Arthroscopic Implantation of Autologous Chondrocytes Transduced with rAAV5-IGF-I Improves Articular Cartilage Repair at 8 Weeks

INTRODUCTION
Articular cartilage injury occurs frequently and often precipitates osteoarthritis, a degenerative condition that is generally debilitating. Cartilage has minimal to no intrinsic healing capabilities, prompting intense investigation into cell-based methods for improving joint surface repair. Autologous chondrocyte implantation (ACI) is being used more extensively to return function and minimize osteoarthritis. Gene enhanced chondrocyte implantation improves repair over naïve cells [1]. IGF-I, an important anabolic protein, has previously been shown to protect and aid in the recovery of the extra-cellular matrix following experimentally induced damage with IL-1 and TNF-α. The transitory nature of growth factor supplementation has led to investigation of gene therapy approaches to allow for persistent transgene expression. AAV is an ideal viral vector as it lacks pathogenicity, can invade dividing and non-dividing cells, has long-term transgene expression (up to 60 days) and appears to be minimally immunogenic. AAV-5 has been shown to provide efficient transgene expression in chondrocytes in vitro. This study evaluated the effects of rAAV5-IGF-I transduced chondrocytes on repair of full-thickness chondral defects in the equine stifle.

METHODS
rAAV-eGFP constructs. The rAAV transfer vector plasmid pHpa-trs-SK contained the gene for eGFP under CMV promoter control, and flanked by ITRs (generous gift from Dr. Ghizzzani, UF). scAAV5-eGFP vectors were prepared by the Research Vector Core Facility of the Children’s Hospital of Philadelphia (CHOP). rAAV-IGF-I constructs. The full-length equine IGF-I cDNA was amplified by PCR, subcloned in-frame into the rAAV transfer vector plasmid pHpa-trs-SK with sacII and NotI sites with the CMV promoter. The rAAV5-IGF-I vector was prepared using a double plasmid helper virus free method by CHOP. Chondrocyte isolation and ex vivo transduction. Cartilage was harvested arthroscopically from the distal lateral and medial trochlear ridges of the talus of 24 two-year-old horses. Chondrocytes were isolated as described [2], stored frozen until needed, and 15-20 million cells thawed and cultured in flasks. 48 hours prior to implantation chondrocytes were infected with 10^5 AAV vg/cell for 2h in Opti-MEM at 37°C. Chondrocytes from 8 horses were transduced with rAAV5-IGF-I, chondrocytes from 8 horses with rAAV5-eGFP as control AAV transduced cells, and chondrocytes from 8 horses remained as untransduced controls. Transduction efficiency of the rAAV-eGFP vector was determined as the relative population of green fluorescent chondrocytes in the cultures by fluorescent microscopy. Chondrocyte implantation. A single 15mm full-thickness chondral defect was created arthroscopically in the lateral trochlear ridge of both femoropatellar joints using a spade bit and protective cannula. The treatment defect was repaired with a mixture of chondrocytes placed in autogenous cryoprecipitated fibrinogen and calcium activated thrombin to form a stable graft while the control defect received fibrin alone (Fig 1).

RESULTS
Arthroscopic implantation of autologous chondrocytes transduced with rAAV5-IGF-I in a fibrin vehicle resulted in significantly better total gross healing scores at 8 week arthroscopic second look surgery when compared to paired control defects repaired with fibrin alone (p=0.03) (Fig 2). Defects repaired with rAAV5-IGF-I transduced chondrocytes also had significantly better total gross healing scores than defects repaired with rAAV5-eGFP (p=0.04).

DISCUSSION
Arthroscopic implantation of autologous chondrocytes transduced with rAAV5-IGF-I improved healing of full-thickness chondral defects in the short-term. rAAV-IGF-I transduced cells produced substantial IGF-I ligand that increased IGF-I concentrations in the synovial fluid compared to pre-implant levels. Long-term results will provide further information on the effect of genetically modified chondrocytes on articular cartilage repair.

SIGNIFICANCE
This study provides evidence that genetically modified chondrocytes can improve healing of articular cartilage. Further investigation into the use of AAV in cartilage repair is warranted.

REFERENCES

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