Introduction: Human intervertebral disc (IVD) degeneration is accompanied by chronic inflammation, particularly seen in the elevated levels of pro-inflammatory cytokines IL-1β and TNF-α [1-3]. Animal models of disc degeneration (DD) using stab or laceration of the disc generally reproduce morphological changes of IVD degeneration. However, inflammatory changes in these models are thought to be acute and transient post injury [4-6]. The goal of this study is to explore the effect of direct inflammatory stimulation of the IVD on disc biochemical and biomechanical properties in vivo. We utilize lipopolysaccharide (LPS), an inflammatory stimulant that provokes secretion of multiple cytokines by disc cells. We have previously shown that direct injection of LPS into the disc results in significantly higher protein levels of IL-1β, TNF-α, HMGB-1 and MIF vs. sham injection up to 7 days post administration [7]. The goal of this study is to explore the dose-dependent response of this inflammatory stimulation on the biochemical and biomechanical properties of IVD in vivo. We hypothesize that LPS stimulation mimics the pathophysiology of DD by triggering a group of cytokines that are associated with IVD degeneration. LPS is administered using micro needles (<10% disc height) in order to minimize the potential disruption of the disc by needle injection.

Methods: Male Sprague Dawley Rats were used (N=15). Rats were anesthetized and an incision was made exposing 4 caudal motion segments (Co 3-4, Co 4-5, Co 5-6, Co 6-7). LPS Injection: A 33G needle was inserted 4mm into the center of the disc with clamp guidance. 2.5μl of LPS (1, 10, or 100μg/mL) or PBS (to serve as a sham control) were injected into alternating discs using a micro-liter syringe. Incision was sutured and animals were allowed unrestricted activity for either 2 or 7 days and then euthanized for biomechanical and biochemical analyses.

Biochemical Content: Individual discs were dissected and separated into annulus fibrosis (AF) and nucleus pulposus (NP). Tissue water content was measured and tissue was digested with papain overnight. Tissue digests were analyzed for DNA content, GAG content (Blyscan assay) and collagen content (hydroxyproline assay). Biochemical content is reported as % of tissue wet weight.

Results: No changes were seen in any of the examined parameters at day 2 post injection. On day 7, significant changes in size, biochemical, and biomechanical properties of segments injected with LPS were found. The DNA content of the AF and NP from discs injected with PBS or LPS were comparable (Figure 1). Motion segment height decreased and CSA increased vs. sham injection at the highest LPS dose (Figure 2). The NP biochemical content changed post LPS injection. The GAG content of the NP was unchanged (not shown), but the collagen content in the NP increased at day 7 post LPS injection at 10 and 100 μg/mL (Figure 3). The collagen content of the AF was unchanged (not shown), however significantly higher levels of GAG were found in the AF in 100 μg/mL LPS group (Figure 4). The creep (equilibrium) modulus of the motion segments was comparable in groups injected with 0 (sham), 1 or 10 μg/mL LPS. Injection of 100 μg/mL LPS resulted in significantly lower creep modulus at day 7 (sham: 3.89±1.7 MPa, 100μg/mL LPS: 2.5±0.58 MPa; Figure 5). A similar response was seen in dynamic modulus, which decreased by ~30% in 100 μg/mL LPS group on day 7 (Figure 6).

Discussion: The goal of this study was to explore the effect of direct inflammatory stimulation of the IVD on biomechanical and biochemical properties in vivo. Our findings indicate that LPS results in dose-dependent loss of ECM constituents and compressive biomechanical properties. We have previously shown that LPS results in increased levels of multiple pro-inflammatory cytokines in vivo on day 1 and day 7 post injection vs. sham [7]. This inflammatory stimulation does not result in loss of disc cell viability (Figure 1), but appears to be sufficiently potent to stimulate early degenerative changes in the IVD. Our findings indicate that LPS results in NP de-differentiation in vivo, with increased levels of collagen content (Figure 3). Similarly, AF tissue was found to have higher GAG content (Figure 4). These changes suggest that LPS stimulation and the resulting inflammation are modulating the microenvironment of the IVD, in a way that mimics the pathophysiology of DD; increases collagen-1 have been previously observed in DD, indicative of degenerative fibrillation [8]. In future studies, we will measure the specific types of collagen present in our motion segments in order to further delineate these changes. We also found that LPS injection results in swelling of the disc (increased CSA) and loss of segment height (Figure 2). These morphological and biochemical changes led to significant loss of compressive mechanical properties of the IVD in dynamic loading and at equilibrium (Figure 5, 6). Our findings indicate that inflammation alone, in the absence of traumatic IVD disruption, may trigger the cascades of disc degeneration.

To our knowledge, this study is the first to examine the effects of inflammation on disc integrity in vivo. This type of model can facilitate the decoupling of the contribution of inflammation from mechanical injury as causes and in propagation of DD.

Significance: This study employs a novel model to induce disc degeneration (DD). Our findings identify a mechanism for DD, where inflammation in the absence of trauma can initiate and propagate the loss of ECM integrity and biomechanical function.