EXPERIMENTAL STUDY

Acta Orthop Traumatol Turc 2012;46(5):393-397
doi:10.3944/AOTT.2012.2770

The effect of dehydration and irrigation on tendon adhesion formation after tendon exposure

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Objective: The aim of this study was to evaluate the effects of dehydration due to tendon exposure on adhesion formation on the tendon surface.

Methods: Achilles tendons of 60 New Zealand white rabbits were surgically exposed and evaluated. In the control group (Group 1), the wound was closed immediately; and in the remaining two groups, Achilles tendons were exposed to air for 60 minutes without (Group 2) or with (Group 3) regular saline irrigation. After undergoing clinical examination, 50% of rabbits in each group were sacrificed 3 weeks postoperatively and 50% at the 6th postoperative week.

Results: All tendons exposed to air exhibited mild or moderate degrees of adhesion. Compared to the control group, the incidence of adhesion formation was significantly higher in the groups where tendons had been exposed to air for 60 minutes, whereas no significant difference was found between the irrigated and non-irrigated groups. No limitations or contractures were detected in the hind limbs of the animals at the clinical examination.

Conclusion: Regardless of irrigation, tendons are not prone to form clinically apparent adhesions during operations under 60 minutes of duration.

Key words: Adhesion; air exposure; drying; irrigation; tendon.

Tendons play a major role in the movement of joints.[1] A smooth sheath surrounding the tendon allows for the gliding movement essential for physiological joint function. However, these structural interactions can be disrupted by injuries and surgical procedures. Among the most frequent complications encountered after tendon surgery are adhesions between the tendon and the tendon sheath.[2] Impairing the gliding properties of the tendon surface, these adhesions can cause limitation in the range of motion (ROM) and contracture of the joint.[2,3]

To date, inflammation, infection, intra- and postoperative bleeding, trauma and foreign body reactions have all been considered to contribute to the formation of adhesions and several biological methods have been employed in order to prevent tendon adhesion.[4,5]

Healthy tendons are protected from external influences by the surrounding tissue and require synovial fluid in order to perform their physiological function. Exposure to air during surgery or in open injuries is known to deleteriously affect connective tissues. In the

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Submitted: October 20, 2011 **Accepted:** February 1, 2012

Available online at
www.aott.org.tr
doi:10.3944/AOTT.2012.2770
QR (Quick Response) Code:

knee joints of rabbits, exposure to air for 60 minutes resulted in the complete necrosis of the articular cartilage.[6] A study investigating cellular activities within the tendon found significant reduction of protein synthesis and abundant cellular proliferation following exposure to air.[7] In order to prevent possible damage due to air exposure, tendons are irrigated with saline, covered with wet gauzes or hidden under adjacent tissues. However, to what extent these procedures are effective has yet to be scientifically investigated.

The aim of this study was to evaluate the effect of air exposure on tendon adhesion formation.

Materials and methods

This study was approved by our Institutional Animal Care and Use Committee. Sixty of a total of 71 New Zealand rabbits weighing 2 to 3 kilograms were included in this experimental study. Eleven rabbits that died before the operation and just after the induction of anesthesia were excluded from the study.

Each animal was anesthetized with an intramuscular injection of 40 mg/kg ketamine HCl and 5 mg/kg xylazine. In preparation for the surgery, 20 mg/kg ampicillin was administered to each animal for infection prophylaxis and the hind legs were shaved from knee to ankle. After application of a tourniquet, skin antisepsis was performed using iodophor followed by an iodine solution. The feet were fixated to the sterile operating table with surgical drapes (Fig. 1). All operations were performed at a room temperature of 21° C and a relative air humidity of approximately 60%.

A longitudinal skin incision of 3 cm was made over the Achilles tendon. The paratenon was opened longitudinally, allowing for the dissection of the Achilles tendon from the surrounding tissues. This procedure was applied to both hind legs of each animal.

In the control group (Group 1), the wound was immediately sutured using absorbable 6/0 monofilament polyglyconate for the paratenon and 4/0 monofilament polypropylene for the skin. In the remaining two groups, a portion of the Achilles tendon, approximately 1 cm in length, was exposed to air 360 degrees circumferentially for 60 minutes with the help of an instrument placed 1.5 cm proximal to the Achilles tendon insertion (Fig. 2). In Group 2, Achilles tendons were left unirrigated, whereas the tendons in Group 3 were kept moist with saline irrigation at intervals of five minutes. After sixty minutes, the layers were closed anatomically as described for the control group. All animals were permitted to resume unlimited activity in their single-cages.

Passive ROM of the ankle joint was examined under general anesthesia 3 weeks postoperatively in 50% of the animals of each group and 6 weeks postoperatively in the other half. Following the examination, animals were sacrificed with a rapid application of 100 mg/kg pentothal sodium through the ear marginal veins. The Achilles tendon segments that had been exposed to air were removed with the adjacent tissues and overlying skin and fixated in 10% buffered formalin solution at room temperature.

Fig. 1. The feet of the rabbit affixed to the table under sterile conditions. [Color figure can be viewed in the online issue, which is available at www.aott.org.tr]

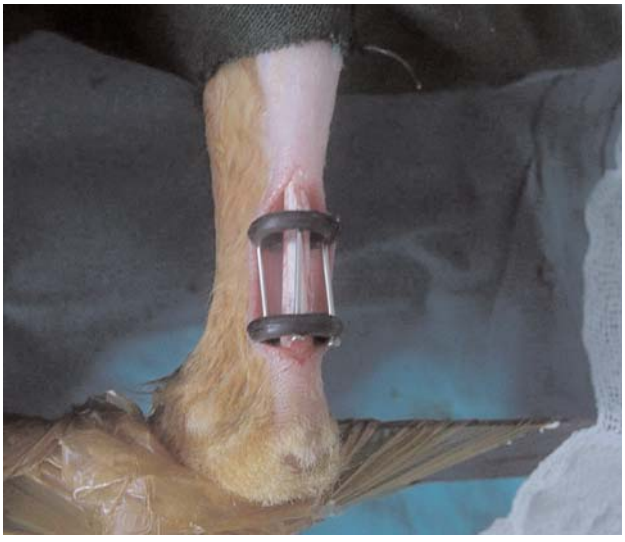

Fig. 2. The instrument which provides air exposure of the tendon. [Color figure can be viewed in the online issue, which is available at www.aott.org.tr]

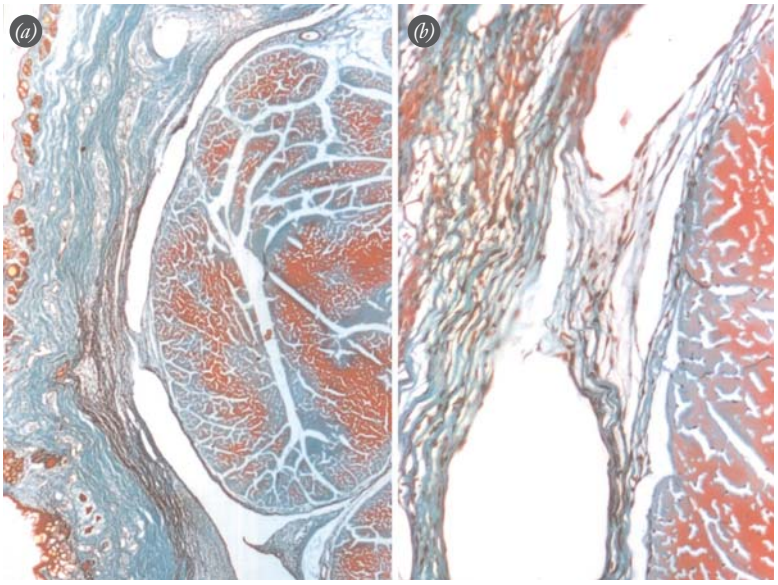

Fig. 3. Microscopic image of a mild degree adhesion with trichrome staining under **(a)** x40 and **(b)** x200 magnification. [Color figure can be viewed in the online issue, which is available at www.aott.org.tr]

The specimens, 1 cm segments of the previously exposed tendons, were dehydrated through a graded series of ethanol and embedded in paraffin wax in order to prepare 10 sections with a thickness of 5 to 7 μm.[5] The sections were stained with hematoxylin/eosin and trichrome. Fibrosis intensity, density of blood capillaries in the tendon tissue and distribution of inflammatory cells were evaluated under a light microscope in order to determine the degree of adhesion. According to the extent of tendon surface involvement, adhesions were classified as severe (>66%), moderate (33 to 66%), mild (<33%) or absent.[4] Of each specimen, the section exhibiting the highest degree of adhesion was used for statistical evaluation.[3] Statistical analysis between and within groups was performed using the chi-square test with a significance level of less than 0.05.

Results

No signs of wound infection were present in any of the animals. The clinical examination did not indicate the presence of contractures or limitations in the hind limbs of the rabbits. Moreover, there was no significant

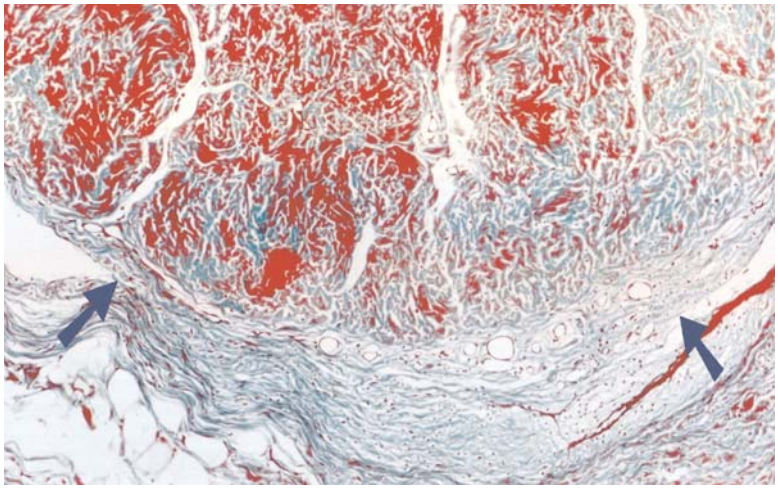

Fig. 4. Microscopic image of a moderate degree adhesion with trichrome staining under x100 magnification. [Color figure can be viewed in the online issue, which is available at www.aott.org.tr]

Table 1. Degrees of adhesion in animals sacrificed 3 weeks postoperatively tested with Pearson's chi-square.

	Absent	Mild	Moderate	Severe
Control (Group 1)	3	16	1	-
Non-irrigated (Group 2)	-	15	5	-
Irrigated (Group 3)	-	14	6	-

p=0.047

Table 2. Degrees of adhesion in animals sacrificed 6 weeks postoperatively tested with Pearson's chi-square.

	Absent	Mild	Moderate	Severe
Control (Group 1)	5	15	-	-
Non-irrigated (Group 2)	-	18	2	-
Irrigated (Group 3)	-	17	3	-

p=0.011

difference between pre- and postoperative ankle ROM. Histological evaluation revealed mild or moderate degrees of adhesion in all tendons exposed to air for 60 minutes (Figs. 3 and 4).

Results of the histological examination of the 60 tendons evaluated 3 weeks postoperatively are shown in Table 1. There was no significant difference between the non-irrigated group (Group 2) and the irrigated group (Group 3). However, the incidence of adhesion formation was significantly higher in these two groups when compared to the control group (Group 1) (p=0.047).

Sixty tendons of the remaining 30 rabbits were evaluated in the same manner (Table 2). Again, while no significant difference could be found between Group 2 and Group 3, the difference between these two groups and the control group was statistically significant (p=0.011).

Statistical analysis did not reveal any significant differences between specimens obtained at 3 and 6 weeks, irrespective of irrigation (p=0.25) (Tables 3 and 4).

Discussion

Although exposure of the tendon surface to air may occur in a wide range of orthopaedic surgeries, irrigation of the tendon in an attempt to prevent subsequent adhesion development is often neglected. The present study investigated whether or not histopathological changes of the tendon surface following exposure to air might be prevented by irrigation. Mitchell and Shepard[6] and Abrahamsson[7] reported harmful effects of air exposure on cartilage tissues and tendons, respectively. In both studies, results were obtained from short-term microscopic and biochemical evaluation. While Abrahamsson observed a decrease of at least 50% in the tendon's protein and collagen synthesis,[7] Mitchell and Shepard reported that exposure to air for

Table 3. Comparison of early and late outcomes in the non-irrigated group.

	Absent	Mild	Moderate	Severe
3 weeks (early)	-	15	5	-
6 weeks (late)	-	18	2	-

p=0.25

Table 4. Comparison of early and late outcomes in the irrigated group.

	Absent	Mild	Moderate	Severe
3 weeks (early)	-	14	6	-
6 weeks (late)	-	17	3	-

p=0.25

60 minutes was adequate for complete necrosis of the cartilage tissue.[6] In contrast, Speer et al. exposed cartilage tissue to air for 3 hours and did not observe any necrotic or degenerative changes 6 weeks postoperatively.[8] In the present study, air exposure did not increase clinically apparent adhesion formation.

Biomechanical studies established that adhesions forming around a tendon increase gliding resistance. Fu et al. induced adhesions in chicken flexor tendon pulleys and observed a significant decrease in flexion angles while gliding resistance significantly increased.[9] In a biomechanical study, Zhao et al. reported increased gliding resistances in repaired flexor tendons exhibiting a gap.[10]

It has previously been shown that immobilization increases the degree of adhesions.[11,12] The absence of immobilization might account for the relatively low rates of severe adhesions in our study. However, air exposure occurs during many orthopaedic procedures, which may not require immobilization after the operation. Nevertheless, as we did not administer immobilization in the control group, we did not examine the effect of immobilization on adhesion formation in the air exposed groups.

Saline irrigation is currently considered the most common way to protect exposed tendons from external effects. Saline solution has served as a gold standard in clinical studies and no adverse effects have been reported to date. However, in our own experience, irrigation with saline was not effective in protecting the tendon from fibrosis formation. We, therefore, neither encourage nor discourage its use as a preventive method in this context.

In conclusion, exposure to air for more than 60 minutes should be regarded as a risk factor for tendon fibrosis. Time-based studies are needed in order to determine when changes in physiologic tendon properties begin to occur and under which circumstances these changes become clinically evident.

Conflicts of Interest: No conflicts declared.

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