Safety of Intra-Articular Use of Atelocollagen for Enhanced Primary Repair of the ACL

Elise Magarian1, Ashley Mastrangelo1, Susan Connolly2, Patrick Vavken1, and Martha Murray1

1Department of Orthopaedic Surgery, Children’s Hospital Boston, 300 Longwood Ave, Boston, MA 02115
2Department of Radiology, Children’s Hospital Boston, 300 Longwood Ave. Boston, MA 02115

Introduction
There has been recent interest in stimulating repair of intra-articular structures, including the anterior cruciate ligament (ACL) using collagen-based hydrogels, typically containing platelets or other forms of growth factors. However, to date, there is little known about the safety of injecting collagen carriers into the joint. As native Type II collagen can cause collagen-induced arthritis (CIA) when injected into the joint, it is important to study whether soluble Type I atelocollagen will cause similar changes in the joint after injection for treatment of ligamentous injury. In this paper, we tested the hypothesis that the addition of atelocollagen to a suture repair of the ACL would result in inflammatory changes in the knee joint. Groups of animals underwent ACL repair with sutures alone or sutures plus atelocollagen in sponge or gel form where the gel form was mixed with autologous platelets before use. Measures of the inflammatory response included physical examination of the knee range of motion, systemic and synovial white blood cell counts, synovial thickness and effusion as measured by MRI at early (4 week) and late (15 week) time points. In addition, histologic analysis of the synovium and capsule and serum levels of two inflammatory cytokines: TNF alpha and IL-1B were measured at 15 weeks after repair.

Methods
After IACUC approval for the study had been obtained, seventeen knees of 30kg pigs underwent primary repair of the ACL. The experimental groups consisted of knees undergoing suture repair alone (SUTURE group, n=6), suture repair augmented with a collagen sponge (SPONGE group, n=5) and suture repair with collagen gel - platelet composite (CPC group, n=6). An additional 16 unoperated knees were used as a control group (INTACT). All knees were evaluated with physical examination, systemic and synovial white blood cell counts, MRI evaluation for the presence of an effusion or capsular thickening at 4 and 15 weeks after surgery (Figure 1). In addition, histologic analysis of the synovium and capsule and serum levels of two inflammatory cytokines: TNF alpha and IL-1B were measured at 15 weeks after repair using ELISA.

Figure 1 SAG T1 (a) and SAG T2 FSE (b) MRI images of a knee treated with SPONGE repair. Measurement sites: 1. suprapatellar synovial thickness, 2. infrapatellar synovial thickness, 3. infrapatellar effusion width, 4. suprapatellar effusion width, and 4. suprapatellar effusion length.

Results
Physical examination showed no significant loss in flexion or extension of the knee in comparing suture repair alone with either of the atelocollagen treatments. This was true after both 4 and 15 weeks in vivo (p > 0.05 for all comparisons). There was an increased loss of flexion in the SUTURE group when compared with the INTACT group at 15 weeks (p < 0.0014); however, there was no difference in the loss of flexion or extension between any of the atelocollagen treatment groups and the intact knees over the same time period of growth (p > 0.40 for all comparisons). At 15 weeks, there was a greater loss of knee flexion in the SUTURE group than in the other three groups, a difference that approached, but did not reach statistical significance due in part to the multiple group comparisons criteria for significance (p = 0.0193 in comparing SUTURE with SPONGE and p = 0.0251 in comparing SUTURE with CPC). In addition, after 15 weeks in vivo there was no significant loss of extension of the knee when comparing any of the treatment groups to each other (p = 0.5428). Finally, there were no significant differences in systemic (p > 0.4 for all comparisons) or synovial (p > 0.1 for all comparisons) white blood cell counts after 15 weeks.

After four weeks in vivo, there was no significant difference in synovial thickness or the amount of effusion between the knees treated with suture repair alone and those treated with the CPC group. After fifteen weeks in vivo the suture group showed significantly less effusion in the suprapatellar region of the knee when compared to the CPC group (p = 0.0046) and intact knees (p = 0.0021). However, after fifteen weeks the suture group showed a significantly greater effusion in the infrapatellar region of the knee when compared to the CPC group (p = 0.0010) and intact knees (p <0.0001; Figure 2). Type I soluble atelocollagen added as either a sponge or a collagen-platelet composite did not have any significant effect on the thickness of the synovium at fifteen weeks. In addition, histological analysis showed no significant differences in the number of synovial cell layers, number of lymphocytes, vascularity, and number of villi of the synovium after 15 weeks in vivo. Serum levels of IL1-β were significantly lower in the PRP group compared to non-PRP (p = 0.002). There was no significant difference in TNF-α levels between the PRP and non-PRP groups (p = 0.836).

Discussion
The introduction of atelocollagen into the knee joint as either a sponge or platelet gel to stimulate healing of the ACL did not appear cause a significant joint effusion, or to result in synovial thickening when compared with suture repair alone after four or fifteen weeks. In addition, no significant differences in synovial cell layers, number of lymphocytes, vascularity, or number of villi were observed after fifteen weeks. These findings are considerably different than those observed when type II collagen fragments are injected into the joint, or when synthetic materials, such as carbon fiber, were used for ACL reconstruction where a large inflammatory reaction occurs. In addition, the use of either a collagen sponge or gel made little difference in the resulting changes induced in the joint capsule or surrounding tissues. Finally, the reduction of IL1-β with the use of PRP suggests the possibility of a decrease in the occurrence of inflammation with enhanced repair, a preliminary finding that deserves additional study to determine whether suture repair enhanced with a collagen-platelet gel may lead to decreased inflammation in the knee when compared with suture repair alone.

Acknowledgements
The authors would like to acknowledge E Abreu, M Palmer, J Hootnick, and M Valenza for their assistance. Funded by NIH Grant AR054099.