Effect of Footprint Preparation on Tendon-to-Bone Healing: A Histologic and Biomechanical Study in a Rat Rotator Cuff Repair Model

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Purpose: To compare the histologic and biomechanical effects of 3 different footprint preparations for repair of tendon-to-bone insertions and to assess the behavior of bone marrow–derived cells in each method of insertion repair. **Methods:** We randomized 81 male Sprague-Dawley rats and green fluorescent protein–bone marrow chimeric rats into 3 groups. In group A, we performed rotator cuff repair after separating the supraspinatus tendon from the greater tuberosity and removing the residual tendon tissue. In group B, we also drilled 3 holes into the footprint. The native fibrocartilage was preserved in groups A and B. In group C, we excavated the footprint until the cancellous bone was exposed. Histologic repair of the tendon-to-bone insertion, behavior of the bone marrow–derived cells, and ultimate force to failure were examined postoperatively. **Results:** The areas of metachromasia in groups A, B, and C were 0.033 ± 0.019, 0.089 ± 0.022, and 0.002 ± 0.001 mm^2/mm^2, respectively, at 4 weeks and 0.029 ± 0.022, 0.090 ± 0.039, and 0.003 ± 0.001 mm^2/mm^2, respectively, at 8 weeks. At 4 and 8 weeks postoperatively, significantly higher cartilage matrix production was observed in group B than in group C (4 weeks, $P = .002$; 8 weeks, $P < .001$). In green fluorescent protein–bone marrow chimeric rats in group B, bone marrow–derived chondrogenic cells infiltrated the fibrocartilage layer. Ultimate force to failure was significantly higher in group B (19.7 ± 3.4 N) than in group C (16.7 ± 2.0 N) at 8 weeks ($P = .031$). **Conclusions:** Drilling into the footprint and preserving the fibrocartilage improved the quality of repair tissue and biomechanical strength at the tendon-to-bone insertion after rotator cuff repair in an animal model. **Clinical Relevance:** Drilling into the footprint and preserving the fibrocartilage can enhance repair of tendon-to-bone insertions. This method may be clinically useful in rotator cuff repair.

The post-repair retear rate of the rotator cuff is reported to be high, at 18% to 94%.[1-5] A normal tendon-to-bone insertion site consists of tendon, uncalcified fibrocartilage, calcified fibrocartilage, and bone.[6,7] In prior animal studies, recovery of the normal 4-zone structure after rotator cuff repair has been difficult[8-10]; thus, better repair of the insertion site is critical.[11-13] Administration of growth factors and stem cell culture transplants has also been reported to enhance tissue repair at the tendon-to-bone insertion.[14-20]

During rotator cuff repair, the torn rotator cuff is sutured to the greater tuberosity (i.e., the footprint); however, only a few reports have investigated the effects of footprint status on tendon-to-bone insertion repair.[21-23] St. Pierre et al.[21] evaluated histologic and biomechanical outcomes of tendon-to-bone healing after reinserting the infraspinatus tendon onto a cortical bone or into a cancellous trough and found no significant difference between groups. Aoki et al.[22] evaluated the fibrous connection on the tendon-to-bone insertion after rotator cuff repair in 3 different footprint groups

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(residual tendon, calcified fibrocartilage layer, and cancellous surface) and reported a secure fibrous connection was observed in the residual tendon group or the cancellous surface group. Recently, Ficklscherer et al.[23] reported that radiofrequency ablation of the footprint resulted in a poor biomechanical and histologic outcome in a rat model.

For rotator cuff repair, bone marrow–derived cells play a critical role.[24] Kida et al.[25] reported that drilling into the footprint contributed to postsurgical rotator cuff healing by inducing bone marrow–derived cell infiltration into the repaired rotator cuff. Levy et al.[26] used a cannulated humeral implant in a rat model to deliver bone marrow to rotator cuff repair sites but did not find a significant improvement. Furthermore, some clinical studies investigating the effect of bone marrow stimulation on rotator cuff repair have been reported.[27-29] Jo et al.[27] reported that multiple channeling significantly decreased the retear rate after arthroscopic rotator cuff repair.

However, it is unclear whether footprint spongialization induces more bone marrow–derived cells and improves healing at the tendon-to-bone insertion. The purposes of this study were to compare the histologic and biomechanical effects of 3 different footprint preparations for repair of tendon-to-bone insertions and to evaluate the behavior of bone marrow–derived cells in each method of tendon-to-bone insertion repair. We hypothesized that drilling into the footprint or footprint spongialization would induce greater bone marrow–derived cell infiltration and improve healing at the tendon-to-bone insertion.

Methods

Animals

Eighty-one male Sprague-Dawley (SD) rats (12 weeks old) were used for H&E and safranin O staining and for biomechanical testing. Three green fluorescent protein (GFP) transgenic rats[30-32] (Japan SLC, Hamamatsu, Japan) and six SD rats were used to prepare GFP–bone marrow chimeric (BMC) rats. This study was reviewed and approved by the institutional animal experiment ethics committee (Laboratory Animal Committee of Kyoto Prefectural University of Medicine, Kyoto, Japan; No. M22-12).

Generation of BMC Rats

GFP rats and SD wild-type rats of the same strain were used to create GFP-BMC rats as described in previous reports.[25,33,34] In brief, recipient SD rats were exposed to 10 Gy of gamma-ray irradiation (Gammacell 40 Exactor; Nordion International, Kanata, Ontario, Canada) under deep anesthesia to achieve bone marrow suppression. Bone marrow–derived cells were subsequently retrieved from the tibia, femur, and humerus of donor GFP rats, under sterile technique. A 70-μm nylon mesh was used to remove impurities from the bone marrow–derived cells collected from the donor GFP rats, the cell count was adjusted to a concentration of 1.0×10^8/mL by use of a hemocytometer, and 1 mL was intravenously transplanted into the irradiated SD rats (GFP-BMC rats). The peripheral blood GFP cell–positive rate in the recipient rats was measured 4 weeks after transplantation by the following method[25,34]: The GFP-chimeric rate in bone marrow is nearly equal to that of the peripheral blood.[34] After washing and hemolysis, samples from the peripheral circulation were suspended in phosphate-buffered saline solution–containing propidium iodide (Sigma-Aldrich, St. Louis, MO) to identify and gate out dead cells. Cell suspensions were analyzed by a FACSCalibur system (Becton Dickinson, Franklin Lakes, NJ) with excitation at 488 nm and fluorescence detection at 530 nm. All flow cytometric data were analyzed with CellQuest software (Becton Dickinson).

Surgical Procedure

SD rats and GFP-BMC rats were assigned to 3 rotator cuff repair model groups (Fig 1). The surgical procedures were performed 4 weeks after transplantation. For all animals, general anesthesia was achieved by intraperitoneal administration of pentobarbital, and the surgical procedures were performed on the right shoulders in a clean environment. In one group, a lateral-to-posterior shoulder incision was made, and to visualize the rotator cuff, the acromion was lifted with an elevator. Then, the supraspinatus tendon was sharply resected from the greater tuberosity. The residual tendon tissue was removed macroscopically by use of a sharp-pointed knife, and the fibrocartilage layer was preserved (control group [group A]) (Fig 1A). In the pilot study, we removed residual tendon tissue grossly, created specimens, and confirmed preserved fibrocartilage under a microscope. The supraspinatus tendon tear underwent a transosseous repair with No. 5-0 nylon thread placed in a modified Mason-Allen configuration[35] through the tendon edge and then brought into approximation with the native insertion site by use of a 0.5-mm burr hole prepared in an anteroposterior direction in the bone cortex of the distal portion of the footprint (Fig 1D). In the second group, we also drilled into the footprint before the supraspinatus tendon was repaired. An electric drill was used to create 3 burr holes (3 mm deep, 0.5 mm in diameter) in the greater tuberosity to reach cancellous bone; bleeding from the bone marrow was confirmed (drilling group [group B], Fig 1B). In the third group, before the supraspinatus tendon was repaired, the footprint was excavated with a fine burr until cancellous bone was exposed (spongialization group [group C], Fig 1C). The acromion was repositioned, and the deltoid split was