ENHANCED HISTOLOGIC REPAIR IN A CENTRAL DEFECT IN THE ANTERIOR CRUCIATE LIGAMENT WITH A COLLAGEN-PLATELET RICH PLASMA SCAFFOLD

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PURPOSE: The anterior cruciate ligament of the knee is an intra-articular ligament that fails to heal after primary repair. Here we hypothesize that intra-articular ligaments can be induced to heal by placing a collagen-platelet rich plasma (collagen-PRP) hydrogel in the wound site.

METHODS: Bilateral defects were created in extra-articular ligaments (medial collateral ligament and/or patellar ligament) and an intra-articular ligament (anterior cruciate ligament or ACL) in canine knees. For the ACL defects, one side was left untreated and one side treated with a collagen-PRP hydrogel. The response to injury for all ligaments was evaluated after 21 days (n=5) and 42 days (n=5) in vivo. Histologic evaluation of the ligament wounds was performed, including immunohistochemistry for detection of PDGF-A, TGF-β1, FGF-2, procollagen I, fibrinogen, fibronectin and von Willebrand’s factor.

RESULTS

INTRA-ARTICULAR LIGAMENT (ACL) HEALING: QUALITATIVE OBSERVATIONS OF THE UNTREATED WOUNDS

In the untreated ACLs, an incompletely filled defect was noted at three and seven days. Immunohistochemistry revealed a paucity of fibrinogen, fibronectin, PDGF-A, TGF-β1 and FGF-2 within the wound site compared with the EA wounds (Figure 1). At three and six weeks, the defects were still visually obvious in the untreated ACL wounds. After six weeks, the average filling of the untreated ACL defects was only 25%. No evidence of immunoreactivity against TGF-β1 or von Willebrand’s factor was present in the ACL wounds at three weeks, and a relative paucity of fibrinogen, fibronectin, PDGF-A, TGF-β1, and FGF-2 was seen within the untreated ACL wounds. At six weeks, von Willebrand’s factor remained absent from the ACL wounds and the decreased presence of the other markers again observed. Polarized light microscopy demonstrated loss of crimp in the ends of the transected fascicles at the three-week and six-week time points.

INTRA-ARTICULAR LIGAMENT (ACL): QUALITATIVE ASSESSMENT OF THE TREATED WOUNDS

Of the treated IA defects, 3 of 4 showed infilling with new cells and matrix of at least 50% at three weeks (20%, 70%, 80% and 100% filling) and 3 of 4 at six weeks (30%, 50%, 70% and 80% filling). The filled defects were distinguishable from the surrounding tissue on hematoxylin and eosin staining initially by the presence of inflammatory cells and at three weeks by their increased fibroblast density and lack of crimp within the defect, as well as decreased crimp in the remnants of the cut fascicles. The formation of crimp with a decreased length was noted in the treated IA defects at the six-week time point. Immunohistochemistry at three and six weeks revealed similar distributions of protein presence in the treated IA wounds and the EA wounds, although TGF-β1 and FGF-2 were distributed more widely within the treated IA wounds at three and six weeks (Figure 3). Endoligamentous hypertrophy was noted in 3 of the 4 treated IA wounds at 21 days (those with 20, 80 and 100% filling) and 2 of 4 IA wounds at six weeks (those with 30 and 70% filling). Epiligamentous hypertrophy was noted in all treated IA ligaments.

THE EFFECT OF TREATMENT ON IA DEFECTS: QUANTITATIVE MEASURES

Effect of Treatment on Ligament Defect Filling

Treatment of the IA wounds resulted in a statistically significant increase in wound filling, although the extent of the filling in the treated defects remained below that of the EA wounds (Figure 2; two-factor ANOVA, p < 0.0001 for ligament group and p > 0.7 for time; Bonferroni-Dunn post hoc testing with p < 0.002 for all comparisons).

Effect of treatment on the composition of tissue filling the defect

Treatment with a collagen-PRP hydrogel had significant effects on the composition of tissue filling the wound, increasing the presence of fibronectin, fibrinogen, PDGF-A, TGF-β1, FGF-2, procollagen I and von Willebrand’s factor to a similar level of that observed in the EA ligament wounds at three and six weeks.

CONCLUSIONS: The use of a collagen-PRP scaffold can ameliorate histologic differences noted between healing extra-articular ligamentous wounds and intra-articular ligamentous wounds that are typically non-healing. Additional work including further scaffold improvements to increase the filling of the intra-articular wound sites, as well as translation into a total transection model of intra-articular healing, are now necessary. However, this study supports the hypothesis that premature scaffold failure may play a key role in the normally expected failure of the ACL to heal after injury. The induction of a reparative phenotype following placement of scaffolding provides an important addition to the understanding of the mechanisms important in intra-articular ligament injury response.

ACKNOWLEDGEMENTS: The authors wish to acknowledge the support by the National Institute of Arthritis and Musculoskeletal and Skin Diseases, grant number K02 AR049346-02 (M.M.M.), the National Football League Medical Charities program (M.M.M.), the Orthopaedic Research and Education Foundation (M.M.M. and K. P. S.), the VUMC Discovery Grant program (K.P.S.) and the Vanderbilt Sports Medicine Research Fund (K. P. S.), each of which provided financial support for portions of this project.

Figure 2: Percent defect filling for untreated intra-articular ligament wounds (IA), intra-articular wounds treated with collagen-PRP hydrogel (TX) and extra-articular wounds (EA) at 21 and 42 days. Bars represent mean, error bars represent the standard error of the mean for each group. All groups were different (two-factor ANOVA, p < 0.0001 for ligament group and p > 0.7 for time; Bonferroni-Dunn post hoc testing with p < 0.002 for all comparisons).

Figure 1: Photomicrographs of extra-articular(EA), untreated intra-articular (IA) and intra-articular ligament defects treated with a collagen-PRP scaffold (IA TX) 42 days after wounding. Again, the untreated IA ligament wounds remain relatively empty of any substratum.

53rd Annual Meeting of the Orthopaedic Research Society
Poster No: 1509