mL penicillin, 200 μg/mL streptomycin, 5 μg/mL Fungizone (PSF, Life Technologies, NY), and centrifuged at 1750 rpm for 15 min at 12°C (3×) until collected into a single suspension. Chondrocytes were resuspended at 5×10^6 cells/mL in complete medium (high glucose DMEM containing 10% Fetal Bovine Serum (FBS), 100 U/mL penicillin, 100 μg/mL streptomycin, 2.5 μg/mL Fungizone, 1% MEM Vitamin Solution (Gibco), 25 mM HEPES buffer, 50 μg/mL ascorbic acid[25]). This cell suspension was plated into ultra-low adhesion (polyHEMA coated) 96 well plates (Corning, NY) at 1×10^6 cells/well, where chondrocytes coalesced within 24 h to form a CTA[25]. CTAs were cultured for a minimum of 14–16 weeks in complete medium prior to injury.

Injurious compression of CTAs and native tissue explants

To determine the level of injury necessary to induce pathological changes in CTAs that mimic changes in cartilage explants, four different injurious compression protocols were applied in a single-sample manner based on previously established injury parameters[5,6,8]. CTAs were subjected to either 50% or 75% strain at one of two strain rates, 10% strain/s or 50% strain/s, followed by a hold period for a total ramp-hold compression time of 10 s. Constructs were then cultured for 5 days after injury, and both CTAs and media were harvested at 12, 24, and 120 hours post-injury for evaluation of biochemical content and presence of soluble catabolic markers as described below. Based on the outcomes of single-sample injury, high-throughput injury was applied to constructs at 75% strain at 50% strain/s. For this, a custom high-throughput mechanical