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Original article

Do adipofascial flaps affect the mechanical properties of a repaired tendon? A biomechanical rat model study

Les lambeaux adipofasciaux affectent-ils les caractéristiques mécaniques d'un tendon réparé? Étude biomécanique sur modèle murin

M. Koc [a], M. Yucens [b,*], N. Aydemir [b], A.C. Yorukoglu [b], K. Guvenc [a], C. Uzun [c], N. Erdal [c], A.F. Demirkan [b]

[a] *Faculty Depth of Orthopaedics and Hand Surgery, Pamukkale University Medical, Denizli, Turkey*
[b] *Faculty Depth of Orthopaedics, Pamukkale University Medical, 20160 Denizli, Turkey*
[c] *Faculty Depth of Biophysics, Mersin University Medical, İhsaniye Mh., 32133 Sokak Çiftlikköy Kampüsü, 33079 Yenişehir-Mersin, Turkey*

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A B S T R A C T

The aim of this study was to evaluate the effect of vascularized and non-vascularized fascial flaps on tendon healing, specifically the maximum strain, maximum stress, elasticity and resistance of the repaired tendon. Rats were randomly divided into 3 groups: Group 1 – primary repair; Group 2 – vascularized pedicled fascial graft; Group 3 – non-vascularized free fascial graft. The rats were euthanized after 2 weeks and 40 mm-long samples were taken from the Achilles tendon and gastrocnemius muscle. To evaluate the mechanical properties of the tendons, maximum load, maximum deformation, energy stored until yield point and stiffness on the load-deformation curve were measured. Based on this mechanical testing, the best group in terms of tissue strength and quality was the primary repair group. When the samples were examined individually, the two samples with the highest breaking force after the control group were in the pedicled graft group. The worst results overall were in the free graft group. We believe that if the blood flow is preserved for the fascial flap in the pedicled graft group, the tendon's breaking force would be higher.

Mots clés :
Lambeaux adipofasciaux
Tendon
Analyse biomécanique

R É S U M É

Le but de cette étude était d'évaluer l'effet des lambeaux fasciaux vascularisés et non vascularisés sur la cicatrisation du tendon et sur la déformation maximale, la contrainte maximale, l'élasticité et la résistance du tendon réparé. Des rats ont été divisés au hasard en 3 groupes: groupe 1, réparations primaires; groupe 2, lambeaux fasciaux pédiculés vascularisés; groupe 3, lambeaux fasciaux libres non vascularisés. Des échantillons de 40 mm de long ont été prélevés sur le tendon d'Achille et le muscle gastrocnémien. Pour évaluer les propriétés biomécaniques des tendons d'Achille, les paramètres biomécaniques mesurés étaient la charge maximale, la déformation maximale, l'énergie stockée jusqu'à la limite d'élasticité et la rigidité selon la courbe de charge-déformation. À la suite des évaluations biomécaniques des tendons d'Achille, le groupe le meilleur en ce qui concerne la qualité de résistance et de la qualité du tissu était le groupe des réparations primaires. À l'examen individuel des échantillons, les deux échantillons avec la force de rupture la plus élevée après le groupe de contrôle appartenaient au groupe des lambeaux pédiculés. Les plus mauvais résultats sur tous les paramètres étaient dans le groupe des greffes libres. On peut considérer que si la circulation du sang était conservée dans le lambeau fascial du groupe lambeau pédiculé, la force de rupture de tendon serait plus élevée.

* Corresponding author: Faculty Depth of Orthopaedics, Pamukkale University Medical, 20160 Denizli, Turkey.
E-mail addresses: mehmetraufk@hotmail.com (M. Koc), aflyucens@yahoo.com (M. Yucens), anaydemir@yahoo.co.uk (N. Aydemir), alicagdasyorukoglu@gmail.com (A.C. Yorukoglu), guvenckenan@hotmail.com (K. Guvenc), cosaruzun@gmail.com (C. Uzun), nerdal@mersin.edu.tr (N. Erdal), fahirdemirkan@yahoo.com (A.F. Demirkan).

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1. Introduction

Tendon injuries are soft tissue injuries that are becoming more prominent in clinical practice. Primary repair of tendon injuries is the gold standard [1,2]. However, the best treatment is controversial and ranges from "wait-and-see" approaches to surgery, with the appropriate treatment generally selected based on the type of injury. In all treatment forms, early rehabilitation and early mobilization are one of the main objectives [3]. Adhesions and poor tendon healing can be seen after tendon repair, when the surrounding tissue is damaged. Tendon grafts placed in a scarred bed are unlikely to function well because tendon gliding requires a non-adherent, unscarred vascular bed. The concept of interposing vascularized tissue (adipofascial flaps) between the tendon and the bed to improve gliding has been used in injuries with poor prognosis, mainly in the acute and subacute period [4,5].

The aim of this study was to evaluate the effect of vascularized and non-vascularized fascial flaps on tendon healing and on the maximum strain (εU, mm/mm), maximum stress (σu; MPa), elasticity (Young's modulus, E, MPa) and resistance of the repaired tendon to determine whether these flaps could be useful in early dynamic rehabilitation.

2. Material and methods

2.1. Animals

The study used a total of 18 Wistar Albino rats, each weighing 210 ± 12 g, which were randomly divided into 3 groups: Group 1 – primary repair; Group 2 – vascularized pedicled fascial graft; Group 3 – non-vascularized free fascial graft. Power analysis, based on biomechanical data from prior studies of rat tendon healing, determined that 6 tendons were required per treatment group per time period for 90% power [6,7]. Throughout the experiment, the rats were fed ad libitum. All animal procedures were approved by the Animal Research Review Committee of the Pamukkale University Medicine Faculty.

2.2. Surgical protocol

Surgical anesthesia was induced with ketamine (90 mg/kg bodyweight) and xylazine (10 mg/kg bodyweight) administered intraperitoneally. The right leg of each rat was sterilized with povidone iodine. An incision was made on the posterior aspect of leg from the gastrocnemius to the heel (Fig. 1A). The gastrocnemius fascia and Achilles tendon were identified and then the Achilles tendon was cut transversely with a scalpel (Fig. 1B). In Group 1, the tendon was sutured with a modified Kessler technique and epitendinous continuous suture. The subcutaneous tissue and skin were sutured closed in order. In Group 2, the gastrocnemius fascia was released from proximal to distal (Fig. 1C) then the Achilles tendon was sutured with a modified Kessler technique and the gastrocnemius fascia was attached to the Achilles tendon with epitendon sutures with a vascularized pedicle (Fig. 1D). The subcutaneous tissue and skin were sutured closed in order. In Group 3, the gastrocnemius fascia was released from proximal to distal and cut from the distal end to obtain a free graft. The Achilles tendon was sutured with a modified Kessler technique and the free gastrocnemius fascia was attached to the Achilles tendon with epitendon sutures, then the subcutaneous tissue and skin were sutured closed in order. The left legs of the Group 1 rats were used as control samples.

2.3. Biomechanical testing

All the rats were euthanized at the end of day 14. Samples 40 mm in length were taken from the Achilles tendon and gastrocnemius muscle (Fig. 2).

The mechanical properties of healing tendon specimens were evaluated according to a previously described procedure using a tensile testing system (Ilfa Electronic San.Tic.Ltd., Turkey). This system had a 1000 N load cell and crosshead speed range of 0–250 mm/min (Fig. 3). Load was applied monoaxially at a constant speed. Then, the load–deformation curve was recorded using the system's software. The load–deformation curve was converted to a stress–strain curve and the saved data was evaluated with Logger Pro® software (V 3.8.3, Vernier Software & Technology, Orlando, FL, USA) (Fig. 4).

The Achilles tendon was placed in the center of the device equidistant from the two clamps and perpendicular to the direction of the loading force. The tendon thickness was measured using digital calipers, then the specimen was clamped between the two computer-controlled crossheads of the tensile testing system. The specimen was stretched to failure point at the displacement rate of 25 mm/min at room temperature [8].

To evaluate the mechanical properties of the Achilles tendons, the maximum load (FU: N), maximum deformation (du: mm), energy stored until yield point (U; mJ) and stiffness (S, N/mm) were measured from the load–deformation curve. The ratio of the load–deformation curve to the standardized stress–strain curve was calculated together with the following parameters: maximum strain (εU, mm/mm), maximum stress (σu; MPa), elasticity (Young's modulus, E, MPa) and resistance (u; MPa).

Conformity of the data to a normal distribution was assessed with the Kolmogorov–Smirnov test; the stored energy ($P = 0.198$), max deformation ($P = 0.200$) and max strain ($P = 0.200$) variables were determined to have a normal distribution. Variance analysis was applied to examine the differences between groups in terms of

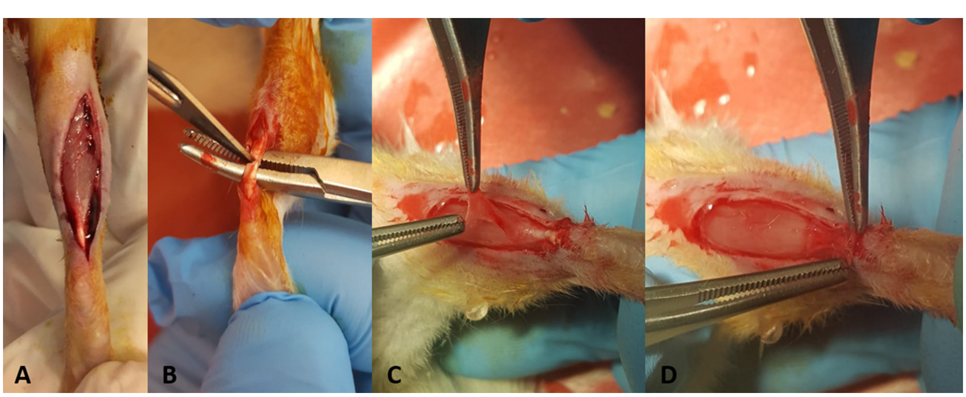

Fig. 1. Surgical procedure. Skin incision (A). Cutting the tendon (B). Elevating the adipofascial flap (C). Covering the flap on the repaired tendon (D).

Fig. 2. Prepared Achilles tendon and gastrocnemius muscle specimens.

stored energy, max deformation and max strain variables. The Kruskal–Wallis test was used to evaluate the stiffness, tensile strength, elasticity, resistance and max stress variables.

3. Results

No statistically significant difference was found between the experimental groups or between the experimental groups and the control group in the stiffness parameter ($P = 0.184$). As expected, a statistically significant decrease was found in the maximum load, Young's modulus, resistance and maximum stress parameters in the experimental groups compared to the control group ($P < 0.05$). No statistically significant difference was found in these parameters between experimental groups (Table 1).

A statistically higher Young's modulus was found in the control group than in the pedicle group. Statistically significantly higher values were found for resistance in the control group compared to the primary repair group and the free fascial graft group. The max stress value was significantly higher in the control group compared to all the experimental groups ($P < 0.05$). No statistically significant difference was found between the groups in the max deformation ($P = 0.380$) and max strain ($P = 0.384$). A statistically significantly higher stored energy value was found in the control group compared to the other groups ($P < 0.05$, Table 1).

When the Achilles tendons were examined relative to their size and shape (extrinsic parameters: maximum load, stiffness, strength, maximum deformation), there was no significant difference between the experimental groups. When the tendons' material properties were determined independent of tissue size (intrinsic parameters: Young's modulus, maximum stress, maximum strain, resistance), the elasticity, resistance to applied force and ability to deform without permanent damage were better in the primary repair group than in the other experimental groups and were similar to those of the tendons in the control group. As a result of the Achilles tendon testing, the best group in terms of the tissue strength and quality was the primary repair group. When the samples were examined individually, the two samples with the highest breaking force after the control group were in the pedicled graft group. The worst results overall were in the free graft group.

4. Discussion

The aim of this study was to evaluate the effect of vascularized and non-vascularized fascial flaps on tendon healing in a rat model. The ultimate goal is to decrease the amount of soft tissue

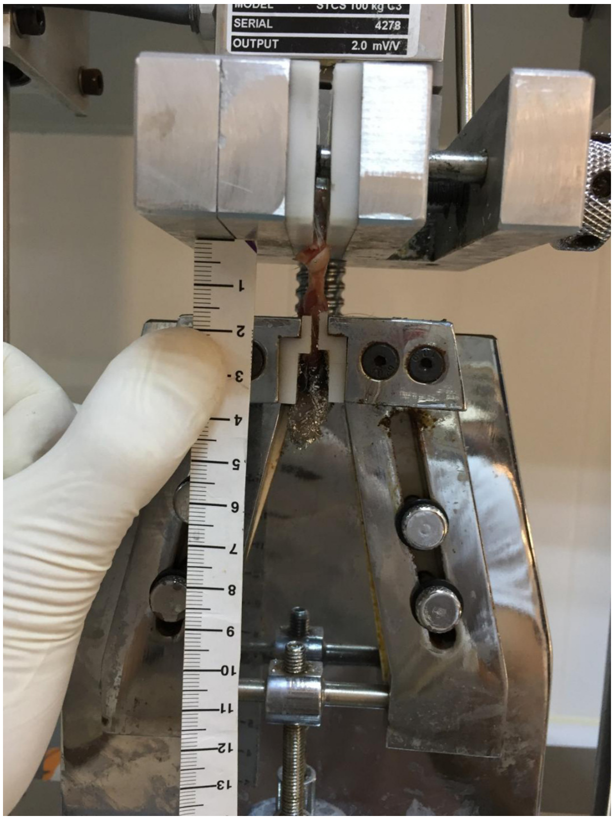

Fig. 3. Biomechanical testing of tendon specimens.

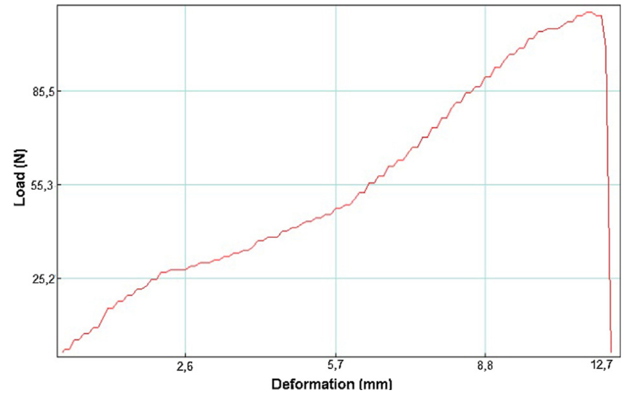

Fig. 4. Load-deformation curve for a specimen in the control group.

debridement surrounding a tendon injury with the use of local adipofascial flaps.

Extrasynovial tendons (Achilles) from mice, rats and rabbits are commonly used to evaluate tendon healing processes. Since no gold standard animal model exists for tendon injuries, species selection for an in vivo assessment of new treatments should be based on initial proof of principle; next, the model should be predictive and accurately reproduce the clinical situation. Among laboratory animals involved in tendon defect or tenotomy repair, rabbits are the most used models (49%) followed by rats (27%) and mice (6%) [9]. Sometimes rats are not preferred because of their small structure. However, they have a faster recovery rate, we are already working with them and their Achilles tendons were compatible with the mechanical testing system used for this study. However, breed variety, high costs, specialized infrastructure and personnel, together with ethical issues are serious disadvantages that need to be considered when using large animals [10]. The choice of animal models is based on scientific criteria as well as on economic and ethical issues. Indeed, animal housing and management costs significantly influence the selection of animal species to obtain reliable results relative to the sample size. Moreover, pressure exerted by public opinion against the use of dogs in animal testing discourages the choice of this species. Therefore, the selection criteria of animal models in proof-of-concept studies has to balance the ethically justifiable, cost-effective model with the features of the model (anatomical site, size, surgical approach, mobilization and biomechanical properties) in order to answer to the scientific question. We preferred using rats because of their easy production, ease of maintenance, lower rank in phylogenetic scale compared to other species, low infection risk and extensive published data [9].

Rats were sacrificed at 2 weeks to investigate the early resilience of tendons because early rehabilitation is needed to decrease adhesions and stiffness. Most studies on different animal species evaluated the neotendon and adhesion formation histologically within the injury site, and biomechanical properties starting from 3 weeks after surgery [11]. Other studies investigated the tendon response to healing and the neovascularization during the early phase within 7 days after surgery. We wanted to know if

Table 1
Achilles tendon mechanical properties after three suture repair methods were compared in rats.

Groups Achilles tendon mechanical parameters	Control [Q1, Q3] Median	Pedicled graft [Q1, Q3] Median	Primary repair [Q1, Q3] Median	Free graft [Q1, Q3] Median
Maximum load (FU: N)	[65.68; 97.75] 72.41	[41.63; 67.50] 49.13	[40.86; 55.34] 52.75	[40.34; 47.06] 44.48[a]
Stiffness (S, N/mm)	[14.43; 34.96] 22.06	[8.23; 19.78] 11.94	[6.26; 17.25] 8.51	[7.53; 12.83] 9.72
Elasticity (Young's modulus) (E, MPa)	[5.75; 15.55] 8.51	[1.25; 2.80] 1.48[a]	[1.28; 3.10] 1.97	[1.51; 2.80] 1.87
Resistance (u; MPa)	[52.17; 87.04] 76.67	[16.85; 26.89] 21.91	[13.13; 19.56] 14.90[a]	[13.87; 15.99] 15.33[a]
Maximum stress (σu; MPa)	[23.04; 34.30] 25.40	[6.15; 9.98] 7.26[a]	[6.72; 8.77] 8.36[a]	[6.87; 8.01] 7.57[a]
	mean ± SD	mean ± SD	mean ± SD	mean ± SD
Energy absorbed (U, mj)	221.20 ± 56.21	156.58 ± 35.16[a]	105.63 ± 25.13[a]	64.30 ± 6.16[a]
Maximum deformation (du: mm)	10.93 ± 2.06	11.19 ± 2.71	8.85 ± 3.27	12.67 ± 4.91
Maximum strain (εU, mm/mm)	8.94 ± 1.87	9.18 ± 2.46	7.05 ± 2.97	10.49 ± 4.42

[a] Significantly different from control group ($P < 0.05$).

the tendon could withstand early movement, so we sacrificed the rats after 2 weeks, to determine which repair type was best suited to early rehabilitation. After an acceptable repair technique in the long term, the most important criteria is tendon rehabilitation [12]. In this study, our aim was to compare the long-term healing potential of the three surgical techniques. The possibility of recovery would be the same regardless of repair type in the next weeks. Two weeks may be early for long-term results, but biomechanical testing in rats at this time point provides some information about durability.

When the Achilles tendons were evaluated biomechanically, the best group in terms of tissue strength and quality was the primary group. However, when the samples were examined individually, the two samples with the highest maximum load after the control group were in the pedicled graft group. The worst results overall were in the free graft group.

In the Yüksel et al. study, no significant difference was found in maximum force (Fmax) values at the 15th and 30th day [13]. In the Dabak et al. study, Young' modulus was the highest in the single-dose phospholipid injection group and lowest in the multiple-dose phospholipid injection group. There was a statistically significant difference between these groups ($P < 0.05$). Ultimate strength was highest in the hyaluronic acid injection group and lowest in the multiple-dose phospholipid injection group, but not statistically different ($P > 0.05$). Energy absorption capacity was highest in the control group and lowest in the multiple-dose phospholipid injection group, but not statistically different ($P > 0.05$).

Adhesions that interfere with tendon gliding are one of the most common problems following tendon repair. Studies have been conducted both by surgeons and physical therapists to explore ways in which soft tissue debridement can be decreased. One way of doing this is by applying an adipofascial flap around the tendon. Del Pinnel et al. studied adipofascial flaps on tendons which have a scarred bed. A total of 11 adipofascial free flaps were applied to 10 patients for sheathed tendons: 3 in the forearm, 5 on the dorsum of the hand, and 3 on the dorsum of a proximal phalanx. The extensors were involved in 8 cases, the digital flexors in the forearm in 2, and a combination of wrist extensors and long thumb tendons in 1 case; all the flaps survived without complications [5]. Temporofascial free flaps have been evaluated in many studies, with good results reported [2,3]. Meimandi-Parizi et al. mentioned the Achilles tendon is not well-suited to studying adhesion formation since it is an extrasynovial structure [14]. But in the Dabak et al. study, adhesions in the rat Achilles tendon were evaluated based on dosage and usage of phospholipids [15]. They found statistically significant differences between the multiple-dose phospholipid injection group and the control group and also the hyaluronic acid group and the control group. The phospholipids could act as a barrier to adhesion even over the extrasynovial rat Achilles tendon. In our study, the adipofascial flap could act as a barrier but adhesions could not be investigated histopathologically in same rats as the ones being used for biomechanical testing. A separate groups of rats is required to study adhesions.

The main blood supply to the middle section of the tendon flows via the paratenon. Vessels within this connective tissue sheath run transversely toward the tendon on its anterior aspect, branch out, and then continue longitudinally along the course of the tendon [16]. In the free graft group in our study, the fascial flap probably did not provide sufficient vascular supply and obstructed the blood supply from the main arterial blood flow of the Achilles tendon. If the blood flow is preserved for the fascial flap in the pedicled graft group, the breaking force of the tendon would be higher than in the primary repair and free graft groups. A limitation of this study was that the blood supply of the tendon and surrounding soft tissues was not analyzed.

5. Conclusion

Tendon primary repair seems to be better for healing of the extrasynovial Achilles tendon in rats compared to the other two flap methods. However, it may be a priority to repair the tendon by rotating with a vascular flap when the surrounding tissue is damaged. For this, complete circular flaps and histopathological evaluation may be more enlightening.

Disclosure of interest

The authors declare that they have no competing interest.

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