Use of Mesenchymal Stem Cells in a Collagen Matrix for Achilles Tendon Repair


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Summary: This investigation tested the hypothesis that delivering mesenchymal stem cell-seeded implants to a tendon gap model results in significantly improved repair biomechanics. Cultured, autologous, marrow-derived mesenchymal stem cells were suspended in a collagen gel delivery vehicle; the cell-gel composite was subsequently contracted onto a pretensioned suture. The resulting tissue prosthesis was then implanted into a 1-cm-long gap defect in the rabbit Achilles tendon. Identical procedures were performed on the contralateral tendon, but only the suture material was implanted. The tendon-implant constructs were evaluated 4, 8, and 12 weeks later by biomechanical and histological criteria. Significantly greater load-related structural and material properties were seen at all time intervals in the mesenchymal stem cell-treated tendons than in the contralateral, treated control repairs (p < 0.05), which contained suture alone with natural cell recruitment. The values were typically twice those for the control tissues at each time interval. Load-related material properties for the treated tissues also increased significantly over time (p < 0.05). The treated tissues had a significantly larger cross-sectional area (p < 0.05), and their collagen fibers appeared to be better aligned than those in the matched controls. The results indicate that delivering mesenchymal stem cell-contracted, organized collagen implants to large tendon defects can significantly improve the biomechanics, structure, and probably the function of the tendon after injury.

Autografts, allografts, xenografts, synthetic polymers, and resorbable biomaterials have been used to repair tendon and ligament defects (1,2,4,10,13,14, 17,19-21,24-26,31). In addition, cell-seeded, resorbable materials have been tried as a means to accelerate the healing process for tendons (8) and ligaments (3,11), on the premise that rapid synthesis and reorganization of the matrix could improve biomechanical competence and functionality (3,11). These resorbable devices were designed to provide an initial load-bearing structure to serve as a scaffold for both the cells introduced into the defect site and those recruited to the area. As these materials degraded, they allowed transfer of mechanical stresses to the newly formed repair tissue, a step that may be critical to increase the diameter of collagen fibrils and, thus, mechanical function of the repair (22,23).

Another promising approach, advocated by Bruder et al. (5), Caplan et al. (9), and Wakitani et al. (30), has utilized noncommitted progenitor cells of musculoskeletal tissues to regenerate soft and hard tissues. These cells, termed mesenchymal stem cells, were isolated from a small volume of bone marrow aspirate and culture-expanded without undergoing differentiation to more committed cell types. Mesenchymal stem cells demonstrated the ability to contract collagen matrices (unpublished data) and showed some promise when applied to a tendon defect site (9).

Cells, when incubated in physically constrained collagen gels, tend to organize their extracellular matrices along lines of tension created by cell-mediated contraction of the gel (15,28). When prepared under such conditions with use of fibroblasts, so-called ligament equivalents had significantly improved biomechanical properties compared with nonorganized matrices (15). Mesenchymal stem cells, in response to physical constraints, also appear to organize collagen in vitro, indicating their fibroblastic lineage potential. This potential and the osteogenic and chondrogenic potentials reported by others (16,18) suggest a pluripotent cell line that could beneficially affect tendon repair. To learn whether such progenitor cells could organize matrix in vitro and improve biomechanical function of tendon repairs in vivo was the motivation to use mesenchymal stem cells in this study.

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produce the contracted cell-matrix construct. The device consists
of a sterile, knotted surgical suture (s) held in tension by a wire
spring (w), which is set into a glass trough (t) to form the cylindrical
shape of the implant.

An autologous mesenchymal stem cell-mediated re-
pair was investigated in a challenging, although not yet
clinically relevant, gap defect model in the gastrocne-
mus tendon of the rabbit. The hypothesis to be tested
was that installation of a cell-seeded, organized con-
struct into a large tendon defect would significantly
improve repair biomechanics and matrix organization
of tissue for as long as 3 months after an operative
procedure compared with a contralateral, natural re-
pair with suture alone.

MATERIALS AND METHODS

Fifty-three female New Zealand White rabbits, 4-6 months old,
were obtained from an approved laboratory animal vendor (Ha-
zelton Research Products, Kalamazoo, MI, U.S.A.). Young animals
were selected to provide a challenging natural repair, given their
inherent regenerative capacity. Normal gastrocnemius tendons
from five animals were used to establish biomechanical properties
of normal tissue. All animals were housed and cared for under
guidelines set forth by the Institutional Animal Care and Use
Committee of Case Western Reserve University. The rabbits were
implanted with autologous, culture expanded mesenchymal stem
cell constructs and assigned in cohorts of 16 animals each to 4, 8,
or 12-week test periods. They were killed at the end of each test
period; this resulted in 13 treated and 13 control tendon samples.
Three pairs of tendons were processed for histology at each time
point.

A power analysis based on preliminary data determined that at
least 12 pairs of tendons were required per time interval to estab-
lish 90% confidence in detecting intra-animal differences in two
structural biomechanical measures (maximum force and stiffness)
with a Type-I error of 5%. The resuling biomechanical data from
all study groups were analyzed by paired Student’s t tests (one-
tailed) to compare treated and contralateral control tendon repairs
at each time interval (p < 0.05). Significance levels were adjusted
to account for multiple comparisons by analysis of variance across
time intervals.

Bone marrow was aspirated from the proximal anterior aspect
of the tibia by a sterile surgical procedure similar to methods
described previously (30). Briefly, an incision was made over the
medial anterior aspect of the tibia, peristeum was elevated, and
a small hole was drilled through the cortex. Several milliliters of
marrow was drawn up through a silastic tubing into a syringe
coated with 3,000 IU of sodium heparin. Cells were cultured in
Dulbecco’s modified Eagle’s medium-low glucose (GIBCO BRL,
Frederick, MD, U.S.A.) with 1% antibiotics and 10% fetal bovine
serum (Intergen, Purchase, NY, U.S.A.), which had been specific-
ally screened to promote proliferation of rabbit mesenchymal stem
cells without differentiation according to the method of Lennon
et al. (18). Primary cultures were seeded at 15-20 million nucleated
cells per 56 cm², underwent a change of medium every 3 or 4 days,
and were maintained for 10-14 days. Nonadherent cells were re-
moved from the cultures by the second or third change of medium.
Confluent colonies were detached from the plate with 4 ml of
0.25% trypsin in 1 mM EDTA. The cells were then collected,
rinsed, centrifuged twice with medium, counted, and replated to
the first passage stage at 3.0-4.5 × 10⁶ cells per 100-mm dish. When
first-passage mesenchymal stem cells were nearly confluent, they
were again detached, counted, and pelleted for construction of the
implant.

The mesenchymal stem cell-matrix implant consisted of a pre-
tensioned, polyglyconate suture (Maxon, Davis and Geck, Manati,
PR) to which cultured mesenchymal stem cells were affixed as they
contracted a collagen gel. This process was performed in a culture
device (Fig. 1) consisting of the suture set into a glass trough, held
in tension between the tips of a bow spring formed from stainless-
steel surgical Kirschner wires (0.89-mm diameter; Zimmer, War-
saw, IN, U.S.A.). Each suture was pretensioned by compressing the
spring so that its ends were 10-mm apart prior to attachment; this
produced a mean restoring force of 4.9 ± 0.7 N on the suture as
measured across seven devices.

Mesenchymal stem cells derived from the first passage were
suspended in serum-free medium at a concentration of 8 × 10⁶
cells/ml. An aliquot of acid-solubilized, type-I bovine skin-derived
collagen dialyzed into hydrochloric acid at approximately 3 mg/ml
(Pancogene-S; Gattefosse S. A., Lyon, France) was combined with
an equal volume of the cell suspension in an ice bath to produce a
final concentration of 4 × 10⁶ cells/ml. The two were mixed well
by pipetting, and a volume of approximately 380 μl was dispensed
onto the tensed suture in the glass trough of the prepared culture
device. After the gel formed at an incubation of 37°C, the entire
construct was covered with serum-free medium and was incubated
at 37°C for approximately 40 hours to form a contracted construct
of adequate stiffness and organization. After mixing, greater than
75% of the cells in analogous constructs were demonstrated to be
viable by the lack of uptake of ethidium homodimer dye (Molec-
ular Probes, Portland, OR, U.S.A.). Each suture was pretensioned by compressing the
spring so that its ends were 10-mm apart prior to attachment; this
produced a mean restoring force of 4.9 ± 0.7 N on the suture as
measured across seven devices.

The rabbits were anesthetized with intramuscular injections of
70 mg/kg of ketamine HCl (Ketaset; Fort Dodge Laboratories, Fort
Dodge, IA, U.S.A.) and 10 mg/kg of xylazine HCl (Rompun; Miles,
Shawnee Mission, KS, U.S.A.) and were prepared for the sterile
operative procedure. From a lateral approach, the gastrocnemius
tendon was separated from the plantaris and soleus tendons and
a 1-cm-long defect was created in the midsubstance of the gastroc-
memius tendon. Implants were sutured in place with use of a mod-
fied Kessler pattern of the 4-0 Maxon incorporated in the implant.
In the control, a suture alone was placed spanning the gap between
the tendon ends. In both the treated and the control groups, the
tension on the tendon was returned to approximately normal by
adjusting the defect to 1 cm in length while maintaining the stifle
and ankle joints in their preoperative joint flexion angles. The
tendon sheath and skin were closed in a routine manner. The
rabbits were allowed movement ad libitum immediately postoper-
atively and throughout the test period in cages measuring 3 ft²
(0.279 m²).

After the animals had been killed, tendons assigned for me-
chanical testing were dissected and frozen at −20°C for shipment.

FIG. 1. Photograph of the pretensioning culture device used to
produce the contracted cell-matrix construct. The device consists
of a sterile, knotted surgical suture (s) held in tension by a wire
spring (w), which is set into a glass trough (t) to form the cylindrical
shape of the implant.

to the University of Cincinnati as previously described (12,26). The length, width, and thickness of the tendon were measured by one author (W.W.) using dial calipers (model 537-120; Mitutoyo, Tokyo, Japan). Local width and thickness were also measured, and their product was calculated for three equally spaced locations in the repair region. All products were then averaged to calculate the cross-sectional area of the repair. No attempt was made to use a weight-applied area micrometer (7) as in previous studies, so as to avoid any damage to the repair site and because the repairs were nearly planar in appearance. Transverse dye lines were also placed at the proximal and distal edges of the repair to visualize where failure occurred during testing.

The proximal fascia of each test specimen was placed between two polypropylene braids (ligament augmentation device: 3M, St. Paul, MN, U.S.A.) and inserted in sinusoidal grips (7). The distal calcaneal bone block was embedded in methylmethacrylate cement in the open cavity of a metal grip as previously described (7). The sinusoidal grip was then secured to the upper, movable actuator of a tensile testing system (model 8501; Instron, Canton, MA, U.S.A.). The specimen was lowered into a bath of warm saline solution (37°C, pH 7.2) mounted on the testing system, and the distal cavity grip was fixed to the immovable load cell at the base of the chamber. Each specimen was equilibrated in the saline solution for 30 minutes before being failed in tension at 20% elongation per second; failure was monitored with a video camera (RCA model CC415; Thomson Electronics, Indianapolis, IN, U.S.A.) (6). Four structural and six material parameters, defined in the Table legends, were measured or calculated as previously described (7,12,26).

Specimens for histology were removed at death of the animal, fixed in 10% buffered formalin, dehydrated through alcohol gradients, cleared, and embedded in polymethylmethacrylate blocks (27). The specimens were cut with a tungsten carbide knife to produce 5-μm sections, which were affixed to glass slides and stained with toluidine blue/basic fuchsin.

RESULTS

When the mesenchymal stem cell-matrix constructs in vitro were observed grossly after 40 hours of incubation in the culture device, the matrix had been contracted to approximately 30% of the original diameter; there was little contraction, and, therefore, minimal
TABLE 1. Structural properties for treated (T) and control (C) repairs

<table>
<thead>
<tr>
<th>Structural properties</th>
<th>Normal (n = 5)</th>
<th>Repair 4 wk (n = 13)</th>
<th>8 wk (n = 13)</th>
<th>12 wk (n = 13)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stiffness (N/mm)</td>
<td>36.5 ± 10.6</td>
<td>T 19.8 ± 1.9</td>
<td>22.9 ± 3.3</td>
<td>23.0 ± 2.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C 9.7 ± 1.9</td>
<td>11.4 ± 1.5</td>
<td>11.5 ± 2.2</td>
</tr>
<tr>
<td>Force_{\text{max}} (N)</td>
<td>189.0 ± 26.8</td>
<td>T 124.0 ± 13.0</td>
<td>114.3 ± 18.3</td>
<td>130.3 ± 16.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C 58.0 ± 11.3</td>
<td>60.3 ± 10.4</td>
<td>57.3 ± 11.7</td>
</tr>
<tr>
<td>Energy_{\text{max-force}} (N•mm)</td>
<td>555.5 ± 79.7</td>
<td>T 525.1 ± 90.5</td>
<td>365.8 ± 84.3</td>
<td>485.6 ± 91.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C 199.9 ± 44.6</td>
<td>196.1 ± 39.4</td>
<td>168.7 ± 36.0</td>
</tr>
<tr>
<td>Energy_{\text{failure}} (N•mm)</td>
<td>901.1 ± 317.2</td>
<td>T 710.5 ± 88.8</td>
<td>539.1 ± 101.0</td>
<td>737.0 ± 182.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C 311.7 ± 47.5</td>
<td>353.4 ± 47.5</td>
<td>290.5 ± 52.1</td>
</tr>
</tbody>
</table>

Structural properties (mean ± 1 SEM) are contrasted to corresponding properties for the normal untreated gastrocnemius tendon. Stiffness represents the increase in force for an incremental increase in elongation in the linear region of the curve; force_{\text{max}} is the largest force developed during failure testing; and energy_{\text{max-force}} and energy_{\text{failure}} are the areas under the force-elongation curve to maximum force and complete unloading, respectively.

"All treated means were significantly greater than controls (p < 0.05)."

decrease in length, along the longitudinal axis. Histological examination of the construct demonstrated an organized structure of elongated cells aligned with the matrix in the direction of tensile load along the longitudinal axis (Fig. 2). For the 4-week postoperative test period, the histology for both the treated and control repairs exhibited a layer of inflammatory cells on the surface of the suture material (Fig. 3a and 3b). At this early time point, the treated repairs exhibited organized bands of collagen with elongated cell morphology reminiscent of tendon fibroblasts compared with variable morphologies in the controls (Fig. 3c and 3d). The volume, density, and crimp pattern of these bands appeared to increase in both groups by 8 and 12 weeks. Some treated repair tissues at 12 weeks had densely packed, highly crimped fibers that were grouped in bundles along the axis of tensile load (Fig. 4). Of the dimensional measurements tabulated, the

FIG. 4. Photomicrographs of treated (a) and control (b) samples at 12 weeks demonstrate bundles of organized cells and matrix under bright-field illumination (examples indicated by brackets marked with b). When viewed under polarizing filters, treated samples (c) exhibit a greater degree of crimp pattern (examples indicated by brackets marked with cr) than the control samples (d). Suture (s) serves as a landmark; arrowheads indicate the tensile load on the tissue. Toluidine blue/basic fuchsin staining, bar = 200 μm.

TABLE 2. Material properties for treated (T) and control (C) repairs

<table>
<thead>
<tr>
<th>Material properties</th>
<th>Normal (n = 5)</th>
<th>Repair 4 wk (n = 13)</th>
<th>8 wk (n = 13)</th>
<th>12 wk (n = 13)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Modulus (MPa)</td>
<td>337.5 ± 205.8</td>
<td>T 53.4 ± 4.9</td>
<td>90.3 ± 10.4</td>
<td>114.4 ± 7.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C 33.5 ± 7.0</td>
<td>62.2 ± 9.2</td>
<td>67.9 ± 9.8</td>
</tr>
<tr>
<td>Stress_max (MPa)</td>
<td>41.6 ± 18.9</td>
<td>T 8.6 ± 0.8</td>
<td>10.5 ± 1.4</td>
<td>15.5 ± 1.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C 4.7 ± 1.1</td>
<td>7.2 ± 1.3</td>
<td>8.0 ± 1.2</td>
</tr>
<tr>
<td>Strain energy density_max</td>
<td>3.9 ± 0.4</td>
<td>T 1.0 ± 0.1</td>
<td>0.8 ± 0.2</td>
<td>1.4 ± 0.2</td>
</tr>
<tr>
<td>Stress (N/mm/mm³)</td>
<td></td>
<td>C 0.4 ± 0.1</td>
<td>0.5 ± 0.1</td>
<td>0.6 ± 0.1</td>
</tr>
<tr>
<td>Strain energy density_fail</td>
<td>6.7 ± 3.6</td>
<td>T 1.3 ± 0.1</td>
<td>1.2 ± 0.2</td>
<td>2.1 ± 0.4</td>
</tr>
<tr>
<td>(N/mm/mm³)</td>
<td></td>
<td>C 0.7 ± 0.1</td>
<td>0.9 ± 0.1</td>
<td>1.0 ± 0.1</td>
</tr>
<tr>
<td>Toe limit stress (MPa)</td>
<td>6.8 ± 0.6</td>
<td>T 2.2 ± 0.3</td>
<td>2.8 ± 0.4</td>
<td>3.9 ± 0.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C 1.4 ± 0.3</td>
<td>1.6 ± 0.2</td>
<td>2.7 ± 0.4</td>
</tr>
<tr>
<td>Toe limit strain (%)</td>
<td>9.0 ± 2.3</td>
<td>T 11.2 ± 1.0</td>
<td>8.9 ± 1.3</td>
<td>10.0 ± 0.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C 11.8 ± 1.3</td>
<td>12.5 ± 5.3</td>
<td>13.8 ± 5.2</td>
</tr>
</tbody>
</table>

Material properties (mean ± 1 SEM) are contrasted to corresponding properties for the normal untreated gastrocnemius tendon. Modulus represents the increase in stress for an incremental increase in strain in the linear region of the curve; stress_max is the largest stress developed during failure testing; strain energy density_max and strain energy density_fail are the areas under the stress-strain curve to maximum stress and complete unloading, respectively; and toe limit stress and toe limit strain are the stress and strain values at the end of the nonlinear, initial loading portion of the stress-strain curve.

*All treated means were significantly greater than controls (p < 0.05), except for toe limit stress, toe limit strain, and 8-week strain energy density values.

Cross-sectional area (mean mm² ± SD) of the treated repair tissues (15.1 ± 6.8, 10.4 ± 3.7, and 7.4 ± 2.8 at 4, 8, and 12 weeks, respectively) was significantly greater than the area for the contralateral control tissues (8.4 ± 1.9, 6.3 ± 1.9, and 5.4 ± 2.6 at 4, 8, and 12 weeks, respectively) at all three time periods (p < 0.05). By comparison, the cross-sectional dimensions of normal, untreated tissue were 4.3 ± 1.3 mm². The cross-sectional areas of both repairs decreased significantly to 12 weeks (p < 0.05), with the treated repair area declining at a significantly faster rate than the control repair area (p < 0.05).

FIG. 5. Average stress-strain curves for treated control and mesenchymal stem cell (MSC)-treated tendon regeneration at 12 weeks postoperatively. The maximum stresses do not correspond to the mean values in Table 2 because these peaks do not occur at exactly the same maximum strain values.
MESENCHYMAL STEM CELL-MEDIATED ACHILLES TENDON REPAIR

Mean values (±1 SEM) for the load-related structural properties of all treated repairs were typically twice the corresponding values for the control repairs at each time period (p < 0.05; Table 1). When expressed as percentages of normal tendon values from a similar population of rabbits, the values for treated and control stiffnesses reached 54.2 and 26.5%, respectively, of normal values by only 4 weeks after the procedure. Maximum forces generated by the treated and control repairs achieved almost two-thirds (65.6%) and one-third (30.7%) of normal values, respectively, after only 4 weeks. These values remained nearly constant 4-12 weeks after the procedure. On average, and over the time periods analyzed, the treated tissues required 60-90% of the energy required for failure of normal tendon, whereas the controls required only 30-40%. In all cases, failure initiated within the repaired gap region of the test sample and propagated into the normal tissue in approximately 60% of the samples.

Mean values (±1 SEM) for four of the load-related material properties (all but toe limit stress and strain) increased significantly with time after the procedure (p < 0.05, Table 2), although the rate of increase was significantly greater for the treated values than for the control values (e.g., modulus: 7.6 compared with 4.3 MPa/wk, respectively; p < 0.05). Both rates were significantly different from zero for all four parameters. When again expressed as percentages of normal tendon values, the modulus for the treated tissues increased from one-seventh (15.8%) of normal values after only 4 weeks to one-quarter (26.8%) and one-third (33.9%) of normal values at 8 and 12 weeks, respectively. The modulus for the controls increased from one-tenth (9.9%) at 4 weeks to one-sixth (18.4%) and one-fifth (20.1%) of normal values at 8 and 12 weeks, respectively. Maximum stress also increased over time for both the treated and control tissues, reaching values of about one-third (37.3%) and one-fifth (19.2%) of normal values, respectively, by 12 weeks.

Figure 5 shows the average stress-strain curves for the treated and control tendon repairs at 12 weeks after the procedure. The treated and contralateral control tendon curves had similar initial toe regions. The toe limit stresses and toe limit strains did not differ significantly with treatment or time after the procedure (p > 0.05; Table 2). Significant left-right differences were observed only after loading proceeded into the near-linear and failure regions.

DISCUSSION

The results of this preliminary study support our hypothesis that applying a cell-contracted, organized, collagen matrix to a defect site significantly improves tendon repair biomechanics compared with a contralateral, natural repair with suture alone as early as 1 month and continuing to 3 months after the procedure. A type-I collagen gel contracted by mesenchymal stem cells onto a tensioned suture not only doubled the load-related structural biomechanics of the gap repair compared with use of suture alone with natural recruitment but also resulted in significant rates of improvement in the material properties of the repair.

The structural properties for the mesenchymal stem cell-seeded repairs were typically twice those for the contralateral controls as early as 4 weeks after the procedure; this suggests the potential for more rapid return to normal function. Specifically, the increases in stiffness and maximum force to one-half and two-thirds of normal tendon values after only 4 weeks indicate the benefits associated with introducing mesenchymal stem cells into the repair construct. This treatment-related benefit could be explained by the significantly larger cross-sectional area of the cell-assisted repairs. The rates of increase in these structural properties did not continue between 4 and 12 weeks for either repair type (Table 1); however, the treated and control repair tissues did maintain stiffness and strength over time. Even though the significant structural properties for the treated tissues could include some contribution from the suture, this effect would also be present in the control samples and would thus likely diminish similarly in both repair groups as the resorbable suture degraded.

The rate of increase in load-related material properties for the treated repair tissues and the relative magnitude of these results compared with those of the controls suggest rapid remodeling of the mesenchymal stem cell-loaded implants. The rate of increase in both modulus and maximum stress for the treated tissue repairs remained significantly greater than corresponding rates for the controls, even as the cross-sectional areas decreased over time. These inherent differences in material quality are independent of tissue mass and might suggest that the resulting matrix ultrastructure (e.g., fibril diameter or alignment, or both) and biochemistry (e.g., permanent crosslink density) are improved by the introduction of mesenchymal stem cells. However, these improvements still do not approach the complex structural organization that is seen in mature normal tendon tissue and, thus, the material properties for even the treated tissues fall short of normal values.

Although only subjective at this stage, the limited histology in this study appears to support the biomechanical findings. For example, the treated tissues showed matrix organization at 4 weeks after the procedure, and at later time points the presence of distinctly organized fibers in the treated and control repair regions is consistent with improved biomechanical properties. Both treated and control tissues showed temporal improvements in cell shape, cell and
matrix alignment, and crimp pattern; however, the treated repairs were qualitatively more mature in appearance than the matching controls. Such time-related increases in tenocyte frequency and collagen crimping have been reported in other studies (14) and are indicative of conversion from neotendon to mature, load-bearing soft tissue.

It could be argued that the significant improvement in the biomechanics of the treated groups was due to the choice of the control. The preferred control would have been the collagen gel placed on the tensioned suture but without cells. However, in our experience, this gel would not contract or adhere to the suture without the presence of cells. Certainly, other cell types such as skin fibroblasts (11) have been studied in cell-seeded implants and might have been used to effect this contraction in the controls. No other cell types (e.g., fibroblasts from another source) or other forms of biomaterials were utilized as controls in this study; instead, treatment with mesenchymal stem cell-contracted collagen gel in the defect was contrasted with a more natural recruitment of reparative cells around the suture as the contralateral control. Although creating a challenging repair model with low biomechanical values, this choice of a suture-alone control does not negate the very rapid increase in material properties or the large load-related structural properties for the treated repairs relative to normal gastrocnemius tendon that has not undergone a procedure.

Another concern in the initial design of the study was the contribution of the degrading suture to the structural and material property values recorded during biomechanical testing. Suture was present in some of the histological samples, although its continuity across the repair site could not be verified. Any attempt to remove this embedded suture prior to mechanical testing certainly would have damaged the test specimen. Others have reported varying degradation rates in sutures precoated with cells (3); however, that study was conducted in a relatively acellular environment. The present study demonstrates cells in treatment and control groups; therefore, differences in degradation rates are not likely to explain the 2-fold treatment-related differences in the biomechanical results at all time periods.

Several groups have assessed how biological and synthetic materials affect repair of the Achilles tendon (1,2,13,14,17,25,29); however, no previous studies that we are aware of have addressed how cell-based therapies influence tendon-repair dynamics. Eight weeks after spanning rabbit Achilles tendon gap defects with a composite of Dacron and degradable polyglycolic acid, Rodkey et al. (25) found that the stiffness and maximum load of the repairs were 48 and 57% of control values, respectively. Their results were better than our results for controls, possibly because of the ability of the composite scaffold they used to recruit more endogenous repair cells than did the suture alone used in our study. However, their results fall below the percentages determined for our treated groups; this might be attributed to the early matrix production and organization occurring in the mesenchymal stem cell-seeded constructs. Filling Achilles defects in the rabbit with autograft tissue has also been shown to produce higher maximum stress values (50% of normal) at 10 weeks (17) than we found in our study at 12 weeks, but the autograft required bridging only of adjacent tissues and therefore required production of a much smaller volume of repair tissue. A more appropriate comparison with the present model might be the portion of the study of Kato et al. that used two collagen scaffolds (17). At 10 weeks, the repair achieved approximately 36% of ultimate stress for the normal tendon, similar to our findings with treated repairs at 12 weeks after the procedure; however, the role of the collagen fibers themselves is difficult to estimate. Other investigators have reported 90-100% of maximum force values for normal rabbit and canine Achilles tendon at 12 weeks using filamentous carbon coated with an absorbable polymer (1) or xenogeneic small intestinal submucosa (2), respectively. However, both studies had small sample sizes (n = 1-3), and concerns persist about graft degradation and long-term biocompatibility of these materials.

The biomechanical functionality of the repair tissue achieved in the current study encourages further investigations into the use of cellss formatted in a pretensioned biomatrix for the repair of large tendon defects. Manipulation of mesenchymal stem cells in a culture system prior to the procedure should produce a better understanding of the effects of physical forces on the lineage progression of stem cells. Moreover, such research should also reveal new techniques for optimizing the configuration of the system and for applying mesenchymal stem cell implants to new, challenging opportunities in the regeneration of soft tissues.

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REFERENCES
2. Badyla B, Tullius R, Kokini K, Shelbourne KD, Klototwyk T, Voytik SL, Kraine MR, Simmons C: The use of xenogeneic small intestinal submucosa as a biomaterial for Achilles ten-


