Journal of Hand Surgery Global Online 2 (2020) 354–358

Contents lists available at ScienceDirect

Journal of Hand Surgery Global Online

journal homepage: www.JHSGO.org

Original Research

Robust Suture Combination for Rat Flexor Tendon Repair Model

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ARTICLE INFO

Article history:
Received for publication July 20, 2020
Accepted in revised form August 5, 2020
Available online September 3, 2020

Key words:
Flexor tendon repair
Rat model
Ultimate strength

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.[1] The difficulty in flexor tendon repair results from to 2 main complications after surgery: rerupture of the repaired tendon and adhesion formation. Many suture techniques have been developed to prevent rerupture of the repaired tendon[2,3] 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.[4]

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,[5] canines,[6] and equines[7] 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.[8–11] However, the murine models used in these studies were different from the flexor tendon repair performed in clinical settings (eg, the Achilles tendon[8] or patellar tendon[9] was used, or the myotendinous junction was transected to reduce strain-induced rupture[10,11]). To date, no flexor tendon repair model with clinical relevance has been established in rats. Oshiro et al[12] performed transection of the flexor digitorum longus tendon and immediate repair with one simple suture; however, they added another transection of the

Declaration of interests: No benefits in any form have been received or will be received by the authors related directly or indirectly to the subject of this article.

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https://doi.org/10.1016/j.jhsg.2020.08.004

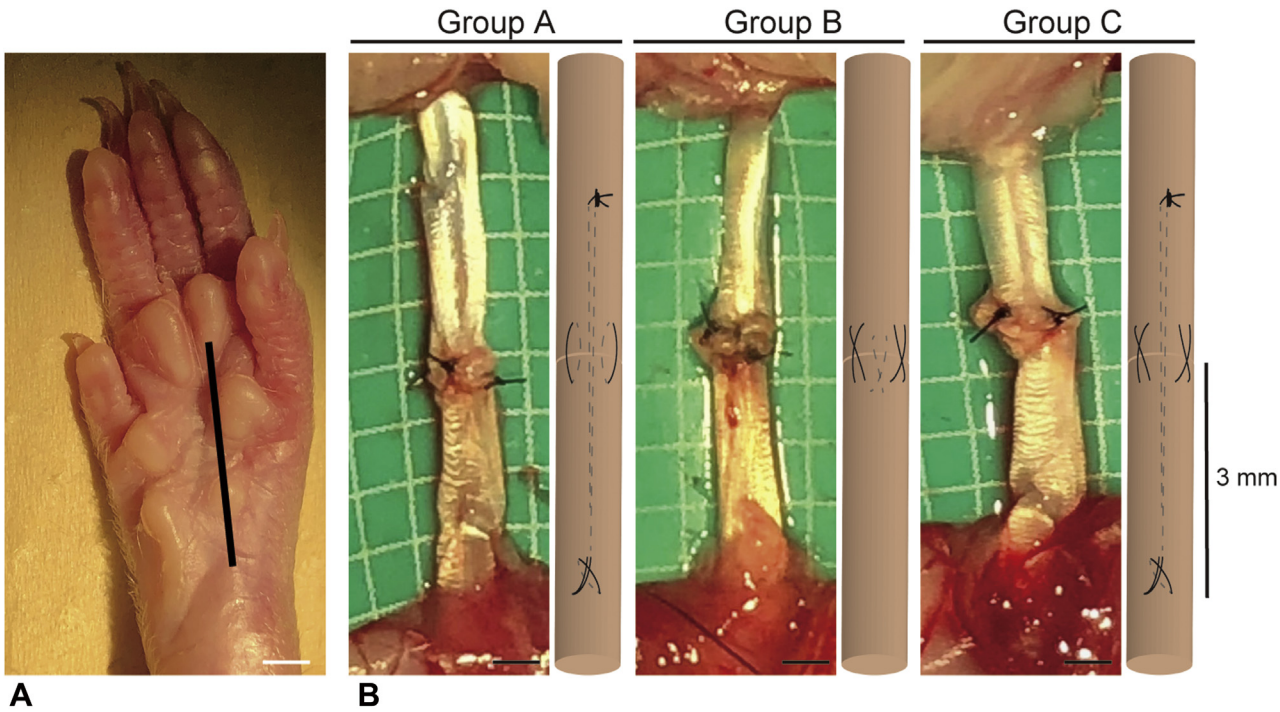

Figure 1. **A** Location of skin incision. Scale bar = 1 mm. **B** Gross appearance and illustration of tendon sutures in each group. Group A had 3 single peripheral sutures plus a 2-strand core suture. Group B had 3 figure-of-eight peripheral sutures alone. Group C had 3 figure-of-eight peripheral sutures plus a 2-strand core suture. The purchase length of the 2-strand core suture was 3 mm. Scale bar = 1 mm.

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 (×300 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).

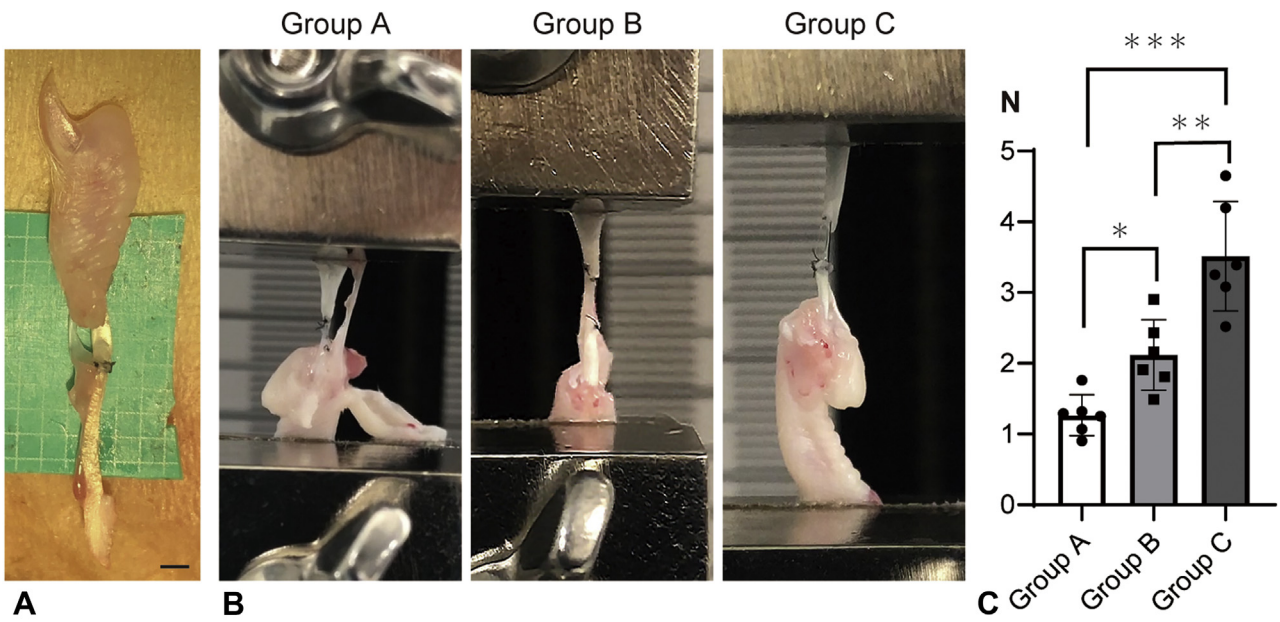

Figure 2. Biomechanical test of rat tendons repaired with 3 different combinations of sutures. **A** Gross appearance of a dissected finger with the metacarpophalangeal joint and repaired flexor tendon. Scale bar = 1 mm. **B** The biomechanical test in the 3 groups. **C** Ultimate strength of the repaired tendons in the 3 groups. *$P = .04$, **$P = .001$, and ***$P < .001$.

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.[13] 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 7-0 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

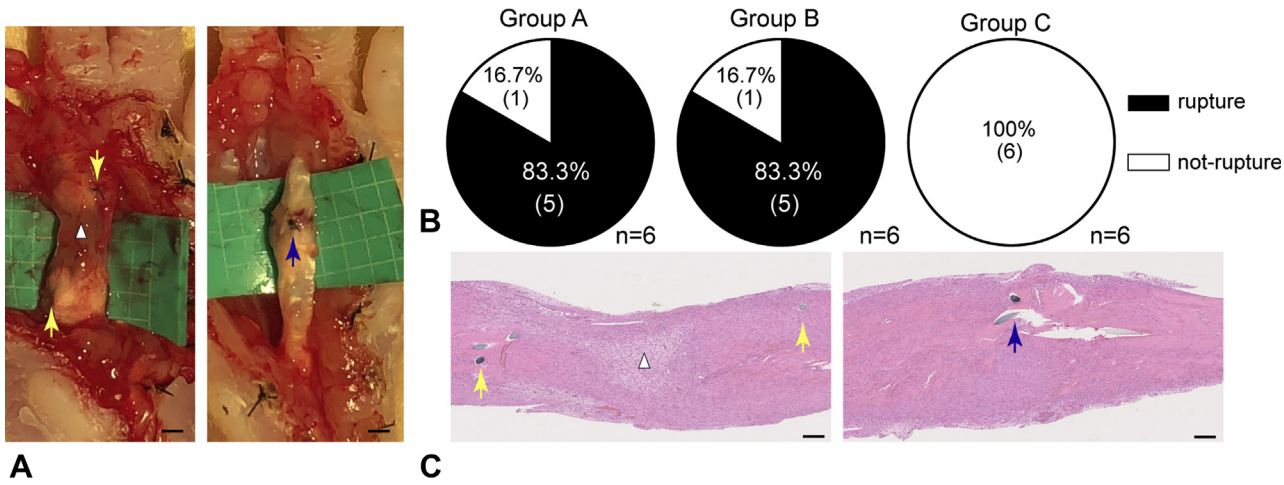

Figure 3. *In vivo* rat tendons repaired with 3 different combinations of sutures. **A** Representative gross appearance of ruptured (left panel, group A) and unruptured tendon (right panel, group C) 3 weeks after surgery. White arrowhead, yellow arrows, and blue arrows indicate fibrous scar tissue between the ruptured tendon stumps, separated suture materials, and tightened suture materials, respectively. Scale bars = 1 mm. **B** Ruptured and unruptured tendons in the 3 groups. Black and white represent ruptured and unruptured tendon, respectively. **C** Representative hematoxylin-eosin–stained images of ruptured (left panel, group A) and unruptured tendon (right panel, group C) 3 weeks after surgery. White arrowhead, yellow arrows, and blue arrows indicate fibrous scar tissue between ruptured tendon stumps, separated suture materials, and tightened suture materials, respectively. Scale bars = 100 μm.

experiments because no other biomechanical study used rat flexor tendons; however, the current *in vivo* experiment indicates the efficacy of this combination of sutures. In our previous experimental study of flexor tendon repair, we used rabbit flexor digitorum longus tendons, which were sutured with a Kessler stitch with 6-0 braided polyester and a continuous adaptation stitch with 8-0 nylon monofilament for circumferential epitenon repair.[14] 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-GFP*[8,16] 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 flexor tendons at the myotendinous junction is adopted.[10,11] These models are probably unsuitable for experimental research of flexor tendon repair because the anatomical structure of large tendons is different from that of flexor tendons, and loss of tensile force adversely affects the tendon healing process. Although the data are not shown here, we were previously unable to develop an appropriate model for flexor tendon repair using mice because of their small size. However, various types of transgenic rats have recently become available with the development of genome editing technology, such as a clustered regularly interspaced short palindromic repeats/clustered regularly interspaced short palindromic repeats–associated proteins system or transcription activator-like effector nucleases. Cell tracking analyses using reporter or *Cre/loxP* rats combined with the current model may reveal the mechanisms underlying actual tendon healing.

We have demonstrated that 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 with no cast fixation or immobilization procedures after surgery. Although surgeons must master microsurgical skills to obtain stable results, the current model will contribute to both an understanding of the molecular mechanisms underlying flexor tendon healing and the development of effective antiadhesive materials or methods for more successful flexor tendon repair.

Acknowledgments

This work was supported by the Nakatomi Foundation, JSPS KAKENHI grant numbers JP18K09019 and JP17K10956. We thank J. Sugita, K. Kaneko, and A. Fujikawa for technical assistance. We thank Angela Morben, DVM, ELS, from Edanz Group (https://en-author-services.edanzgroup.com/) for editing a draft of the manuscript.

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