Introduction: Progression to osteoarthritis after joint injury involves multiple tissue interactions. The combination of cartilage mechanical injury (INJ) in vitro followed by co-culture with excised joint capsule (Co) has been used as a model to physiologically mimic an acute injury [1]. Previous studies [2] have shown that INJ+Co increases catabolic gene expression and aggrecan fragment levels. We hypothesized that treatment of growth factors will mitigate the effects of injury on gene, protein, and cell vitality levels. Our objective was to test whether OP-1 and IGF-1 can protect cartilage and joint capsule (JC) following injury by measuring gene expression, proteoglycan synthesis, and apoptosis.

Materials and Methods: Tissue Harvest: Bovine calf cartilage disks were harvested from the patello-femoral groove and equilibrated for 2 days under free-swell (FS) conditions in serum-free DMEM supplemented with 1% ITS. JC explants were excised adjacent to the femoral condyles and equilibrated for two days. Injury and Time Course: At time = 0, cartilage disks were allocated into one of 8 conditions: (1) FS, (2) normal cartilage co-cultured with JC (Co), (3) cartilage mechanically injured (INJ, 50% strain at 1mm/sec), and (4) injured cartilage co-cultured with joint capsule (INJ+Co). (5-8) 100ng/ml OP-1 + 300ng/ml IGF-1 (GFs) added throughout culture duration. Gene Expression: Cartilage disks and JC were flash frozen 2, 8, 24, 48, and 72 hours after injury for short term, and frozen at days 1, 4, 8, and 16 days for longer term; RNA was extracted, reverse-transcribed, and mRNA levels of 48 cartridge relevant genes measured using qPCR and normalized to 18s. K-means-Clustering analysis was performed to determine gene co-expression pattern. Protein Bioynthesis: 35S-sulfate and 3H-proline incorporation (for proteoglycans and total protein synthesis) were measured in the last 24 hours of 1, 4, 8, 12, or 16 day cultures post injury. Apoptosis: Plugs were fixed in 4% paraformaldahyde at 1, 4, 8, and 16 days post injury and were sectioned to evaluate apoptosis via nuclear blebbing by light microscopy. Statistics: Significance was determined using the Wilcoxon-Sign rank test and ANOVA at the p < 0.05 level.

Results: Cartilage Short Term Gene Expression: Key ECM molecules (aggrecan, collagens) were significantly down-regulated in the presence of Co, INJ, and INJ+Co. Proteinases (MMP-1,3,9,13; ADAMTS-1,4,5) and TIMP-1,2,3 were maximally expressed compared to controls by INJ+Co. GF failed to rescue ECM down-regulation, while up-regulating proteinase transcripts. iNOS and caspace-3 were maximally expressed under Co alone; iNOS reached values of 2000x FS. Clustering analysis revealed 5 distinct gene expression profiles (Fig 1): Group-1 (ECM molecules) was down-regulated with Co, INJ, and INJ+Co. Group-2 (iNOS, Cas-3) was up-regulated by Co. Groups-3, 5 (proteases) were maximally expressed by INJ+Co. Group-4 (oxidation genes) was responsive to GF treatment. Joint Capsule Gene Expression: Relative gene expression levels within JC explants while in co-culture with normal cartilage explants showed proportionally higher levels of protease abundance compared to normal cartilage (Fig 2). All genes in JC (except ADAMTS-5) were stimulated by GF treatment. Cartilage Longer Term Gene Expression: While most genes measured (Fig 2B) returned to FS values after 16 days, MMP-9 showed a consistent upregulation even at day 16 for Co (147x) and INJ+Co (45x). Chondrocyte Biosynthesis: 35S-sulfate and 3H-proline incorporation was significantly reduced by Co and INJ+Co. GF treatment slightly increased 35S-sulfate incorporation for all conditions (Figure 3A). Apoptosis: Significant apoptosis was observed at days 4, 8, and 16. At Day 8, INJ caused significantly higher levels of apoptosis (47%) compared with FS (<1%). GF treatment significantly decreased apoptosis in INJ alone (Fig 3B).

Discussion: GF treatment stimulated most of the genes measured, suggesting that the growth factor combination upregulated general remodeling rather than just anabolic stimulation of ECM. Aggrecan and collagen transcript levels were severely down regulated under each injury stimuli, and GF treatment failed to protect such decreases in transcript levels. GF was able to increase protein synthesis (ongoing studies using IHC are to identify specific anabolic and catabolic events). The joint capsule expressed proteases at levels orders of magnitude greater than non-injured cartilage (Fig 2), suggesting the probability of cartilage-JC communication. GF down-regulated ADAMTS-5 while up-regulating all other genes; suggesting a potentially important protective effect. Caspase-3, a key signaling molecule in apoptosis, was significantly up-regulated (12s) under Co alone. As seen before [3], apoptosis was significantly increased following INJ, while there is a trend towards an increase in cell death under Co. While previous studies showed increased apoptosis due to mechanical injury, we have now shown a direct link between chondrocyte apoptosis in normal cartilage due to co-culture with excised joint capsule. GF treatment protected cartilage against apoptosis under INJ alone; when JC was present the effect was less dramatic. Thus, GFs could party reverse injury-induced changes in biosynthesis and apoptosis, but not transcript levels.