Stem Cell Loaded Sutures Improve Tendon Healing and Repair Strength in a Rat Model

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INTRODUCTION:
Primary surgical repair of torn or lacerated tendons is the standard of care. Although suture repairs provide stability to the healing tendon, patients are often left with deficits in strength and/or function. Healing tissue composition and collagen organization are known to influence repair strength. It may be possible to improve outcomes if biological aspects of repair such as tissue structure, organization, and composition can be enhanced by the use of biologic materials.

The addition of mesenchymal stem cells (MSCs) to tendon repair sites, via injection or through tissue-engineered carriers, has demonstrated accelerated healing, increased expression of tendon-specific genes, and better organized repair tissue compared to controls.

A suture has been developed in which MSCs are embedded within the fibers of the suture, thereby allowing the delivery of MSCs to the repair site. The purpose of this study was to determine the efficacy of a mesenchymal stem cell loaded suture to contribute to tendon healing properties in a rat Achilles tendon repair model.

METHODS:
This study was approved by our Institutional Review Board and Institutional Animal Care and Use Committee.

Iliac crest bone marrow aspirate (BMA) was obtained from a human undergoing foot and ankle surgery. The BMA was centrifuged and the platelets were removed. The remaining aspirate was cultured until the MSCs were at confluence. All MSCs used in this experiment were from passage two or three and from the same donor.

Bilateral hind limbs from 54 Sprague-Dawley rats (n=108) were used in this study. Each hind limb underwent transection of the Achilles tendon 3mm distal to the musculotendinous junction. Next, a section of the tendon was removed, creating a 3-mm gap. The hind limb was then randomized to one of three groups: suture repair, suture repair with an injection of 1x10^6 MSCs around the repair site, or the stem cell suture loaded with 1x10^6 MSCs (n=36 per group). In all cases, the 3-mm gap was maintained during the suture repair and the same type of suture (size #2) was used.

One-half of the animals were randomly sacrificed at two and four weeks. The Achilles tendon was harvested. At each timepoint and from each repair group, 12 hind-limbs were randomized to biomechanical testing, and 6 hind-limbs were randomized to histological analysis.

Specimens undergoing biomechanical testing were measured with digital calipers and attached to custom fabricated grippers secured to a linear stage with a 200N load cell. The suture was cut and removed from the tendon to ensure testing of the integrity of the biological repair only. No pre-load was applied. A displacement rate of 0.17mm/s was applied to the tendon. The maximum load to failure (N) was recorded and peak stress (MPa), and stiffness (N/mm) were calculated.

Specimens undergoing histological analysis were placed in formalin. Three sections (6 microns thick), 100 microns apart were taken from the midsubstance of the tendon in the sagittal plane. The sections were stained with hematoxylin and eosin and Masson’s trichrome stains. A board certified pathologist, blinded to the repair method, scored the histology using a previously published 11-point scoring system with subcategories of collagen orientation, degree of angiogenesis, and cartilage formation. The scores from each of the three sections were averaged to create a final combined score for each tendon.

One-way ANOVA with Tukey HSD post-hoc analysis was performed on all parameters using SPSS statistical software (IBM, Armonk, NY).

RESULTS:
All tendons were intact at the time of harvest without evidence of the previously created 3-mm gap. Gross observation demonstrated decreased tendon size in the MSC repair groups compared to suture alone. There was no difference in the maximum load to failure among the three groups at either of the time points. However, cross-sectional area in the MSC groups was significantly lower than the suture only group at 2 and 4 weeks (Figure 1). Additionally, the peak stress of the stem cell suture group was significantly higher than the suture only repair group at both time points (Figure 2). There was no difference in stiffness among the three groups at either time point.

Histological analysis demonstrated a significantly better total tendon healing grading score and collagen orientation for the stem cell suture group at both time points over both suture only and suture plus stem cell injection groups. Representative images of the tendon histology are shown in Figure 3.

DISCUSSION:
This study demonstrated that a suture loaded with MSCs can provide increased tendon repair strength and decreased cross-sectional area of the repaired tendon compared to a suture only repair. Additionally, a stem cell loaded suture provided better tissue organization at the repair site over suture alone or suture plus stem cell injection. Although stem cell tracking was not performed, it is likely that the embedded nature of the MSCs within the suture allows for longer retention of these cells at the repair site. This could allow for a longer time for MSC differentiation or the promotion of autologous stem cells and growth factors to the repair site.

SIGNIFICANCE:
These data support the efficacy of the addition of biologics to traditional suture repair of tendons. A stronger, better organized and smaller cross sectional area tendon could allow for decreased adhesions, faster mobilization, and less resulting deficit in strength or function.

FIGURES:

![Figure 1. Cross-Sectional Area (mm^2).](image1)

![Figure 2. Peak Stress (MPa).](image2)

![Figure 3. Representative histology images.](image3)