INTRODUCTION
Proinflammatory cytokines including interleukin-1 (IL-1) are upregulated in cartilage and synovial fluid during the pathogenesis of osteoarthritis (OA)\textsuperscript{[4,5]}, and IL-1 treatment of cartilage tissue in vitro has been widely used as a model of OA degradation\textsuperscript{[3,4]}. The current study targets the effects of IL-1 on the fibrocartilaginous meniscus of the knee, major contributors to load distribution, lubrication, and overall joint stability\textsuperscript{[9]}. Recent evidence that degenerative meniscal lesions precede cartilage degeneration both in early stage OA patients and in asymptomatic population\textsuperscript{[6,7]}, suggests that meniscal degeneration may be an early event in the development of idiopathic osteoarthritis of the knee. Despite the growing indications of its importance, however, relatively little is currently known regarding the causes and implications of meniscal degeneration. The goal of this study was to characterize the biochemical and subsequent biophysical effects of exposure of meniscal tissue explants to IL-1 over a time-course of 14 days.

METHODS
Tissue Culture
Meniscus cores (4mm dia) were excised from both menisci of immature (2-4 week) bovine stifte joints and trimmed to a thickness of 2mm by removing both surface layers. Explants were precultured for 3 days in basal serum-free medium (DMEM, 50g/mL Gentamicin, 0.1mM NEAA, 50g/mL ascorbate) and then an additional 14 days with or without 20ng/mL IL-1. Media were changed and collected every 2 days. Explants (n=8/group/time point) were removed after 0, 7, or 14 days and stored at -20°C for subsequent analysis. Mechanical Testing: Explants were tested at room temperature in 0.15M PBS plus protease inhibitors in shear and in unconfined compression as previously described\textsuperscript{[1]}. Briefly, complex shear moduli ($G^*$) were obtained through oscillatory torsion testing (10% compressive strain, 0.5% shear strain; 0.01, 0.1, 1, 10Hz). Next, equilibrium moduli ($E_{eq}$) were obtained from equilibrium stress-strain values from a step compression-relaxation protocol (5% steps to 20% max strain), and dynamic moduli ($E_{dyn}$) were determined from a composite frequency sweep (10 ± 1.5% strain; 0.001, 0.01, 0.1, and 1Hz). Explants were then digested in Proteinase K. Biochemical Assays: Explant digests and conditioned media were assayed for sulfated glycosaminoglycan (sGAG) content via the DMMB assay and for collagen content via the chloramine-T/pDAB hydroxyproline assay using a collagen:hypro-ratio of 8:1. Media nitrite levels (a measure of nitric oxide release) were measured by the Griess reaction. Analysis: Media biochemical data and mechanical testing data were log-transformed to equalize variances. At each time point, data were analyzed with Student’s t-test ($p<0.05$) and are plotted as mean ± s.e.m.

RESULTS
Depletion of meniscal extracellular matrix was evident through increased release of matrix constituents to the media and corresponding decreases in residual biochemical content. Control samples exhibited a steady, basal sGAG release rate, but released virtually no collagen. Peak release of sGAG from IL-1-treated meniscus explants was detected at day 2 ($p<0.005$ v. control, Fig 1A), followed by a gradual drop to control levels by day 7. Collagen release began at day 6 and accelerated to a peak at day 14 ($p<0.001$, Fig 1B). The kinetics of media nitrite levels paralleled sGAG release with a peak at day 2 ($p<0.001$, Fig 1C). Of note, each of these occurred substantially earlier for meniscus explants than has previously been reported for articular cartilage (Fig 2)\textsuperscript{[7]}. At day 7, residual sGAG and collagen contents of IL-1 treated samples were not significantly different from serum-free controls ($p<0.172$ and $p>0.878$, respectively), but by day 14 there were significant decreases in both sGAG and collagen contents ($p<0.002$ and $p<0.026$, respectively, Fig 3B, 3C).

DISCUSSION
In vivo models of OA, differential effects of OA development on cartilage and meniscus tissues have been identified. This in vitro study revealed that while the overall patterns of IL-1 induced degradation are similar for meniscus and cartilage explants, peak release of sGAG and collagen occurs earlier (by 2-4 days) for meniscus. Likewise, meniscus explants exhibited a more rapid loss of material properties than previously reported for cartilage explants. Interestingly, changes in all fibrocartilage material properties appeared to follow the sGAG depletion. While the compressive properties ($E_{eq}$, $E_{dyn}$) were expected to depend on proteoglycan abundance, the behavior under shear ($G^*$) was expected to depend strongly on the abundance and integrity of the collagen network. The strong dependence of the shear modulus on PG content suggests that despite the relatively low PG content of meniscal tissue, osmotic "inflation" of the collagen network is an important determinant of fibrocartilage mechanical behavior.

ACKNOWLEDGEMENTS
Supported by an Arthritis Foundation Arthritis Investigator grant, NSF and Medtronic Foundation fellowship to SMI, and through the GT/NIH Training Program in Cellular and Tissue Engineering for CGW.

REFERENCES