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What is This?
Stem Cell-Bearing Suture Improves Achilles Tendon Healing in a Rat Model

Samuel B. Adams, Jr, MD1, Margaret A. Thorpe, BS2, Brent G. Parks, MSc1, Gary Aghazarian, BS1, Elizabeth Allen, MD3, and Lew C. Schon, MD1

Abstract
Background: Tendon healing is a slow and complicated process that results in inferior structural and functional properties when compared to healthy tendon tissue. It may be possible to improve outcomes of tendon healing with enhancement of biological aspects of the repair including tissue structure, organization, and composition. The purpose of this study was to determine whether use of a stem cell-bearing suture improves Achilles tendon healing in a rat model.

Methods: The Achilles tendon was transected in 108 bilateral hind limbs from 54 rats. Each limb was randomized to repair with suture only (SO), suture plus injection (SI) of mesenchymal stem cells (MSCs) at the repair site, or suture loaded with MSCs (suture with stem cells, SCS). One half of the animals were randomly sacrificed at 14 and 28 days after surgery and the Achilles tendon was harvested. From each repair group at each time point, 12 limbs were randomized to biomechanical testing and 6 to histologic analysis. Tendons were loaded using a 223-N load cell at 0.17 mm/s. A blinded pathologist scored the histology sections.

Results: Ultimate failure strength (N/mm²) was significantly higher in the SI and SCS groups versus the SO group. In the SI group, ultimate failure strength decreased significantly at 28 days versus 14 days. Histology score in the SCS group was significantly lower (better) than in both other groups (P ≤ .001). Histology findings at day 28 were significantly higher versus day 14 for all groups (P = .01).

Conclusions: Both the SI and the SCS groups had significantly higher ultimate failure strength versus the SO group, and strength was maintained at 28 days in the SCS group but not in the SI group. Histology in the SCS group was significantly better than in both other groups.

Clinical Relevance: These findings in a rat model suggest that the use of stem cells enhances healing after Achilles repair and that embedding of stem cells directly into suture offers sustained early benefit to tendon healing.

Keywords: stem cell-bearing suture, Achilles tendon, tendon repair, bone marrow, mesenchymal stem cells

Introduction
Repair of acute or chronically injured tendons and tendon transfers are commonplace in foot and ankle surgery. Tendon healing is a slow and complicated process that results in inferior structural and functional properties when compared to healthy tendon tissue.1 Repaired or tenodesed tendons are at risk for rerupture or loss of fixation if exposed to high tensile loads early in the postoperative period. However, during the postoperative immobilization period, inadequate limb movement promotes adhesions that can result in deficits in strength and function. Therefore, future advances in tendon repair should focus on improving early postoperative tendon repair quality to maximize functional outcome.

Modern postoperative rehabilitation protocols for Achilles tendon repair call for accelerated weight-bearing, range of motion, and return to sport.1,2,13,14 However, early motion before healing may promote excessive gap formation at the repair site and an elongated tendon, leading to a reduction in tensile strength and a delay in healing. Separation of tendon ends has been clearly demonstrated after Achilles repair.16,18 Also, weakness in end-range plantarflexion after Achilles tendon repair has been attributed to excess tendon lengthening.17 These data further support the need for improved early tendon repair quality.
Tendon tissue produced during healing exhibits inferior structural and functional properties when compared to healthy tendon. It may be possible to improve outcomes if biological aspects of the repair including tissue structure, organization, and composition can be enhanced by the use of biologic materials such as growth factors or exogenous cells. The addition of bone marrow-derived mesenchymal stem cells (MSCs) to tendon repair sites, via injection or through tissue-engineered cell-scaffold constructs, has demonstrated accelerated healing, increased expression of tendon-specific genes, and better organized repair tissue compared to controls. The positive effects of local delivery of MSCs are thought to be threefold: direct differentiation of the stem cells, release of growth factors and cytokines, and host stem cell recruitment. Therefore, introduction of MSCs to the repair site may increase initial tendon repair strength, allow for more aggressive rehabilitation, and reduce adhesions associated with current tendon repair techniques.

Many different tissue-engineered scaffolds have been employed as cellular delivery vehicles and structural support for the healing tendon. Currently, a woven suture is used to span the repair site, sometimes multiple times, in nearly every tendon repair or tenodesis. Therefore, it would seem logical to employ the suture as both a mechanical stabilizer and an exogenous cell carrier/scaffold for the generation of new tendon tissue. We tested a suture in which MSCs were embedded within the core of the suture with protected delivery of MSCs to the repair site. The purpose of this study was to determine whether use of an MSC-bearing suture was associated with higher ultimate failure load, lower cross-sectional area, and better histology after Achilles tendon repair in a rat model.

Methods

Study Design

Three different suture repair types were tested in this study: suture only (suture-only group, SO), the same suture plus the injection of $1 \times 10^5$ stem cells around the repaired tendon (suture + stem cell injection group, SI), or the same suture impregnated with $1 \times 10^5$ stem cells (stem cell-bearing suture group, SCS). The stem cell-bearing suture (Bioactive Surgical, Inc., Clarksville, MD) differs from the coating method employed by Yao et al. in that the MSCs are within the core of the proprietary braided suture at a high concentration. The structural biomechanical design features are intended to enhance cell delivery, retention, and function. The same stem cell-bearing suture was used in all of the groups for control purposes. However, stem cells were embedded in the suture only in the SCS group. Histologic and biomechanical testing was performed at 14 and 28 days after repair. This study was approved by our Institutional Review Board and Institutional Animal Care and Use Committee.

The primary outcome of this study was biomechanical integrity of the repair. Power analysis, based on biomechanical data from prior reports of rat tendon healing, determined that 12 tendons were required per treatment group per time period for 90% power to identify a significant difference with a Type I error of .05. An additional 6 tendons per treatment group per time period were required for histologic evaluation. A total of 18 tendons was used per treatment group per time period, totaling 54 animals and 108 hind limbs. All animals were adult male Sprague Dawley rats aged 96 ± 22 days with a mean weight of 370 ± 34 g.

Bone Marrow-Derived Mesenchymal Stem Cell Harvest, Isolation, and Expansion

Iliac crest bone marrow was aspirated from 1 human donor. Human stem cells have been used to study effects on tendon repair in a rat model. The bone marrow was then processed to remove the red blood cells and isolate mononuclear cells. This processing was achieved using Ficoll-Paque PLUS (STEMCELL Technologies, Vancouver, BC, Canada), which is a density gradient medium for isolation of mononuclear cells. The fresh bone marrow aspirate was diluted at a 1:1 ratio with phosphate buffered saline (PBS), pH 7.4 (Sigma-Aldrich, St Louis, MO), with 2% fetal bovine serum (FBS) for human MSCs (STEMCELL Technologies). The diluted bone marrow aspirate was then layered on top of the Ficoll-Paque PLUS density gradient medium in a 50-mL conical tube and centrifuged at room temperature for 30 minutes at 400 g with the centrifuge brake off. The MSC-containing mononuclear cell layer at the plasma-Ficoll interface was then removed. The mononuclear cells were washed once with PBS containing 2% FBS before being plated in T-75 cm² tissue culture flasks at a density of 4000 cells per cm². The isolated MSCs were cultured in MesenCult MSC Basal Medium (STEMCELL Technologies) supplemented with MesenCult Mesenchymal Stem Cell Stimulatory Supplements (STEMCELL Technologies). The medium was changed every 2 to 3 days. The cells were expanded in a 37°C humidified incubator with 5% CO₂ in air and greater than 95% humidity until they were 60% to 80% confluent, at which point the adherent cell monolayer was dissociated from the cell culture flask using 0.25% Trypsin-EDTA (GIBCO Invitrogen, Cat no. 15050-057). The dissociated MSCs were then centrifuged for 10 minutes at 500 g, counted, and replated in new T-75 cm² tissue culture flasks at a density of 4000 cells per cm². Second-passage cells were used for implantation. Previous studies have shown that MSCs maintain their pluripotency through the third passage.
Animal Surgery

Prior to the start of the procedure, each hind limb was randomized to receive 1 of the 3 repair types. The animals were anesthetized with an intraperitoneal injection of Nembutal (pentobarbital, 50 mg/kg). Bilateral hind limbs were shaved and prepped with alternating alcohol and chlorhexidine scrubs in triplicate. Next, a longitudinal posterior midline incision was made along the Achilles tendon. Medial and lateral full-thickness skin flaps were developed, allowing the Achilles tendon to be identified and isolated. The Achilles tendon was transected 3 mm distal to the musculotendinous junction. A second transection was performed 3 mm distal to the first, resulting in the removal of a 3-mm section of the tendon. Next, the Achilles tendon ends were loosely approximated, using the appropriate suture repair type, with a figure-of-eight stitch. Prior to tying the suture knots, an instrument 3 mm in width was placed between the tendon ends to preserve the 3-mm gap. The gap model was chosen to isolate the effect of treatment from the high innate ability of the animal to heal. The generation of tissue across the gap was considered to reflect the process expected in healing. The wound was irrigated and closed with 4-0 monofilament suture. If the hind limb was randomized to receive the stem cell injection, the wound was almost completely closed. Then, $1 \times 10^6$ stem cells were delivered through the wound in a volume of 25 µL and a final stitch was placed. Next, the same procedure was performed on the contralateral hind limb. The limbs were not immobilized and the animals were allowed ad libitum activity.

Stem Cell-Bearing Suture

For those tendons that were randomized to the stem cell suture repair, $1 \times 10^6$ stem cells in 25 µL of medium were loaded into a 2-cm portion of the suture immediately prior to implantation. This 2-cm portion was marked on the suture and used for the figure-of-eight repair suture and knot.

Achilles Tendon Harvest

The animals were randomized to be sacrificed at either 14 or 28 days, and each hind limb was randomized to be sent for histological or biomechanical processing. After CO$_2$ and cervical dislocation euthanasia, each hind limb was approached through a posterior midline incision. The Achilles tendon was identified. The gastrocnemious and soleus muscle bellies were transected, and the tendon was reflected posteriorly. The tendon was then sharply peeled off of its insertion into the calcaneus.

Biomechanical Testing

Tendons that were randomized for biomechanical testing were wrapped in saline-soaked gauze and stored at $-80^\circ$C until analysis was performed. All testing was performed in blinded fashion. After the tendons were thawed to room temperature, any remaining suture material was removed from the tendon to ensure testing of only the integrity of the repair and not the suture. Digital calipers were used to measure the cross-sectional area of the tendon at the repair site. The tendon was then transferred to a custom uniaxial testing system with a 223-N load cell (MLP-50; Transducer Techniques, Temecula, CA) and secured into clamps at each end with ethyl cyanoacrylate. The tendon was then loaded at a rate of 0.17 mm/s until ultimate failure. Failure was defined as tearing of the tendon. The ultimate failure strength was calculated by dividing the ultimate failure load by the original cross-sectional area.

Histologic Processing and Grading

Specimens collected for histology were fixed in 4% paraformaldehyde in phosphate buffer, pH 7.2, at room temperature. The tendons were dehydrated in ethanol and embedded in paraffin. They were then sectioned (5 µm) in the sagittal plane across the midline of the tendon and stained with hematoxylin and eosin. Three sections from each specimen were chosen at random for grading. The repair sites of the 3 sections were graded according to the Hospital for Special Surgery (HSS) tendon histological scoring system by a board-certified pathologist blinded to the repair method used. In this scoring system, a lower score correlates with a more normal tendon appearance. The grades from the 3 slides of each tendon were averaged to obtain a final overall grade.

Statistical Analysis

Biomechanical and averaged histologic data were analyzed across the 3 treatment groups and 2 time points using a 2-factor analysis of variance (ANOVA) with Tukey post hoc analysis. Multifactor ANOVA was used to determine whether there was any interaction of groups and days (interaction of individual groups across time points) for any factor tested. Statistical significance was set at $P < .05$. Because we had only 1 pathologist, 20 slides were chosen at random and blindly graded twice. A Cohen’s kappa coefficient for intra-rater reliability was calculated. Statistical analysis was performed using Statgraphics (Warrenton, VA, version 15.2).

Results

Ultimate failure load was significantly higher in the SI and SCS groups versus the SO group (Table 1). Ultimate failure load decreased significantly at 28 days in the SI group but not in the SCS group. Cross-sectional area was nonsignificantly lower (better, indicating lower scar tissue formation).
Histology scores in the SCS group were significantly lower (indicating better collagen orientation and fewer fibroblasts) than in the SI and the SO groups (Table 1). The histology findings at day 28 were significantly higher (worse) than at day 14 for all groups. The Cohen’s kappa coefficient for the histology grader was 0.958.

No other tissue types were identified by histology except for chondrocytes in 1 tendon sample from the SI group at 28 days. Photopictomicrographs of the tendon repair sites from the 3 groups at each time point and an example of uninjured rat tendon are shown in Figure 1. Visual differences in amount of collagen organization and number of fibroblasts can be observed among the groups.

### Discussion

In our tendon generation model, the use of stem-cell suture was associated with significant improvements in important aspects of tendon healing. Ultimate failure load was significantly higher in the SI and SCS groups than in the SO group. Ultimate failure load was maintained in the SCS group over time but decreased significantly in the SI group at 28 days versus 14 days. The SCS group had significantly better histology than both other groups. Cross-sectional area was nonsignificantly lower in the SI and SCS groups versus the SO groups, indicating nonsignificantly improved tendon architecture in that lower area reflects the formation of tendon tissue without scarring. A lack of scarring may be of benefit with regard to improved gliding and decreased adhesion formation within a tendon sheath. These findings suggest that the stem cell-bearing suture tested in this study had a positive effect on some aspects of early tendon generation and architecture.

Although traditional suture repair provides stability to the healing tendon, operative intervention and prolonged postoperative immobilization can result in adhesions and scar tissue formation that can lead to deficits in strength and/or function. Collagen that stays unstressed during the proliferative and remodeling phases of tendon healing remains haphazard in organization and is weaker than stressed collagen. Therefore, methods to increase the quality of early tendon healing would allow for safe initiation of early protected motion and could have important implications for tendon repairs and tenodesed tendons where early mobilization is desirable.

The addition of bone marrow-derived MSCs to tendon repair sites, via injection or through tissue-engineered cell-scaffold constructs, has demonstrated accelerated healing, increased expression of tendon-specific genes, and better organized repair tissue compared to controls. The exact role that the MSCs played in tendon healing in the current study is not known. No stem cell tracking was done in our study, and therefore we were unable to differentiate between host and transplanted tissue at the repair site.

Suture has been used previously to deliver growth factors to a healing tendon. Dines et al demonstrated that tendons repaired with sutures coated with growth differentiation factor-5 showed a significantly higher ultimate tensile load and stiffness at 3 weeks compared with tendons repaired with control sutures. Rohrich et al reported on covalently bound epidermal growth factor on Mersiline suture that led to an increase in cellular proliferation along the suture in a rat flexor tendon repair model. Hamada et al developed a monofilament nylon suture that eluted basic fibroblast growth factor over the course of 1 week. A suture has also been used to deliver stem cells in vitro. Yao et al coated FiberWire suture segments with poly-L-lysine, subsequently

### Table 1. Comparison of Ultimate Failure Load, Cross-Sectional Area, and Histology Data between Groups and at 14 Versus 28 Days.

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Day</th>
<th>n</th>
<th>Mean ± SE</th>
<th>Day</th>
<th>n</th>
<th>Mean ± SE</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Suture Only</td>
<td></td>
<td></td>
<td>Suture + Injection</td>
<td>Suture + MSCs</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>13</td>
<td>1.3 ± 0.4</td>
<td>10</td>
<td>3.0 ± 1.8</td>
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<td>3.2 ± 2.6</td>
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<tr>
<td></td>
<td>28</td>
<td>13</td>
<td>1.3 ± 0.4</td>
<td>12</td>
<td>1.6 ± 0.8</td>
<td>11</td>
<td>2.3 ± 0.8</td>
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<td>18.8 ± 1.4</td>
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<td>9.7 ± 1.6</td>
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<td>8.2 ± 1.4</td>
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<td>18.3 ± 1.1</td>
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<td>11</td>
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<tr>
<td></td>
<td>28</td>
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<td>3.39 ± 0.2</td>
<td>6</td>
<td>2.83 ± 0.3</td>
<td>6</td>
<td>2.06 ± 0.1</td>
</tr>
</tbody>
</table>

Abbreviations: MSCs, mesenchymal stem cells; SCS, suture + MSCs; SI, suture + injection; SO, suture only.

*Multifactor analysis of variance (ANOVA) and Tukey post hoc test were used.

bSI and SCS were significantly higher than SO group.

cA subsequent detailed comparison found that the significant decrease in failure strength over time was primarily attributed to the SI group (P = .02).

SO, P = .82; SCS, P = .26.

dSCS was significantly lower (better) than SO and SI groups.

eHistology score was significantly higher (poorer) at 28 days than at 14 days for all groups.

![Image](fai.sagepub.com)
Adams et al

cultured them with pluripotent embryo cells, and passed them through harvested rabbit Achilles tendons. The authors found that the cells survive the trauma of suture passage and remain metabolically active in the tendon tissue.

Differentiation of the MSCs into chondrocytes was seen in 1 specimen, and no other non-tendon tissue types were identified. With the addition of stem cells, the potential for unregulated differentiation of the cells into various lineages is a concern. The fact that this phenomenon was not seen in this study lends credence to the philosophy of the allogenic MSCs as “growth factor pumps” or instigators of the host repair system rather than the primary cells involved in the tendon repair.

We studied ultimate failure load as an indicator of tendon biomechanical integrity. Other important factors that remain to be investigated are elasticity and ratio of more elastic Type I collagen to Type III collagen in tendon tissue. Elasticity is important to tendon integrity in that it allows accommodation of a load over time.

Our study design incorporated a gap between tendon ends to isolate the effect of treatment from the natural high healing ability of the animals, based on the rationale that generation of tendon is analogous to the healing process. This design allowed us to observe differences between the treatments but was based on a model not used in clinical practice. We did not perform histological analysis on the suture to confirm that all stem cells in the stem cell suture were incorporated into the tendon. Allocation of specimens to test groups was inadvertently uneven, and therefore 1 group at both time points had more and 1 group had fewer specimens than required by power analysis for biomechanical testing. It is possible that the correct allocation of specimens would have resulted in differences among the groups tested.

**Conclusion**

Both the SI and the SCS groups had significantly higher ultimate failure load versus the SO group, and strength was maintained at 28 days in the SCS group but not in the SI group. Histology in the SCS group was significantly better than in both other groups. These findings in a rat model...
suggest that the use of stem cells enhances healing after Achilles repair and that embedding of stem cells directly into suture offers sustained early benefit to tendon healing.

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Declaration of Conflicting Interests
The author(s) declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: L.C.S. is a co-owner and M.A.T. is a full-time employee of Bioactive Surgical, Inc.

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