Acta of Bioengineering and Biomechanics
Vol. 20, No. 2, 2018

Original paper
DOI: 10.5277/ABB-01084-2018-02

Comparison of different suture techniques for Achilles tendon repair in a rat model using collagen scaffolds

CAROLIN GABLER*, SUSANN GIERSCHNER, THOMAS TISCHER, RAINER BADER

[1] University Medicine Rostock, Department of Orthopedics,
Biomechanics and Implant Technology Laboratory, Rostock, Germany.

Purpose: Tendon injury is an increasing problem in orthopedic and trauma surgery due to aging of the population and increased activity demands. Many rodent animal models are used in order to evaluate tendon reconstruction. Although tendon reruptures are a well-known clinical problem, the outcomes of tendon repair in animal models are rarely discussed in the literature. The goal of the present experimental study was to compare the primary fixation stability of three suture techniques for repair of Achilles tendon defects in a rat model using a collagen scaffold. *Methods:* Cadaveric left hind limbs of Sprague-Dawley rats were prepared with an Achilles tendon defect of 3 mm and rejoined using a collagen scaffold. Three suture configurations (simple, simple stitch with additional framing suture, and modified Mason–Allen stitch; $n = 5$ each) underwent tensile testing until complete failure was observed. *Results:* Under a load of a mean value of 6.6 N, the failure load of simple stitches was the significantly lowest ($p < 0.01$). Both, modified Mason–Allen stitches and simple stitches with additional framing suture showed a mean failure load of more than 14 N. Regardless of the suture technique, most of the samples showed failure of tendon due to suture tear-out. The suture material as well as the scaffold remained mostly intact. *Conclusions:* Although simple end-to-end suture techniques are common in the literature, stitches with more suture strands should be preferred. Using techniques like an additional framing suture or modified Mason–Allen stitch, maximum failure load can be doubled and the risk of tendon rerupture may be decreased within *in vivo* testing.

Key words: *tendon repair, suture techniques, scaffolds, defect model*

1. Introduction

Tendon injury (e.g., in rotator cuff or Achilles tendon) is an increasing problem in orthopedic and trauma surgery due to aging of the population and increased activity demands. To augment or replace damaged tendons, tissue engineering applications and scaffold development have become a major focus of research [21]–[23]. Usually, new scaffold materials are tested in animal models before they are used clinically. Thereby, biofunctionality of the scaffolds and their effect on the healing process can be observed in a relatively short time [13]. Some animal models have been established in order to evaluate the quality and functionality of these materials. Models range from large animals (e.g., dogs or sheep) to small animals (e.g., rabbits or rats) [6]. Certainly, animal models have their shortcomings including anatomic, metabolic and hormonal differences from humans. However, they provide an appropriate tool to investigate novel ideas and theories [6], [13]. Mostly, the investigation of scaffolds as an augmentation material is executed on the M. supraspinatus tendon as a part of the rotator cuff [5], [6], [19]. Defect models, in which the scaffold serves as interposition, were usually carried out in small animal models using the Achilles tendon [3], [24], but the surgical techniques themselves showed large variances. For tendon repair of the Achilles tendon, defect sizes range from 3 mm [1] to 5 mm [24]. The supplementary excision of the M. plantaris tendon is recommended in the rat model by some working groups

* Corresponding author: Carolin Gabler, Biomechanics and Implant Technology Research Laboratory, Department of Orthopedics, University Medicine Rostock, Doberaner Strasse 142, 18057 Rostock, Germany. Phone: +49 381 494-9375, e-mail: carolin.gabler@med.uni-rostock.de
Received: January 9th, 2018
Accepted for publication: May 25th, 2018

[2], [8], [18]. Numerous suture techniques are available, such as simple end-to-end [24], [25] and modified Kessler stitches [8], [12].

Although reruptures are a well-known problem in tendon repair in humans [21]–[23], the outcomes of tendon repair in animal models are rarely discussed in the literature. It is important to know whether the initial restoration was sufficient to evaluate the data adequately. Some models with postoperative immobilization were also present [4], [7], [17], but it remained controversial whether or not they result in better outcomes [10], [13]. However, most of these immobilization procedures may cause problems, such as skin irritation, weight loss, and slipping out of the cast [4], [10]. Mostly, animals are allowed to move free in their cages after surgery. Therefore, a secure initial suture technique is important to prevent suture pullout of the scaffolds or adjacent tendon tissue directly after the operation.

Hence, the goal of the present experimental study was to compare the primary fixation stability of three suture techniques (simple stitch, simple stitch with additional framing suture, and modified Mason–Allen stitch) for repair of Achilles tendon defects in a rat model using a collagen scaffold to serve as a basis for future animal experiments.

2. Materials and methods

Sample preparation

In total, 15 cadaveric left hind limbs of male Sprague-Dawley rats were harvested, wrapped in gauze, soaked with saline solution, and kept frozen (–20 °C) until the day prior to testing. The hind limbs were thawed in a bath of saline solution at 4 °C overnight and stored at room temperature for at least four hours before *ex vivo* operation and six hours before final testing.

Each sample underwent a transection of the Achilles tendon with creation of a tendon defect of 3 mm. The plantaris tendon was removed. The remaining tendon ends were rejoined with a scaffold (5 mm length × 3.5 mm width × 0.88 mm thickness) based on bovine stabilized collagen chemically cross-linked with oriented collagenous fibers [9]. The fiber direction of the scaffold was oriented along the longitudinal direction of the Achilles tendon. Three suture configurations were randomly selected and placed into 3 groups (Fig. 1): group 1: two simple stitches at each end with Vicryl® 4-0 (Ethicon, Somerville, NJ, USA); group 2: two simple stitches at each end (Vicryl® 4-0) augmented with an additional framing suture (Vicryl® 2-0); and group 3: two modified Mason–Allen stitches (Vicryl® 4-0) at each end. Five specimens were tested for each suture configuration.

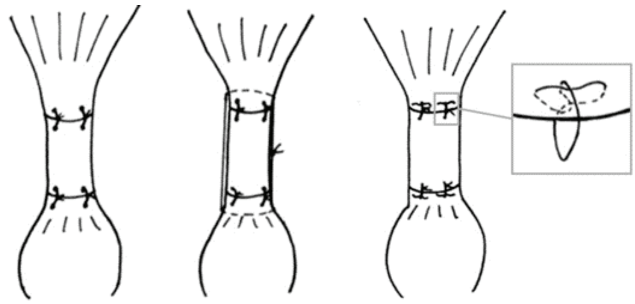

Fig. 1. Schematic illustration of the suture techniques.
Left to right: simple stitches (group 1),
simple stitches with framing suture (group 2),
and modified Mason–Allen stitches (group 3)

Load-to-failure tensile test

The Achilles tendon-calcaneus-foot complex was freed from all hard and soft tissue before testing. The foot was mounted with a cyanoacrylate adhesive (LOCTITE® 4902™, Henkel, Düsseldorf, Germany) at 45° to the surface of a custom-made aluminum block and additionally fixed due to a clamping unit via screws. For tensile testing the specimen-block-complex was fixed in a material testing machine (Z1.0, Zwick, Ulm, Germany). Specimens were preloaded with 1 N and subsequently stretched at a rate of 1 mm/s until complete failure was observed. The load-displacement curve was recorded and maximum failure load and stiffness were evaluated. Additionally, video recordings were made to evaluate the mode of failure macroscopically. Specimens were kept moist during the whole procedure.

Statistics

Statistical analysis was performed using IBM SPSS Statistics 22 software (IBM, Ehningen, Germany). All values showed normal distribution within the Kolmogorow–Smirnow test. Because of the low number of samples, statistical significance of differences between the groups was calculated using Mann–Whitney *U*-test. The level of significance was set to $p < 0.05$.

3. Results

The simple stitch suture showed significantly lower maximum failure loads (F_{max}) with 6.65 ± 2.28 N, compared to simple stitches with framing suture ($p < 0.01$) and modified Mason–Allen stitches ($p < 0.01$). Dif-

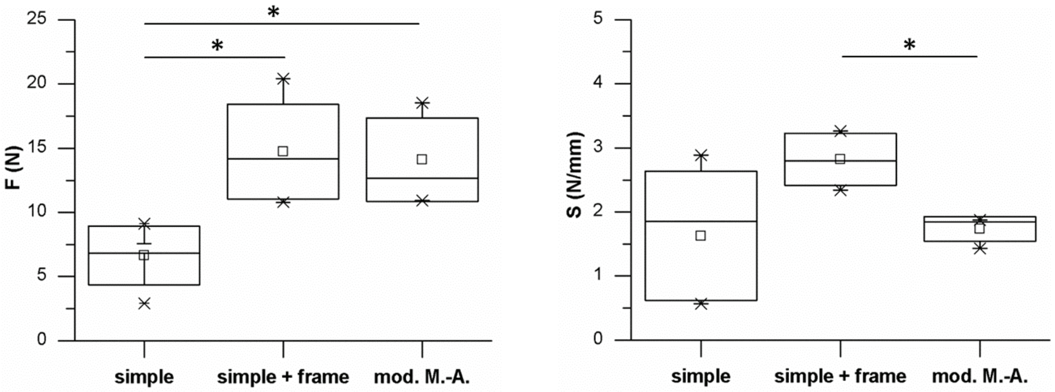

Fig. 2. Results of biomechanical testing. Left: Maximum failure load (*F* [N]).
* Simple stitch suture showed the significantly lowest maximum failure load ($p < 0.01$). Right: Stiffness (*S* [N/mm]).
*Modified Mason–Allen stitch showed significantly lower stiffness compared to simple stitches with framing suture ($p < 0.01$)

Table 1. Failure characterization

Suture technique	Failure			Location	
	suture	scaffold	tendon	proximal	distal
Simple stitches			5	5	
Simple stitches with framing suture			5	4	1
Modified Mason–Allen stitches	1*	1	4*	4	1

* One case: distal stitches failed: one due to loose knot and the other due to suture tear-out of the tendon. Therefore $n = 6$ in failure column.

ferences in maximum failure load of simple stitches with framing suture ($F_{max} = 14.72 \pm 3.70$ N) and modified Mason–Allen stitches ($F_{max} = 14.09 \pm 3.24$ N) were not recorded ($p > 0.05$). Stiffness of the simple stitches with framing suture was highest with 2.82 ± 0.41 N/mm. Simple stitches and modified Mason –Allen stitches showed lower values of stiffness with 1.63 ± 1.01 N/mm (slightly not significant, $p = 0.056$) and 1.73 ± 0.19 N/mm (significant with $p < 0.01$), respectively. No differences in stiffness of simple stitches and modified Mason–Allen stitches could be observed ($p > 0.05$). Results are presented in Fig. 2.

Table 1 shows the distribution of failure modes in tendons and sutures for the individual suture techniques. The evaluation shows that most of the samples (14 out of 15) finally failed due to suture tear-out of the tendon. The suture material as well as the scaffold remained mostly intact. All samples in group 1 (simple stitches) failed due to suture tear-out at the proximal tendon side. In group 2 (simple stitches with framing suture), two samples showed tear-out of the framing suture at the proximal tendon side. Two more samples showed tear-out of the simple stitches at the distal side, but also finally failed due to tear-out of the framing suture at the proximal tendon side. One specimen showed suture tear-out (simple stitches with framing suture) at the distal tendon side. The highest diversity in failure characterization was seen in group 3 (modified Mason–Allen stitches). One specimen showed migration of the distal sutures out of the tendon, but finally failed because the scaffold broke down at the proximal suture. Another sample showed migration of the sutures at both tendon sides, but final failure was recorded at the proximal tendon side. In one case, migration of the sutures at both tendon sides was also recorded. Finally, the distal sutures failed consecutively due to one loose knot and one suture tear-out of the tendon. The other two specimens of the modified Mason–Allen suture group showed a suture tear-out at the proximal tendon side.

4. Discussion

The healing after tendon repair still remains a clinical challenge with high rerupture rates, depending on factors including patient's age, tendon quality, and tear size [21]. Regeneration of the tendon is a slow and complex process. Biologic or synthetic, scaffold devices for tendon augmentation are used to provide more effective management option, with increased healing rates [23]. To overcome current limitations, such as lack of biocompatibility or mechanical strength during the remodeling process, development of new implantable scaffold materials has been the focus of research in the field of tissue engineering for tendon repair [22], [23]. For this purpose, several approaches with animal trials were analyzed. However, animal models as well as surgical techniques have high variability in the literature.

In the present study, we investigated the primary fixation stability of three suture techniques for bridging an Achilles tendon defect in a rat model with a collagen scaffold to avoid early postsurgical failure. No statistical differences between modified Mason–Allen stitches and simple stitches with framing suture were found, but both techniques showed more than twofold failure loads and, therefore, significantly higher values, compared to simple stitches (>14 N vs. 6.6 N). One sample from group 1 (simple stitches) had the lowest failure load of 2.92 N. It was likely that the proximal suture was placed (not quite optimally) in the surrounding connective tissue rather than in the tendon. Since the risk of misalignment during surgery in small animal experiments exists, the sample was not excluded from the evaluation. Most of the samples showed failure of tendon due to suture tear-out. The suture material as well as the scaffold remained mostly intact. These results support the hypothesis that an increased number of suture strands can improve the repair characteristics [13].

Animal experiments, especially with small animals, enable relatively fast insight into the functionality of new scaffold material, since the animals have a high regeneration potential [23]. Suture techniques for several tendons, such as Achilles tendon, flexor tendon, or rotator cuff repair in humans are controversially discussed [15], [16], [20]. Complex stitches and a high number of suture strands are known to provide better results in failure tests [11], [14]. In small animals space is limited and, therefore, simplification of suture techniques right up to simple stitches [24], [25] is often required.

The problem of high rerupture rates is clinically well-known in the restoration of tendon injuries. However, in the literature regarding outcome of tendon repair in animal models these problems are rarely discussed and data about rerupture rates have not been provided so far. In a previous animal experiment, we tested collagen scaffolds for tendon repair in a rat model were tested [9]. Before mechanical testing was carried out, tendon repair was examined via magnetic resonance imaging (MRI). A failure rate of 31% was observed. Most of these failures were not visible after preparation of samples. There was no correlation of biomechanical values (i.e., failure load or stiffness) of the tested Achilles repairs regarding whether they failed *in vivo* or not. MRI indicated that scaffolds remained intact and dislocation of scaffolds seems to be caused by suture tear-out of the tendon. It has been reported, that mechanical properties of tendon repairs with different suture techniques did not show clear differences after healing *in vivo* [6]. Aspenberg and Virchenko [1] showed that a 3 mm Achilles tendon defect can achieve nearly 70% of maximum failure load after 28 days of healing, compared to non-operated control. On the other hand, we assume that the remodeling process is dependent on the junction to native tendon tissue and transferred tensile loads, so the Achilles tendon repair was intact over time or not. Therefore, it is important to use a secure suture technique to prevent suture tear-out or other defects. Although simple end-to-end suture techniques are common in the literature, other stitches with more suture strands should be preferred, even in small animal models. Beyond higher breaking strength, the framing suture of the modified percutaneous stitch can act as a partial immobilization, as described by Güngörmüs et al. [10]. In their work, they could show a stimulatory effect of that partial immobilization on tendon healing. With this, overloading could be prevented without damaging the surgical repair sides, so that and animals are enabled to move freely at the same time.

The present study was limited by small sample size. Nevertheless, the results support our findings in MRI that failure was mainly caused by suture tear-out. To investigate Achilles tendon repair in rat, especially with the use of scaffolds it is important to avoid early postsurgical failure. In this context, imaging techniques like MRI are important auxiliary tools to verify the outcome of tendon repair in animal models by showing the healing in the right place.

5. Conclusion

Many rodent animal models are used in order to evaluate tendon repair and reconstruction in the literature, but rerupture rates in animal models are rarely discussed. Because postoperative immobilization can implicate many problems and may lead to dropout results, many animals are allowed to move freely after surgery. Therefore, secure suture techniques are required. Our results showed that simple sutures performed poorly against techniques with more suture strands, in this particular case simple stitches with an augmenting framing suture vs. modified Mason–Allen stitches. Using these techniques, the maximum failure load can be doubled and the risk of rerupture may be decreased within *in vivo* testing.

Acknowledgment

We thank MedSkin Solutions Dr. Suwelack AG (Billerbeck, Germany) for providing the test materials made of bovine collagen. There are no disclosures to declare. This work was supported by the Federal Ministry for Economic Affairs and Energy within the ZIM program (KF2100703AJ2).

References

[1] Aspenberg P., Virchenko O., *Platelet concentrate injection improves Achilles tendon repair in rats*, Acta Orthop. Scand., 2004, 75(1), 93–99.

[2] Best T.M., Collins A., Lilly E.G., Seaber A.V., Goldner R., Murrell G.A.C., *Achilles tendon healing: A correlation between functional and mechanical performance in the rat*, J. Orthop. Res., 1993, 11(6), 897–906.

[3] Bolt P., Clerk A.N., Luu H.H. et al., *BMP-14 gene therapy increases tendon tensile strength in a rat model of Achilles tendon injury*, J. Bone Joint Surg. Am., 2007, 89(6), 1315–1320.

[4] Coutinho E.L., Gomes A.R.S., França C.N., Salvini T.F., *A new model for the immobilization of the rat hind limb*, Braz. J. Med. Biol. Res., 2002, 35(11), 1329–1332.

[5] Derwin K.A., Codsi M.J., Milks R.A., Baker A.R., McCarron J.A., Iannotti J.P., *Rotator Cuff Repair Augmentation in a Canine Model with Use of a Woven Poly-L-Lactide Device*, J. Bone Joint Surg. Am., 2009, 91(5), 1159–1171.

[6] Edelstein L., Thomas S.J., Soslowsky L.J., *Rotator cuff tears: what have we learned from animal models?*, J. Musculoskelet Neuronal Interact., 2011, 11(2), 150–162.

[7] Eliasson P., Andersson T., Aspenberg P., *Achilles tendon healing in rats is improved by intermittent mechanical loading during the inflammatory phase*, J. Orthop. Res., 2012, 30(2), 274–279.

[8] Freedman B.R., Gordon J.A., Bhatt P.R. et al., *Nonsurgical treatment and early return to activity leads to improved Achilles tendon fatigue mechanics and functional outcomes during early healing in an animal model*, J. Orthop. Res., 2016, 34(12), 2172–2180.

[9] Gabler C., Gierschner S., Lindner T., Tischer T., Bader R., *Magnetic Resonance Imaging as an auxiliary tool for evaluation of tendon repair in an animal model using colagen-based scaffolds*, EORS 2017, Munich, September 13–15.

[10] Güngörmüş C., Çetİnkaya M.A., Demİrutku A., *A new model for partial immobilization of rat hind limb after Achilles tendon excision/reinterposition*, Turk. J. Vet. Anim. Sci., 2013, 37(5), 546–552.

[11] Ikemoto R.Y., Murachovsky J., Nascimento L.G.P., Bueno R.S., Almeida L.H., Strose E., *Study on the resistance of the supraspinous tendon using simple, matress and mason allen stitches*, Acta Ortopédica Bras., 2010, 18(2), 100–103.

[12] Kaymaz B., Gölge U.H., Ozyalvacli G. et al., *Effects of boric acid on the healing of Achilles tendons of rats*, Knee Surg. Sports Traumatol. Arthrosc., 2016, 24(12), 3738–3744.

[13] Lin T.W., Cardenas L., Soslowsky L.J., *Biomechanics of tendon injury and repair*, J. Biomech., 2004, 37(6), 865–877.

[14] Ma C.B., MacGillivray J.D., Clabeaux J., Lee S., Otis J.C., *Biomechanical evaluation of arthroscopic rotator cuff stitches*, J. Bone Joint Surg. Am., 2004, 86-A(6), 1211–1216.

[15] McCoy B.W., Haddad S.L., *The Strength of Achilles Tendon Repair: A Comparison of Three Suture Techniques in Human Cadaver Tendons*, Foot Ankle Int., 2010, 31(8), 701–705.

[16] Momose T., Amadio P.C., Zhao C., Zobitz M.E., Couvreur P.J., *An K-N., Suture techniques with high breaking strength and low gliding resistance: Experiments in the dog flexor digitorum profundus tendon*, Acta Orthop. Scand., 2001, 72(6), 635–641.

[17] Murrell G.A.C., Lilly E.G., Goldner R.D., Seaber A.V., Best T.M., *Effects of immobilization on achilles tendon healing in a rat model*, J. Orthop. Res., 1994, 12(4), 582–591.

[18] Ouyang H.W., Goh J.C.H., Thambyah A., Teoh S.H., Lee E.H., *Knitted Poly-lactide-co-glycolide Scaffold Loaded with Bone Marrow Stromal Cells in Repair and Regeneration of Rabbit Achilles Tendon*, Tissue Eng., 2003, 9(3), 431–439.

[19] Perry S.M., Gupta R.R., Van Kleunen J., Ramsey M.L., Soslowsky L.J., Glaser D.L., *Use of small intestine submucosa in a rat model of acute and chronic rotator cuff tear*, J. Shoulder Elbow Surg., 2007, 16(5), S179–S183.

[20] Rebeccato A., Santini S., Salmaso G., Nogarin L., *Repair of the achilles tendon rupture: A functional comparison of three surgical techniques*, J. Foot Ankle Surg., 2001, 40(4), 188–194.

[21] Ricchetti E.T., Aurora A., Iannotti J.P., Derwin K.A., *Scaffold devices for rotator cuff repair*, J. Shoulder Elbow Surg., 2012, 21(2), 251–265.

[22] Rodrigues M.T., Reis R.L., Gomes M.E., *Engineering tendon and ligament tissues: present developments towards successful clinical products*, J. Tissue Eng. Regen. Med., 2013, 7(9), 673–686.

[23] Walden G., Liao X., Donell S., Raxworthy M.J., Riley G.P., Saeed A., *A Clinical, Biological, and Biomaterials Perspective into Tendon Injuries and Regeneration*, Tissue Eng. Part B Rev., 2016, 23(1), 44–58.

[24] Webb W.R., Dale T.P., Lomas A.J. et al., *The application of poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) scaffolds for tendon repair in the rat model*, Biomaterials, 2013, 34(28), 6683–6694.

[25] Zantop T., Gilbert T.W., Yoder M.C., Badylak S.F., *Extracellular matrix scaffolds are repopulated by bone marrow-derived cells in a mouse model of achilles tendon reconstruction*, J. Orthop. Res., 2006, 24(6), 1299–1309.