Abstract introduction: Pro-inflammatory cytokines in the inflamed joint disrupt the catabolic and anabolic balance of the chondrocytes, leading to the destruction of cartilage matrix [1-3]. Two most major pro-inflammatory cytokines in the joint are IL1β and TNFα [4]. They cause joint cartilage degradation by elevating the levels of transcription factors such as NFκB, GADD45β, ESE-1 and API1, which in turn activate the expression of Cox-2 and cartilage degradation enzymes MMPs [4]. Tissues surrounding the articular cartilage contribute to chondrocyte homeostasis and the manifestation of arthritis. Several factors (including TGFβ and IGF-I) have shown antagonistic effects toward pro-inflammatory cytokine-induced cartilage degradation, although they don’t often inhibit all aspects of pro-inflammatory cytokine signaling [5]. Therefore, much investigation is still needed to develop novel strategies for inhibiting cartilage destruction, which would lead to the regenerating stable cartilage.

Muscle lies in close proximity to cartilage and may regulate cartilage development and homeostasis by releasing biochemical signals into the interstitial fluid or blood in addition to providing biomechanical stimuli [6, 7]. A variety of growth factors are expressed in the muscle cells [8, 9]. In addition, muscle has been suggested to be an immune organ because it secretes a number of cytokines into the blood stream and may contribute to exercise-induced immune regulation [10]. Indeed, weakness of the quadriceps muscle is common in people with knee arthritis [11]. The objective of this study is to examine the effect of muscle cells on the response to pro-inflammatory cytokines in chondrocytes by co-culturing muscle-cartilage cells.

Methods: Murine myoblast (C2C12) cell line was purchased from ATCC. Rat cartilage cell line (RCS) was a gift courtesy of Dr. Lassar (Harvard Medical School). C2C12 muscle cells were cultured at a density of 60-90%. The cells were seeded at a density of 10^5/well of a 24 well plate. The cells were cultured for 3 days and were subsequently treated with TNFα (Peperotech) for another 3 days before 4% PFA fixation. Protein expression was analyzed by immunocytochemistry (antibodies from DSHB and Abcam) and Western Blot. qRT-PCR was performed on BioRad real time PCR machine. Statistically significant differences (i.e. P<0.05, denoted as “**”) were determined by one-factor ANOVA with post-hoc Tukey test using the statistics software SYSTAT12 (Systat).

Results section:
1. Co-culturing with muscle cells leads to enhanced-resistance to TNFα-induced damage in chondrocytes. We evaluated the effect of muscle cells on TNFα-induced cartilage damage by co-culturing muscle and cartilage cells in the presence of pro-inflammatory cytokine TNFα. A common mouse muscle cell line C2C12 was co-cultured with a rat chondrocyte cell line RCS [8, 12]. We found that when RCS chondrocytes were exposed to 2ng/ml of TNFα, the levels of both Collagen II and Collagen IX proteins were significantly reduced (Fig.1). However, chondrocytes co-cultured with C2C12 muscle cells exhibited much higher expression of Collagen proteins (Fig.1).

2. Co-culturing with muscle cells leads to a reduced-inflammatory state upon TNFα-treatment. We further evaluated the expression of factors that mediate the cartilage-damaging effect of pro-inflammatory cytokines by RT-PCR. We found that chondrocytes co-cultured with C2C12 muscle cells exhibit lower levels of NFκB, ESE-1, Cox2 and GADD45β (Fig.3).

Discussion: We found that co-culturing with muscle cells caused a much-reduced inflammatory state in the presence of TNFα in chondrocytes, which led to more stable collagen expression. Similar results were found when we applied IL1β to our cultures (data not shown). Our control experiments using non-muscle cell type NIH3T3 or Cos7 cells did not have this effect (data not shown), suggesting that muscle cell may provide certain signals that enhance the resistance to pro-inflammatory cytokine-induced cartilage damage. This study may lead to the discovery of novel muscle-derived signals in cartilage regulation or the progression of arthritis and provide insights into engineering stable cartilage for clinical applications.