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BASIC SCIENCE

# Rotator cuff repair augmentation in a rat model that combines a multilayer xenograft tendon scaffold with bone marrow stromal cells

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**Hypothesis:** A composite of multilayer tendon slices (COMTS) seeded with bone marrow stromal cells (BMSCs) may impart mechanical and biologic augmentation effects on supraspinatus tendon repair under tension, thereby improving the healing process after surgery in rats.

**Methods:** Adult female Lewis rats (n = 39) underwent transection of the supraspinatus tendon and a 2-mm tendon resection at the distal end, followed by immediate repair to its bony insertion site under tension. Animals received 1 of 3 treatments at the repair site: (1) no augmentation, (2) COMTS augmentation alone, or (3) BMSC-seeded COMTS augmentation. BMSCs were labeled with a fluorescent cell marker. Animals were euthanized 6 weeks after surgery, and the extent of healing of the repaired supraspinatus tendon was evaluated with biomechanical testing and histologic analysis.

**Results:** Histologic analysis showed gap formation between the repaired tendon and bone in all specimens, regardless of treatment. Robust fibrous tissue was observed in rats with BMSC-seeded COMTS augmentation; however, fibrous tissue was scarce within the gap in rats with no augmentation or COMTS-only augmentation. Labeled transplanted BMSCs were observed throughout the repair site. Biomechanical analysis showed that the repairs augmented with BMSC-seeded COMTS had significantly greater ultimate load to failure and stiffness compared with other treatments. However, baseline (time 0) data showed that COMTS-only augmentation did not increase mechanical strength of the repair site.

**Conclusion:** Although the COMTS scaffold did not increase the initial repair strength, the BMSC-seeded scaffold increased healing strength and stiffness 6 weeks after rotator cuff repair in a rat model.

**Level of evidence:** Basic Science Study, Animal Model.



**Keywords:** Bone marrow stromal cell; composite of multilayer tendon slices; rotator cuff tear; scaffold; xenograft; tendon; biomechanics; animal model

Despite advances in repair techniques, the repair of large-to-massive rotator cuff tears is still challenging. Multiple factors are associated with low healing rates after these repairs, including increased age, tendon quality, muscle atrophy, size of the tear, and gap formation at the repair site shortly after surgery.[22] The increased tension

The protocol for this study was approved by the Mayo Clinic Institutional Animal Care and Use Committee (protocol number A26113).

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needed to hold the once-retracted tendon in the repaired position is a primary cause of gap formation and failure to heal after surgery.[9,14]

Efforts to improve clinical outcomes have been reported on mechanical augmentation with graft materials, such as human dermal graft,[5] porcine dermal graft,[3] small intestine submucosa,[27] and autologous biceps tendon,[30] with promising results. Nevertheless, grafting materials used clinically may not provide sufficient mechanical support to promote sound healing, thereby resulting in high failure rates.[18,27] Researchers are exploring biologic augmentation of the rotator cuff using bone marrow stromal cells (BMSCs), which have the potential to differentiate into various tissue types.[8] Currently, the effectiveness of BMSC treatment remains controversial.[15,34]

Omae et al[25] previously reported significant healing in a composite of multilayer tendon slices (COMTS) model to increase the surface area seeded with BMSCs in vitro. In a subsequent in vivo study, Omae et al[24] also demonstrated that a xenograft COMTS scaffold seeded with BMSCs survived for 2 weeks after transplantation and could be incorporated in a rabbit patellar tendon defect model, with the transplanted BMSCs expressing a tendon phenotype. On the basis of our experience in tendon engineering, we posited that the fundamental approach to repairing large rotator cuff tears potentially can shift to using BMSC-seeded COMTS xenograft scaffolds.

The purpose of the present study was to evaluate the mechanical and biologic effects of BMSC-seeded COMTS scaffolds on the repair of supraspinatus tendons under tension in rats. We hypothesized that BMSC-seeded COMTS would mechanically improve the initial repair strength to prevent gap formation and biologically augment the postoperative healing process.

## Materials and methods

### Study design

Shoulder surgery was performed in 39 adult female Lewis rats. Lewis rats were chosen because they are inbred to the point of being essentially syngeneic. Therefore, transplantation of cells from one rat to another is analogous to an autograft transplantation, which limits the risk of graft rejection.[16] Rats underwent transection of the supraspinatus tendon and a 2-mm tendon resection at the distal end, followed by immediate repair to its bony insertion site under tension. The animals received 1 of 3 treatments at the repair site: (1) no augmentation, (2) COMTS augmentation alone (COMTS-only group), or (3) BMSC-seeded COMTS augmentation (BMSC-COMTS group), with 13 animals per group. Six weeks after surgery, the animals were humanely killed with $CO_2$ asphyxiation (11 for biomechanical testing and 2 for histologic analysis). The same procedure was performed in 22 rat cadaveric shoulders, with or without COMTS augmentation, and served as baseline (time 0) controls for the no-augmentation and COMTS-only groups during biomechanical testing.

### COMTS scaffold preparation

Deep digital flexor tendons were obtained from the hind limbs of 8 mixed-breed dogs (weight, 21-26 kg) that had been euthanized for other approved studies. Harvested tendons were trimmed into segments ~20 mm in length, immersed in liquid nitrogen for 2 minutes, and then thawed in saline at 37°C for 10 minutes. This procedure was repeated 5 times to kill residual cells in the tendon.[24] After washing in phosphate-buffered saline (3 × 30 minutes), tendon segments were incubated in 20 mL nuclease solution (RNase from bovine pancreas, 1.5 U/mL; Roche Diagnostics, Indianapolis, IN, USA) for 12 hours at 37°C. Finally, the tendon segments were rinsed in 50 mL phosphate-buffered saline (3 × 30 minutes) at room temperature with gentle agitation.

Each tendon segment was frozen at −20°C, fixed on a Leica CM1850 cryostat (Leica Biosystems, Buffalo Grove, IL, USA) with optimum Tissue-Tek cutting temperature compound (Sakura Finetek, Torrance, CA, USA), and sliced longitudinally into 5 layers (each 100-μm thick), leaving a ~5 mm portion intact on one end of the tendon segment. This specific slicing method was termed the *tendon-book technique*. Sliced COMTS were rinsed twice by immersing in saline to the remove cutting compound. COMTS were then dried in a lyophilizer (Benchtop Manifold Freeze Dryer [BT48]; Millrock Technology Inc, Kingston, NY, USA) for 24 hours. Each dried COMTS was trimmed to a 4-mm × 10-mm rectangle, leaving a 2-mm attachment site (ie, the tendon book "spine"; Fig. 1). Finally, COMTS were sterilized with ethylene oxide gas.

### BMSC harvesting

BMSCs were collected from 6 adult Lewis rats. Bilateral femora and tibiae were harvested under sterile conditions. The intramedullary canals of the long bones were washed with 10 mL of cell culture medium with 20% heparin. Culture medium consisted of the minimal essential medium with Earle's salts (Thermo Fischer Scientific, GIBCO, Waltham, MA, USA), 10% fetal bovine serum, and 1% antibiotics (antibiotic-antimycotic; GIBCO). Harvested bone marrow cells were transferred to a 50-mL centrifuge tube and filtered through a Falcon 70-μm Cell Strainer (Corning Life Sciences DL, Corning, NY, USA). Cells were centrifuged at 380*g* (1500 rpm) for 5 minutes at room temperature, heparin was removed, and the cell pellet was resuspended in 20 mL of cell culture medium and divided into two 100-mm dishes. Bone marrow cells were incubated at 37°C with 5% $CO_2$ at 100% humidity. After 3 days, the medium containing floating cells was removed, and fresh medium was added to the adherent cells. These adherent cells were defined as BMSCs.[28] Culture medium was changed every third day. After BMSCs reached confluence, they were harvested using trypsin-ethylenediaminetetraacetic acid (EDTA) 0.25% with phenol red (GIBCO) and subcultured. Cells from passage 2 or 3 were used for the experiments.

### Engineered tendon preparation, with or without BMSC seeding

On the day of surgery, adherent BMSCs were trypsinized and centrifuged at 380*g* (1500 rpm) for 5 minutes to remove the trypsin-EDTA solution. Cells were counted using a hemocytometer and mixed with 0.5 mg/mL bovine collagen gel (PureCol; Advanced BioMatrix, Carlsbad, CA, USA), following an