Rotator Cuff Repair Augmentation in a Rat Model with Use of a Combination of Multilayer Xenograft Tendon Scaffold and Bone Marrow Stromal Cells

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Introduction: Re-tears have been recognized as one of the major complications that cause the high failure rate after the repair of large to massive rotator cuff tears. A tissue engineering technique using novel scaffold would offer potential alternatives for managing this issue. The purpose of the present study was to evaluate the mechanical and biological augmentation effect of the composite of multilayer tendon slices (COMTS) with seeded bone marrow stromal cells (BMSCs) on the supraspinatus tendon repair under tension in rats. We hypothesized that the COMTS with seeded BMSCs may impart a mechanical augmentation effect as well as a biological augmentation effect on the healing process after the surgery.

Methods: Thirty-nine adult female Lewis rats underwent a transection of the supraspinatus tendon and a tendon resection at its distal end by two millimeter followed by immediate repair to its bony insertion under tension. The animals received one of three treatments at the repair site: (1) no augmentation, (2) COMTS augmentation alone, and (3) BMSC-seeded COMTS augmentation. In order to make COMTS scaffold, the deep digital flexor tendons of dogs’ hind limbs were harvested and acellularized. They were then sliced into the shape of a book, which has five 100 µm tendon layers bound together in one end (Figure 1). The animals were euthanized at six weeks after the surgery, and the healing and gene expression of the repaired supraspinatus tendon was evaluated with biomechanical testing, histological analysis and RT-PCR. For results of biomechanical testing (ultimate load-to-failure and stiffness), the differences among five groups including two time-zero groups (no augmentation and COMTS augmentation alone) were statistically evaluated using one-way analysis of variance (ANOVA), and subsequent comparisons were made with the Tukey-Kramer method. For the results of RT-PCR (fold change), the difference among three treatment groups at six weeks were statistically evaluated in the same way. The level of significance was set at p = 0.05.

Results: Histological analysis showed a gap formation between the repaired tendon and the bone in all specimens regardless of the treatment. While the fibrous tissue within the gap was scarce in rats with no augmentation and COMTS augmentation alone, a more robust fibrous tissue was observed in the gap in rats treated with BMSC-seeded COMTS augmentation (Figure 2). In addition, the labeled transplanted BMSCs migrated throughout the repair site including tendon-to-bone insertion. Biomechanical analysis showed the repairs augmented with the BMSC-seeded COMTS had significantly greater ultimate load-to-failure (p < 0.0001) and stiffness (p < 0.05) compared to the other treatments (Figure 3). However time zero data showed that an augmentation with COMTS alone did not increase the mechanical strength of the repair site. RT-PCR analysis did not show any significant difference in gene expression among those treatments.
Discussion: BMSCs have the potential to differentiate into a variety of cell types including osteocytes, chondrocytes, tenocytes, and adipocytes. In order to maximize the ability of BMSCs as biological augmentation source in conjunction with a mechanical augmentation, we have focused on developing a scaffold that is able to carry a large number of BMSCs efficiently. With the COMTS formed into a book, we were able to maximize the number of BMSCs to be transplanted without causing subacromial impingement. Although the COMTS scaffold did not increase the strength immediately after rotator repair as was shown by time-zero data, the scaffold in combination with BMSCs increased healing strength and stiffness after six weeks of rotator cuff repair in the rat model.

Significance: Biological augmentation effect of BMSCs in combination with the multilayered xenograft tendon scaffold may provide a clinically important improvement in rotator cuff tear treatment. Further studies with use of larger animal model may support the use of COMTS concept in xenograft scaffold to maximize the regenerative potential of BMSC in rotator cuff repair surgery.

Figure 1: Composite of multilayer tendon slices (COMTS) was made from the deep digital flexor tendon in dog’s hind leg using acellularization and the tendon book slicing method. Acellularized xenogenic tendon was sliced into 100 μm thickness five times leaving a binding portion in one end followed by trimming into the size of 10 mm in length including 2 mm of binding portion, 4 mm in width, and 0.5 mm in thickness.
Figure 2: Representative hematoxylin-eosin (H&E) stained sections of the repaired supraspinatus tendon and its bony insertion (A-C, original magnification ×20), fibrous scar tissue in the gap, the location of which is indicated by asterisk in the upper row image of each (D-F, original magnification ×100), and tendon-to-bone insertion (G-I, original magnification ×100), showing specimens with no augmentation (left column), with COMTS augmentation alone (middle column), and with BMSC-seeded COMTS augmentation (right column) at 6 weeks after surgery. T = tendon, H = humeral head.

Figure 3: Graphs showing (A) the ultimate load-to-failure and (B) stiffness. Results are shown as mean and the error bars represent the standard deviation. The ultimate load-to-failure and stiffness increased significantly between time zero and six weeks, regardless of treatment (a, b p < 0.05). The ultimate load-to-failure and stiffness were significantly higher in the BMSC-seeded COMTS augmentation group compared with both control groups at six weeks (* p < 0.05). At time zero, there were no differences in ultimate load-to-failure and stiffness between the treatments with and without COMTS augmentation. N = 11 for each group.
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