Robust Suture Combination for Rat Flexor Tendon Repair Model

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Purpose: We aimed to develop a rat flexor tendon repair model that could be applied to experiments in similar clinical settings.

Methods: We prepared 3 different combinations of sutures in rat flexor tendons: group A had 3 single peripheral sutures plus a 2-strand core suture; group B had 3 figure-of-eight peripheral sutures alone; and group C had 3 figure-of-eight peripheral sutures plus a 2-strand core suture. We examined the in vitro tensile strength of the repaired tendons by a biomechanical test, the rerupture rate within 3 weeks, and histological findings in vivo.

Results: Group C displayed the greatest ultimate strength by the mechanical test. The flexor tendons in group C did not rerupture within 3 weeks after surgery, whereas many of those in groups A and B reruptured. Fibrous scar tissue was observed in the gap of the tendon stumps in groups A and B, but not in group C.

Conclusions: The combination of figure-of-eight peripheral sutures and a 2-strand core suture provided the repaired rat flexor tendon with enough strength to prevent rerupture without cast fixation or immobilization after surgery.

Clinical relevance: This combination of sutures is useful to reproduce flexor tendon repair similar to that performed in clinical settings and will contribute to various translational experiments in vivo.

Flexor tendon injury remains a challenging problem in hand surgery despite improvements in surgical techniques of tendon repair and postoperative rehabilitation protocols. The difficulty in flexor tendon repair results from to 2 main complications after surgery: rerupture of the repaired tendon and adhesion formation. Many surgical techniques have been developed to prevent rerupture of the repaired tendon and all have contributed to improvements in clinical outcomes. However, postoperative peritendinous adhesion formation remains an unresolved issue. Despite efforts to prevent peritendinous adhesion formation during the past several decades, no effective antiadhesive materials or methods have been developed.

To improve the outcomes of flexor tendon repair, the molecular mechanisms underlying the healing of repaired tendons and adhesion formation must be revealed using experimental animal models. Large animals such as rabbits, canines, and equines have been employed in previous experiments of flexor tendon repair, whereas rats and mice have been widely used for research in molecular biology because genetic modification techniques for these animals have been well-developed, and they have additional advantages in terms of cost and housing space. Several recent studies revealed contributions of progenitors or stem cell populations to tendon healing using reporter mice and tendon repair models. However, the murine models used in these studies were different from the flexor tendon repair performed in clinical settings (eg, the Achilles tendon or patellar tendon was used, or the myotendinous junction was transected to reduce strain-induced rupture). To date, no flexor tendon repair model with clinical relevance has been established in rats. Oshiro et al performed transection of the flexor digitorum longus tendon and immediate repair with one simple suture; however, they added another transection of the
tendon at a proximal site to reduce the tensile force across the repair site. Considering that tensile force is essential for homeostasis and functions of tendons, this modification is expected to affect their natural healing adversely.

In the current study, we aimed to develop a rat model that could be applied to experiments designed to examine the efficacy of novel materials or methods for flexor tendon repair. We hypothesized that optimization of the suturing technique would prevent rerupture of the flexor tendon without using immobilization procedures. We prepared 3 different combinations of sutures in rat flexor tendons and examined the in vitro tensile strength of the repaired tendons by a biomechanical test, the rerupture rate within 3 weeks, and histological findings in vivo.

Materials and Methods

Animals

All animal experiments were performed according to the protocol approved by the Animal Care and Use Committee of the University of Tokyo. We used 21 adult Wistar rats weighing 170 to 200 g, 3 of which were assigned to the biomechanical test and 18 flexor digitorum profundus tendons of which were from both hind paws (6 flexor tendons/rat). The other 18 rats were assigned to the in vivo experiment. The rats were housed and given laboratory rat chow and water ad libitum and exposed to a 12-hour light–dark cycle at a room temperature of 22°C.

Surgical procedure

A combination anesthetic composed of 0.3 mg/kg medetomidine, 4.0 mg/kg midazolam, and 5.0 mg/kg butorphanol was intraperitoneally administered to each rat at 0.5 mL/100 g of body weight. A 1-cm central volar incision was made from the third metatarsophalangeal joint toward the proximal direction (Fig. 1A). The third flexor digitorum superficialis was removed for a clear operative field, and the third flexor digitorum profundus was exposed by opening the most proximal pulley. The tendon was transected in the middle portion and then repaired by 3 different procedures. In group A, the tendon stumps were first repaired with a single peripheral suture in the back side using 8-0 suture (T04A08N15-15M, Bear Medic Corporation, Tokyo, Japan), and a 2-strand core suture was placed using an 8-0 Tsuge looped suture (NBB008, Kono Seisakusho, Ichikawa, Japan). A single peripheral suture was then added in the anterolateral and anteromedial sides using 8-0 suture (Fig. 1B). In group B, only 3 figure-of-eight peripheral sutures were placed without a 2-strand core suture (Fig. 1B). In group C, we placed 3 figure-of-eight peripheral sutures and a 2-strand core suture using 8-0 nylon (Fig. 1B). All surgical procedures were conducted using a microscope (x300 magnification, Konan Medical, Hyogo, Japan).

Biomechanical test

The ultimate strength of the repaired tendons was quantified in vitro using a rheometer (CR-500-DX-LII, Sun Scientific, Tokyo, Japan). The repaired tendon was freed from the surrounding tissue and transected at 5 mm proximal to the surgical site. The third finger was then dissected through the metacarpophalangeal joint (Fig. 2A). The distal site of the third finger was secured to the moving jaw of the rheometer, and the proximal site of the flexor tendon was secured to the stationary jaw of the rheometer. The flexor tendon was pulled at 20 mm/min until the repair site ruptured and the breaking force was recorded (Fig. 2B).
Histology

The rats were killed 3 weeks after surgery, and the repaired tendons were harvested and fixed in 4% paraformaldehyde buffered with phosphate-buffered saline for 2 hours. The samples were embedded in paraffin and cut into 4-μm sagittal slices. The tissue specimens were stained with hematoxylin-eosin.

Statistics

We analyzed the ultimate strength of the 3 different suture techniques using one-way analysis of variance. When the result indicated significance, multiple comparisons were performed with the Tukey–Kramer test. In all tests, a \( P < .05 \) was considered significant. Values are expressed as mean ± SD.

Results

Biomechanical test

We first performed a biomechanical test of rat tendons repaired with the 3 different combinations of sutures (Fig. 2A, B). The ultimate strength of the repaired tendons in groups A, B, and C was 1.26 ± 0.10, 2.11 ± 0.20, and 3.51 ± 0.30 N, respectively (Fig. 2C). One-way analysis of variance indicated a significant difference among the groups (\( P < .001 \)), and Tukey's multiple-comparisons test showed the most significant difference between groups A and C (\( P < .001 \)). Moreover, the ultimate strength of the repaired tendons in group C was significantly greater than that in group B (\( P = .001 \)), and that in group B was significantly greater than that in group A (\( P = .04 \)) (Fig. 2C).

In vivo experiment

Next, we evaluated the repaired flexor tendons in each group 3 weeks after surgery. In groups A and B, 5 repaired tendons were ruptured macroscopically; only one tendon remained intact (Fig. 3A, B). Notably, all 6 repaired tendons were intact in group C (Fig. 3A, B). Histological examination showed that the ruptured tendon stumps were apart from each other and that the gap was filled with fibrous scar tissue (Fig. 3C). In contrast, the tendon stumps were tightly connected to each other without fibrous tissue in the unruptured tendons (Fig. 3C).

Discussion

In this study, we compared 3 different combinations of sutures for rat flexor tendon repair and found that the repair with 3 figure-of-eight peripheral sutures and a 2-strand core suture displayed the greatest ultimate strength by the mechanical test. The flexor tendons repaired by this suture method did not rerupture within 3 weeks after surgery, whereas many of them repaired by other techniques reruptured.

Generally, the tensile strength of a repaired tendon is proportional to the number of core sutures and the caliber of the suture threads. Nevertheless, the width of a rat flexor tendon is less than 1 mm, and it is difficult to place more than a 4-strand core suture. Moreover, it is difficult to use nylon threads thicker than 70 because of the needle size. Therefore, we planned to increase the tensile strength of the repaired tendon by adding peritendinous sutures. Several techniques are available for placing peritendinous sutures in transected tendons, including simple continuous, cross-stitch, and interlocking horizontal mattress peritendinous sutures. Among these, we employed the figure-of-eight technique for the peripheral sutures because it is easier to perform this technique in thin tendons. Notably, using only 3 figure-of-eight peripheral sutures (group B) provided a stronger connection than using 3 single peripheral sutures and a 2-strand core suture (group A) (Fig. 2C), indicating the efficacy of the figure-of-eight technique. Furthermore, the ultimate strength produced by 3 figure-of-eight peripheral sutures and a 2-strand core suture (group C) was 3.51 N, approximately 3-fold higher than that produced by the 3 single peripheral sutures and 2-strand core suture (group A) (Fig. 2C). We cannot compare the current data with the results of other rat
about 3 N.14 Because a rabbit is about 10 times heavier than a rat, the ultimate strength of the repaired tendons 1 week after surgery was about 3 N.15 The ultimate strength of the repaired tendons 1 week after surgery was about 3 N.14 Because a rabbit is about 10 times heavier than a rat, the strength of 3.51 N in the current study seems satisfactory. A recent article describing a canine flexor tendon repair model also showed that adding peritendinous sutures reduces gap formation and increases the ultimate strength of the repaired tendons.15

Novel findings of the mechanisms underlying tendon healing were recently produced by studies using transgenic mice, including Scx-GFP16 and Scx-Cre mice.10 In the future, various genetically modified mice may further contribute to an understanding of the biology of tendon homeostasis and pathology of tendon injury. Despite these advantages, however, murine flexor tendons are too small to suture tightly. For mouse experiments, larger tendons such as the patella or Achilles tendons are usually used,8,9 or transection of the exor tendon repair model also supports that the mechanisms underlying tendon healing and the development of effective antiadhesive materials or methods for more successful flexor tendon repair.

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