INTRODUCTION
Arthritic degeneration of articular cartilage, marked by degradation of the extracellular matrix (ECM) and loss of mechanical properties, can be cell-mediated and triggered by proinflammatory cytokines such as interleukin-1 (IL-1). Small-molecule inhibitors of matrix metalloproteinase (MMP) and a disintegrin and metalloproteinase with thrombospondin-type motif (ADAMTS)-class enzymes can delay or block IL-1-induced loss of sulfated glycosaminoglycans (sGAG) and collagen from articular cartilage explants in vitro [1-3] with a corresponding protection of mechanical properties [1,3]. While fibrocartilage degeneration has received less attention, there is growing evidence that meniscal degeneration can be an early event in the development of knee arthritis [4,5] and that IL-1 stimulation of meniscal fibrocartilage triggers ECM destruction mediated by MMPs and ADAMTSs [6-8]. However, the specific contributions of these enzyme systems to fibrocartilage degeneration are not well understood. The aim of this study was to determine the ability of selective and non-selective metalloproteinase inhibitors to mitigate cell-mediated sGAG depletion and loss of tissue mechanical properties in meniscal fibrocartilage.

METHODS

Tissue Culture
Bovine fibrocartilage explants (n=114, 3mm dia, 2mm thick) were harvested aseptically from the menisci of a 1-2 week old calf. Explants were preincubated in basal serum free medium for 3 days followed by up to 12 days with or without stimulation by 20ng/mL rhIL-1α. Additional groups were further treated with synthetic inhibitors selective [3] for ADAMTS-4/5 (RO3310769, 20μM ‘TS4/5 Inh’), MMPs but not ADAMTS-4/5 (RO023555, 5μM ‘MMP Inh’), or a non-selective inhibitor of ADAMTS-4/5 and MMPs (RO4002855, 5μM ‘NS Inh’). Media were collected and changed every 2 days. Explants were removed after 4, 8, and 12 days (n=6/group/time point) and stored at –20°C in PBS with protease inhibitors (PBS+PI) for subsequent analysis. Mechanical Testing
Explants were tested in PBS+PI at room temperature. Dynamic shear moduli G’ were determined via oscillatory torsional shear testing with a 0.5% shear strain at 0.001, 0.01, and 0.1Hz at a 10% compressive offset. Dynamic compressive moduli E’ were determined via sinusoidal unconfined compression with a 1.5% strain at 0.001, 0.01, and 0.1Hz at a 10% compressive offset. Biochemical Assays
Explant digests and conditioned media were assayed for sGAG content via the DMMB assay. Statistical Analysis
Explant sGAG and mechanical properties were compared to the IL-1 group using Student’s t-test (p<0.05).

RESULTS
Consistent with previously reported data [6], sGAG release in IL-1-stimulated fibrocartilage peaked at day 2, substantially earlier than in articular cartilage explants under identical conditions [3]. Low, basal levels of sGAG release were measured in untreated controls. Treatment with the non-selective, TS4/5–, or MMP-selective inhibitor did not significantly alter release kinetics (Fig. 1A). Interestingly, unstimulated explants treated with the non-selective inhibitor had significantly reduced sGAG release through day 4 and significantly higher sGAG content at day 12 than controls (p<0.05, data not shown). After twelve days of IL-1-stimulation, explants had significantly lower sGAG content than unstimulated controls (Fig. 1B). The non-selective and MMP-selective inhibitors blocked sGAG depletion from the explants through day 12, but the TS4/5-selective inhibitor did not. Dynamic shear and compressive moduli of the fibrocartilage explants, indicators of tissue function, were significantly reduced in IL-1-stimulated explants (Fig. 2A&B); addition of the non-selective or MMP-selective inhibitor, however, mitigated this loss of mechanical properties through day 12. In contrast, the TS4/5-selective inhibitor did not prevent IL-1-induced loss of tissue mechanical properties.

DISCUSSION
Selective and non-selective metalloproteinase inhibitors were shown to have significant effects on IL-1-induced changes in explant sGAG content and mechanical properties. More specifically, inhibition of MMPs with the non-selective and MMP-selective inhibitors but not the ADAMTS selective inhibitor demonstrated “protective” effects on fibrocartilage sGAG content and mechanical function during IL-1 stimulation. This suggests that, unlike articular cartilage for which ADAMTS mediated cleavage is responsible for early IL-1 induced proteoglycan release, MMPs but not ADAMTSs play a key role in IL-1-induced proteoglycan release from meniscal tissue explants. Immunohistochemical studies in our lab [8] have shown that in vitro IL-1 stimulation of meniscal fibrocartilage leads to generation of the G1- NITEGE aggrecan cleavage product, indicative of activated aggrecanase activity. MMPs can serve as upstream regulators of aggrecanase activation [9], and it is possible that these doses of non-selective and MMP-selective inhibitors are sufficient to prevent MMP-mediated activation of the aggrecanases and subsequent proteoglycan depletion. Interestingly, preservation of the collagen network through MMP inhibition – by the non-selective and MMP-selective inhibitors – may contribute substantially to retention of proteoglycans in meniscus fibrocartilage. Differences in enzyme activation and activity between articular cartilage and meniscal fibrocartilage may require different therapeutic strategies for these two tissues.

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REFERENCES

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