MSC-Collagen Gel Augmentation to Enhance Osteotendinous Integration of a Patellar Tendon Autograft

INTRODUCTION
Proper formation of a tendon-to-bone insertion site is critical for successful repair of many clinical tendon injuries. A lack of tissue integration at the insertion site can compromise joint stability and function as well as long-term clinical outcome. The high failure rate of rotator cuff repair is often attributed to poor tissue incorporation at the tendon-to-bone insertion site.

When a soft tissue patellar tendon autograft (PTA) was used to repair a central-third PT defect in a rabbit, a normal zonal insertion site was not regenerated at the patellar or tibial insertion by 12 weeks post-op (Fig. 1). It is possible that the biological cues necessary to promote bone ingrowth and tendon incorporation may have been absent or present at insufficient levels in the repair site. We questioned whether a mesenchymal stem cell (MSC)-collagen gel biologic augmentation, secured in the bone troughs at the patellar and tibial insertion sites, might provide a medium to enhance autograft integration into bone.

Figure 1. Tendon-to-bone insertion site of the PTA and native struts. H&E and immunohistochemical staining for collagen type II (col2) demonstrate the presence of a fibrocartilage-like region that is organized in the native strut but disorganized in the PTA repair at 12 weeks post-op.

The objective of this study was to improve tendon-to-bone integration and repair tissue biomechanics of the patellar tendon autograft (PTA). We hypothesized that implanting MSC-collagen gel biologic augmentations at both insertions would improve repair tissue biomechanics over autograft repair alone at 12 weeks post-surgery.

METHODS
All procedures were approved by the University of Cincinnati Institutional Animal Care and Use Committee. MSC Harvest: Autologous MSCs were harvested from the iliac crest of skeletally mature, female New Zealand white (NZW) rabbits (n = 5). Bone marrow-derived MSCs were sub-cultured to passage two (P2). MSC-Collagen Gel Biologic Augmentation Creation: The collagen gel was created at a concentration of 2.6mg/ml according to Invitrogen protocol 5024 with the exception that de-ionized water was replaced with a mixture of phosphatebuffered saline (PBS), Dulbecco’s Modified Eagle’s Medium (DMEM) and MSCs at a concentration of 100K cells/ml. The cell-gel mixture was pipetted into the wells of a silicone dish (1.4 ml/well) and allowed to set for two to four hours before being supplemented with MSC feeding medium (DMEM, 5% FBS, 1% antibiotic/antimycotic, 1% Gluta-Max™). MSC-collagen gel biologic augmentations were fed three times weekly for two weeks.

MSC-Collagen Gel Biologic Augmentation Implantation: Under aseptic conditions, central-third PT tissue was excised from both limbs of each NZW rabbit. Bone defects were created at the proximal and distal insertions. In one limb, a biologic augmentation was secured in each of the proximal and distal bone defects before reattaching the resected PTA to the remaining native struts (PTA+Gel). In the contralateral limb, the excised PTA was also re-attached to the PT native struts but with no biologic augmentations (PTA). Following closure of the wound sites, rabbits were permitted 12 weeks of recovery. After sacrifice, each limb was harvested and frozen at -20°C for later biomechanical evaluation.

Biomechanical Evaluation: After recording whole PT dimensions, the central-third repair tissue was isolated and measured. Patella-repair tissue-tibia samples were then failed in uniaxial tension at a rate of 20%/sec as previously described.

Statistical Analysis: Treated and control groups were contrasted and also compared with normal PT properties. Statistical significance was established using ANOVA at a p<0.05.

RESULTS
MSC-collagen gel augmentation did not significantly improve PTA repair tissue biomechanics after 12 weeks of recovery (Fig. 3). The linear stiffness of PTA+Gel and PTA repairs reached 35.0% and 42.7% of normal central-third PT, respectively. The maximum force sustained by PTA+Gel and PTA repairs was 26.9% and 33.3% of normal, respectively.

Figure 3. Force-elongation plots for PTA and PTA+Gel repair tissues after 12 weeks of recovery relative to normal central-third PT.

DISCUSSION
There are several potential explanations as to why the MSC-collagen gel biologic augmentation did not improve PTA repair tissue biomechanics. First, the implanted augmentations may not have remained in the bone defects. To determine if this is the case, MSC-collagen gel biologic augmentations have recently been created with fluorescently labeled cells and implanted in vivo (n = 4). Histological sectioning of the resulting repair tissues will indicate the location of the implanted cells. Second, the augmentations may have remained in place but did not possess the correct biological cues to promote tendon-to-bone integration at the insertion site. In vitro studies are currently examining the alkaline phosphatase activity (ALP, an early marker for bone formation) and the mRNA expression levels of collagen type II (col2) and bone morphogenetic protein-7 (BMP-7) of MSC-collagen gel biologic augmentations. Parallel studies are examining methods to promote ALP activity and mRNA expression of col2 and BMP-7 such as increasing the cell concentration of the augmentations and/or changing the number of days in culture. We expect that this strategy to augment autograft incorporation using MSC technology will also be useful in promoting osteotendinous integration in the ACL and rotator cuff.

REFERENCES

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