Introduction
Controlling cartilage degradation involves maintaining balance between anabolic growth factors like IGF-I and catabolic proteins, including interleukin-1. IGF-I is well known for increased proliferation and metabolism of chondrocytes and also has been shown to promote cartilage healing when administered intra-articularly, but has limited effects due to a short half-life. Gene transduction of IGF-I to the synovial membrane would be less invasive, allowing longer ligand production. Interleukin-1 receptor antagonist gene has been shown to be effective in the control of the catabolic cascade associated with osteoarthritis. Combination therapy of IGF-I and IL-1Ra may provide dual axis therapy that has the potential to concurrently improve cartilage resurfacing techniques and control osteoarthritic progression and healing. This study investigated the possibility that dual transduction of synoviurn with IGF-I and IL-1Ra would increase the production of IGF-I and IL-1Ra and concurrently control cartilage matrix degradation and promote cartilage restoration.

Materials and Methods
Recombinant viral vectors containing either the coding sequence of equime IGF-I (Ad IGF-I) or equine IL-1Ra (Ad IL-1Ra) were constructed using the Cre-lox system and propagated in a transformed embryonic kidney line. Cartilage explants and synovial membrane were harvested from two 14 month-old horses. The synovial membrane was digested in 0.15% collagenase and 0.015% DNase and cultured in 6 well plates at 1.5x10⁶ cells per well. Monolayers were established and transduced with both Ad IGF-I (200 moi), Ad IL-1Ra (100 moi) or the combination of Ad IGF-I and Ad IL-1Ra. Following transduction, cartilage explants were exposed to the synovial monolayer medium by a co-culture system. Cultures were maintained with either serum-free medium or medium containing 10 ng/mL recombinant human IGF-I for 6 days, with medium exchanged every 48 hours. At 6 days, the monolayers were harvested for gene expression analysis measured using Taqman® PCR analysis of IGF-I, IL-1Ra, Collagen type I, Decorin, IL-1β, IL-1α, and Stromelysin expression. Cartilage explants were snap frozen in liquid nitrogen for gene expression analysis of IGF-I, Collagen type II, IL-1β, IL-1α, Tissue inhibitors of metalloproteinase-I (TIMP-1) and TIMP-3. Explants were also rinsed in protease inhibitors and snap frozen for proteoglycan determination using DMMB dye-binding assay, or fixed in 4% paraformaldehyde, paraffin embedded and sectioned for H&E and toluidine blue histochemical staining and in situ hybridization for IGF-I, and Collagen type II expression. Media samples were analyzed by DMMB dye-binding assay for proteoglycan content, radioimmunoassay for IGF-I ligand concentration, and ELISA for IL-1Ra ligand concentration.

Results
Synovial monolayer transduction was confirmed by analysis IGF-I and IL-1Ra transgene expression using Taqman® PCR (Table 1).

Table 1. Results of Taqman® PCR of synovial monolayers for IGF-I and IL-1Ra.

<table>
<thead>
<tr>
<th>Synovial Treatment</th>
<th>Normal Medium</th>
<th>Interleukin-1 Medium</th>
<th>Normal Medium</th>
<th>Interleukin-1 Medium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>68 ± 47</td>
<td>254 ± 137</td>
<td>106 ± 97</td>
<td>107 ± 51</td>
</tr>
<tr>
<td>Ad IGF-I</td>
<td>64016 ± 121193</td>
<td>1704000 ± 49069</td>
<td>133 ± 49</td>
<td>106 ± 100</td>
</tr>
<tr>
<td>Ad IL-1Ra</td>
<td>20 ± 12</td>
<td>210 ± 111</td>
<td>631259 ± 127441</td>
<td>361096 ± 132315</td>
</tr>
<tr>
<td>Ad IGF-I/Ad IL-1Ra</td>
<td>446100 ± 62974</td>
<td>576541 ± 259684</td>
<td>762450 ± 88579</td>
<td>582507 ± 90518</td>
</tr>
</tbody>
</table>

*Samples were normalized to 18S copy number.

Synovial IL-1β expression was increased in samples maintained in IL-1β medium and control wells transduced with Ad IL-1Ra. Message expression for IL-1α was increased in samples treated with IL-1β medium and samples transduced with Ad IL-1Ra and co-transduced with Ad IGF-I/Ad IL-1Ra. Stromelysin expression was elevated in the control treatment monolayers containing IL-1β medium, but was maintained at basal levels when adding the IGF-I and IL-1Ra transgene, irrelevant of medium content. Decorin expression was highest in control wells and wells transduced with Ad IL-1Ra in both medium treatments. Collagen type I expression was generally higher in the control medium treatment, with highest expression in the Ad IGF-I transduced monolayers. Conversely, Collagen type I expression in the IL-1β medium was up-regulated in the Ad IGF-I/Ad IL-1Ra co-transduced monolayers (Figure 1).

Discussion
Synovial monolayers were readily transduced with both Ad IGF-I and Ad IL-1Ra. IGF-I ligand was secreted at moderate levels. Transduction with IGF-I stimulated the synovial production of connective tissue molecules, such as collagen type I. The beneficial effects of the combination of anabolic growth factors and catabolic blockers were evident in increased proteoglycan content of cartilage explants when exposed to IL-1. These results show that gene therapy combining anabolic growth factors to stimulate matrix synthesis and catabolic blockers to prevent matrix degradation by IL-1, protects and causes partial restoration of cartilage matrix, and suggest the benefit of combination therapy for cartilage healing.

References
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