Introduction
Intra-articular (IA) injections with hyaluronan (HA) have been used clinically to manage osteoarthritis (OA) pain. The symptom-modifying effects of HA with a course of 3 or 5 injections may last over 6 months to a year in some patients, which exceeds the relatively short residence time of exogenous HA in the injected joints. Accumulating evidence has also demonstrated disease-modifying activity of HA in animal models of OA. These findings led us to hypothesize that HA may play a biological role by affecting gene expression in OA joints, resulting in pain relief and possibly some joint protection. In our previous study, we found that 3-weekly injections with hylan G-F 20, a cross-linked high molecular weight HA (6-8 MDa), improved the structural score of cartilage in a rabbit instability model of OA at 1-week post treatment regimen. In this study, we performed a time course study to examine the sustainability of the chondro-protective effect using the same rabbit model. Moreover, to probe for the mechanism of the chondro-protective effect, we analyzed expression of genes involved in cartilage matrix metabolism in normal, OA, and hylan-treated OA cartilage and synovium. These studies provide insights into the therapeutic effects and molecular mechanism of viscosupplementation therapy.

Methods
Study design: Male New Zealand White rabbits (8-12 months old, 3.2-4.5 kg body weight, 10 per group) underwent either anterior cruciate ligament transaction (ACLT) to induce OA or a sham surgery in one randomly selected knee. Four weeks following the operation, a series of 3-weekly IA injections was performed in the operated knee of 0.5 ml of hylan G-F 20 (8 mg/ml) or Lactated Ringer’s Solution (LRS) as injection control. Animals were sacrificed 1 week after the last injection for histological assessment. Some animals were sacrificed 1-week after the last injection for gene expression analysis.

Histological assessment: The knee joints were collected, fixed in 4% paraformaldehyde, decalcified, processed, and stained with H&E and toluidine blue. Histological sections were scored by a blinded board certified veterinary pathologist using both the Mankin and Carlson scoring systems. The Carlson cartilage structure score, and Carlson PG content score were also examined separately using a relative quantitative Carlson scoring system on H&E and toluidine blue stained sections, respectively. Osteophyte size determination: The formation of osteophytes was quantified by tracing the osteophyte area on the perimeter of the trochlear ridges using Meta Morph image analysis of gross morphological photos.

RNA isolation and real-time PCR analysis: Total RNA was isolated from articular cartilage and synovium using a modified TRIzol method. Twenty nanograms of RNA were used to produce amplified cDNA (NuGen Ovation). Rabbit gene specific primers and probes were designed using Primer Express software (Applied Biosystems). The gene specificity of the amplicons was confirmed by BLASTN search detected in the synovium, but the levels were unaltered across the 1-week time point, the disease group (ACLT-LRS) developed more significant lower (better) Carlson cartilage structure score (8.0 vs. 9.7; p = 0.0119; n = 10) and smaller osteophytes (1.42 x 10^5 vs. 3.58 x 10^5 pixels; p< 0.05) than OA control groups. At 8-week post injections, the 3 scores of the OA control group were higher (worse) than those at 4-week. At this time point, no significant difference was observed in the parameters examined between hylan treated and non-treated OA groups.

To explore the mechanism of the early chondro-protective effect of IA injections with hylan G-F 20, we examined the expression patterns of several genes involved in cartilage matrix metabolism including extracellular matrix proteins, matrix-degradation proteinases, and pro-inflammatory cytokines. Type I (Col I) and type II collagen (Col II) mRNA levels were increased in OA cartilage compared with normal controls, which were further increased in hylan treated OA cartilage compared with OA controls. The ratio of Col II/COL I, as a degeneration index of cartilage, was lower in both non-treated and treated OA cartilage than normal cartilage (control = 199±81, OA = 25±5, and OA + hyalan = 19±7), reflective of the degenerative nature of OA. MMP13 expression was dramatically increased in OA cartilage by >10-fold, and similar expression levels were also detected in hylan-treated cartilage. Significantly, MMP16 was identified as one of the hylan-responsive gene that was altered in both cartilage and synovium compared with the control and was changed back toward normal in hylan treated tissues. A similar expression pattern of MMP16 was also found in the synovium. IL-1β is known to play an important role in cartilage matrix metabolism. We found that IL-1β mRNA levels were low or undetectable in cartilage in control, OA, and OA + hylan groups. Low levels of IL-1β message were detected in the synovium, but the levels were unaltered across the groups.

Discussion
Viscosupplementation therapy has shown efficacy in relieving OA symptoms in clinical patients and having short-term effects on modifying OA progression in animal models. The mechanism of action of both effects remains unclear. Using a rabbit ACLT model of OA, we found that 3-weekly IA injections with hylan G-F 20 improved the integrity of cartilage structure, but not its PG content. This chondro-protective effect was observed as early as 1-week, sustained at 4-week, and undetectable at 8-week post-treatment. We then examined if hylan exerted its role by affecting gene expression in joint tissue. Because of its specific role in cartilage structure, we focused on the genes that are potentially involved in cartilage matrix metabolism. Our findings of type II collagen up-regulation and MMP16 down-regulation in hylan-treated joint tissues suggest that hylan G-F 20 may protect the cartilage structure by both stimulating the repair and inhibiting the degradation processes at the molecular level. Interestingly, we also found that hylan treatment reduced osteophyte formation in this rabbit model. Whether these effects contribute to clinically reported long-term pain relief associated with hylan G-F 20 treatment requires further investigation. Analyses of the genes that may contribute to osteoarthritic pain are currently underway.


Poster No: 0703