INSULIN-LIKE GROWTH FACTOR-I ENHANCES CELL-BASED ARTICULAR CARTILAGE RESURFACING

*Fortier, L.A., **Lust, G., +Nixon, A.J. *+Comparative Orthopaedics Laboratory and James A. Baker Institute for Animal Health, Cornell University, Ithaca, New York 14853, (607)253-3050, FAX (607)253-3271, ajn1@cornell.edu.

Introduction—Traumatic articular cartilage injuries in adults are common but given the minimal repair response of cartilage, the injuries may lead to the development of osteoarthritis. Several grafting methods have been used in an attempt to augment the normal healing response and prevent osteoarthritis. Insulin-like growth factor-I (IGF-I) enhances the phenotypic expression of cartilage matrix molecules when added to cartilage explants or isolated chondrocytes.[1,2] IGF-I may be incorporated into fibrin during polymerization from fibrinogen, and elutes from fibrin for 22 days at concentrations that effectively enhance chondrocyte phenotypic expression.[3] Using fibrin as a graft vehicle has the added advantage of being arthroscopically applicable thereby avoiding the morbidity of an arthrotomy. Our hypothesis for this study was that IGF-I, added to chondrocyte-fibrin grafts would significantly enhance the repair tissue formed compared to chondrocyte fibrin grafts alone.

Methods—Eight horses were anesthetized and 15mm full-thickness cartilage defects were made on the lateral trochlear ridge in each femoropatellar joint using arthroscopic guidance. All protocols were approved by the IACUC. The defects were grafted with fibrin containing autologous chondrocytes ($1x10^7$/ ml of fibrin), anchored to the base of the defect by 2 Biofix® Minitacks . One defect was grafted with fibrin + chondrocytes + 25μg IGF-I, and the control defect was grafted with fibrin + chondrocytes without added IGF-I.

The horses were rested for 6 weeks followed by a graded exercise program. Synovial fluid was sampled from each joint on postoperative days 4, 7, 10, 14, 21, 28, and 240 and analyzed for differential cell counts, total protein, and hyaluronan content. All horses were euthanized at 240 days. Cartilage was harvested from the lesion, perilesional (1 cm from defect) and remote (>2 cm) to the defect for histologic and biochemical analyses, and synovial membrane was harvested for histologic analysis. Histology specimens were stained with H&E to evaluate morphology; cartilage-bone specimens were also stained with toluidine blue to assess proteoglycan distribution. Histologic specimens were evaluated blindly and assigned an overall score based on several criteria evaluated. Cartilage biochemical analyses included total glycosaminoglycans (GAGs) by DMMB spectrophotometry, total DNA by bisBenzimide fluorescence, total collagen by rpHPLC determination of hydroxyproline content, and percent type II collagen content by CNBr- cleavage and SDS-PAGE analysis. Statistical analyses for differences in synovial fluid cell count, total protein and hyaluronan content between IGF-I treated and control joints were performed by analysis of covariance using time as a covariate to account for the expected changes following surgery. Histologic scores and biochemical data were analyzed using a Student's paired-T test since each animal served as its own control. For control and IGF-I containing grafts, a one-way analysis of variance was used to determine differences between lesional, perilesional, and remote sites within each animal.

Results—The IGF-I treated joints had a significantly improved synovial membrane score and in the decalcified sections there was better integration of cell and matrix patterns between repair and surrounding tissues (*Fig. 1*).

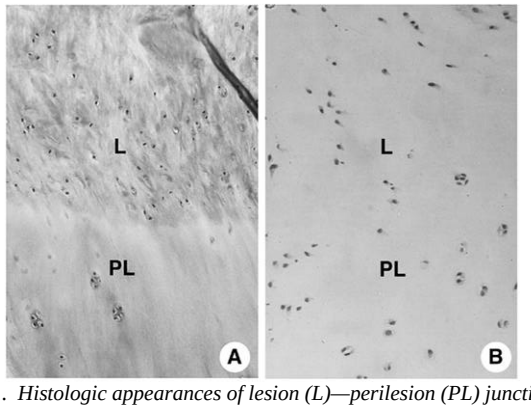

Figure 1. Histologic appearances of lesion (L)—perilesion (PL) junctional cartilage tissues in control (A) *and IGF-I grafted joints* (B). *There is better integration between the tissues in the IGF-I grafted* (B) *than in the control joint* (A). *(H&E)*

The overall cartilage histologic score in the IGF-I treated joints was not significantly increased compared to control joint scores. There were no differences in synovial fluid parameters between control and IGF-I grafted femoropatellar joints. The IGF-I treated joints contained increased amounts of total GAGs and DNA compared to control joints. The addition of IGF-I to the graft material also significantly increased the percent of type II collagen as determined by CNBr- cleavage and SDS-PAGE analysis (*Fig 2.).*

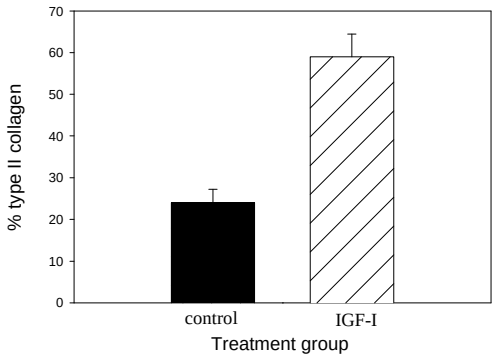

Figure 2. Cyanogen bromide cleavage and SDS-PAGE analysis of cartilage tissue from grafted sites. The addition of IGF-I to the graft material significantly increases the percent of type II collagen produced. Each bar represents the mean of n=8 +/- sem.

Discussion—The addition of IGF-I to chondrocyte-fibrin graft material significantly enhanced the quality of repair tissue formed in large, full-thickness acute cartilage injuries. There were no indications of adverse reactions to the IGF-I in any of the anaylses performed on the synovial fluid, synovial membrane, or cartilage tissues. The significantly improved synovial membrane histologic score in the IGF-I treated joint likely indicates a reduced synovial membrane response during the initial 28 days since all synovial fluid parameters measured were similar between IGF-I treated and control joints. These data suggest that exogenous IGF-I increased cell proliferation and phenotypic expression of the chondrocytes as indicated by the increases in total DNA, total GAG and percent type II collagen content in the IGF-I containing grafts compared to the control grafts. Grafting full thickness articular cartilage defects with fibrin containing chondrocytes and IGF-I enhances the endogenous healing reponse, forming tissue that resembles but does not entirely duplicate the structural or biochemical properties of normal articular cartilage.

References—

1. Luyten FP, Hascall VC, Nissley SP, et al. *Arch Biochem Biophys* 267:416-425, 1988.
2. Malemud CJ. *Rheum Dis Clin North Amer* 19:569-580, 1993.
3. Foley RL, Nixon AJ. *Am J Vet Res* 58:1431-1435, 1997.

Acknowledgements—Funded by National Institutes of Health AR08360-02 and the Harry M. Zweig Fund for Equine Research.

☐ One or more of the authors have received something of value from a commercial or other party related directly or indirectly to the subject of my presentation.

☒ The authors have not received anything of value from a commercial or other party related directly or indirectly to the subject of my presentation.