The effect of organic silicon injection on Achilles tendon healing in rats

Arel GERELİ[1], Umut AKGÜN[1], Serap USLU[2], İsmail AĞIR[3], Filiz ATEŞ[4], Ufuk NALBANTOĞLU[1]

[1]Department of Orthopedics and Traumatology, Acıbadem University, Faculty of Medicine, Istanbul, Turkey;
[2]Vocational School of Health Services, Acıbadem University, Istanbul, Turkey;
[3]Department of Orthopedics and Traumatology, Adıyaman University, Faculty of Medicine, Adıyaman, Turkey;
[4]Institute of Biomedical Sciences, Boğaziçi University, Istanbul, Turkey

Objective: The aim of this study was to assess the efficacy of a soluble absorbable silicon compound on healing of the Achilles tendon.

Methods: The Achilles tendons of 21 Wistar albino rats were cut and repaired. A 0.01 ml organic silicon solution (silanol) was injected peri/intratendinously into the left leg of all rats and the same dose of saline into the right leg postoperatively. Rats were randomly divided into 3 groups for biomechanical testing on Day 10 (7 rats) and Day 20 (7 rats) and histological and immunohistochemical assessment on Day 20 (7 rats). Fibroblast cell count and diameter, tissue vascularity and blood vessel diameter were evaluated by histomorphometry. Basic fibroblast growth factor (bFGF) immunoreactivity was analyzed with immunohistochemistry on Day 20. Failure load and stiffness of the repaired tendons were measured on Days 10 and 20.

Results: The number of fibroblasts per area, average fibroblast diameter, number of vessels parallel to collagen bundles per area and average vessel diameter were significantly higher in the organic silicon group than in the control group (p<0.05). Strong immunoreactivity of bFGF in the silicon group was detected. Failure load was significantly higher in the silicon group than in the control group on Day 10 (p=0.041). On Day 20, while a difference still existed, this difference was not significant. There was no effect of the silicon injection on stiffness of healing tendons.

Conclusion: Organic silicon appears to have a positive effect on tendon healing and is suitable for further studies on host healing response modification.

Key words: Achilles tendon; organic silicon; tendon healing.

Tendon injuries are a common clinical condition. Even when repaired, the healing process of an injured tendon takes a long time and some sequelae usually remain. It has been reported that mobilization in the early period of healing resulted in improved tensile properties of the repair site, enhanced gliding function and reduced morbidity.[1-4] Since the tendon should be able to withstand early active mobilization, the initial strength of the healing tissue is important. Therefore, most research to date has focused on the stimulation of tendon healing in the early period.

In an attempt to better understand tendon healing

mechanisms and to improve the healing properties, researchers have investigated a broad range of factors shown to affect tendon healing, such as growth factors, mesenchymal stem cells, cytokines,[2,5] gene-therapeutic approaches,[6] sodium hyaluronate,[7,8] platelet concentrate,[9] anticoagulants[10] and hyperbaric oxygen.[11] However, as of yet, no current gold standard related to improved tendon healing with exogenous substances has been established.

Silicon compounds are currently used for such medical purposes as prosthetic replacement and scar treatment.[12-14] They have also been implicated as important for collagen synthesis in both skin/fibroblast[15-19] and bone/cartilage.[12-15,18-21] Soluble silicon (organic silicon) stimulates collagen Type 1 synthesis in human osteoblast-like cells and skin fibroblasts by modulating prolyl hydroxylase activity, which is an important catalyzer for collagen production.[18] A positive correlation has been shown between the serum silicon concentration and the collagen concentration in cartilage, which suggests the involvement of silicon in the formation of extracellular matrix components.[15] In addition, it has been suggested that silicon increases the basic fibroblast growth factor (bFGF) level,[16] which may stimulate cell proliferation, angiogenesis and collagen synthesis.[3,22-24] To the best of our knowledge, no study related to silicon effect on the tendon healing response has been reported to date.

The aim of this study was to assess the efficacy of a soluble absorbable silicon compound on tendon healing in terms of the number and diameter of fibroblasts and blood vessels, bFGF immunoreactivity and load at failure and stiffness.

Materials and methods

The study included 21 8-month old female Wistar albino rats weighing between 350 and 450 grams. The left legs of all rats were prepared as the silicon group (21 tendons) and the right legs as the control group (21 tendons). Power analysis with an effect size of 1, α error probability of 0.05, β error probability of 0.20 and power 0.8, indicated a sample size of 6 rats in each group.[25] Rats were randomly divided into three groups for biomechanical testing on Day 10 (7 rats) and Day 20 (7 rats) and histological and immunohistochemical assessment on Day 20 (7 rats). Consent was given for the use of laboratory animals by the Research Ethics Commission for animal experiments (Marmara University, no. 17.12.2009-60.2009.mar). Animal care complied with the principles of laboratory animal care and international laws regarding the care and use of laboratory animals.

Anesthesia was performed using an intraperitoneal

injection of sodium thiopental (50 mg/kg Pentothal; Abbott Laboratories, Abbott Park, IL, USA). A preoperative dose of intramuscular cefazolin sodium (0.1 mg/kg Cefozin; Bilim İlaç, Istanbul, Turkey) was administered for infection prophylaxis. Rats were positioned prone on the operating table and both hind limbs of each rat were prepared with a povidone-iodine (Betadine®) solution. A posterior longitudinal skin incision of 2 cm was made in both legs sequentially. The Achilles and plantaris tendons were exposed and full-thickness transverse tenotomy with a number 15 blade was made at a point 0.5 cm proximal to the distal insertion of the tendons. The Achilles tendon was repaired using a modified Kessler technique and 5/0 monofilament polypropylene (Prolene®; Ethicon Inc., Bridgewater, NJ, USA) sutures. Tendon sheaths were not repaired. The skin was then sutured using 4/0 monofilament polypropylene sutures. A separate suture was used as a skin marker indicating repair level in order to standardize the injection site. Wound dressings or casts were not used postoperatively. Paracetamol tablets were added into the tap water to control pain.

Animals had free access to tap water and standard laboratory chow. They were housed in wire-topped cages in groups of four and 12-hour light/12-hour dark cycle. To achieve the comparison of silicon and saline-injected tendons on the same rat, all left legs were prepared as the silicon group (n=21) and all right legs as the control group (n=21). Silicon injection was started at the end of the inflammatory phase on Day 3.[26] A 0.01 ml sterile, soluble, absorbable silanol solution (Conjonctyl®; Sédifa Laboratoire, Monaco) was injected at the marked level peri/intratendinously on postoperative days 3, 5, 7, 9, 11, 13, 16, and 19 in each left leg for the silicon group using a 26G syringe. The same dose of sterile saline solution was injected on the same days and in the same manner in each right leg for the control group. Rats were randomly divided into 3 groups of 7 rats/14 tendons. Rats were sacrificed on postoperative Day 10 and 20 for biomechanical testing and on Day 20 for histological and immunohistochemical assessment. No signs of systemic toxicity or injection site infection were seen in any of the animals during the study period.

Animals were anaesthetized with an intraperitoneal injection of 50 mg/kg sodium thiopental for the histology group. Healing tendons were removed at both ends by transverse sharp dissection while the rats were still alive and under anesthesia. After tendon removal, animals were sacrificed with a lethal dose of pentobarbital (150 mg/kg). Fresh tendons were immediately fixed in 10% neutral-buffered formalin for 24 hours at room

temperature and processed for embedding in paraffin wax. Serial sections, 5 μm thick, were cut using a microtome (Thermo Scientific Shandon; Fisher Scientific UK Ltd., Leicester, UK). All sections were stained with hematoxylin-eosin for histomorphometric analyses and Masson's trichrome for the detection of collagen fibers.

Histomorphometric analysis was undertaken with hematoxylin-eosin and actin-stained sections using the semiautomatic UTHSCSA (The University of Texas Health Science Center at San Antonio) Image Tool for Windows v1.28 software. Five serial sections for each paraffin block were used as samples. Five microscopic areas adjacent to the repair/injection zone from each serial histological section were evaluated. Areas found to be hypervascular and partly containing a thin vessel at the periphery were selected in every sample for standardization of the histomorphometric working area. The number of fibroblasts, the diameter of fibroblasts, the number of blood vessels parallel to the collagen bundles, and the diameter of vessels were all measured, recorded and statistically compared in both silicon and control groups.[27,28] Measurements were performed by two independent researchers, who were blind to the experimental groups. A fibroblast located between the collagen fibers, having an abundant and irregularly branched cytoplasm with an ovoid, large and pale-staining nucleus, fine chromatin and a prominent nucleolus was defined as active.[27]

Serial sections 2-μm thick were cut using a microtome in each paraffin block and used as samples in immunohistochemistry analysis. The microscopic areas ad-
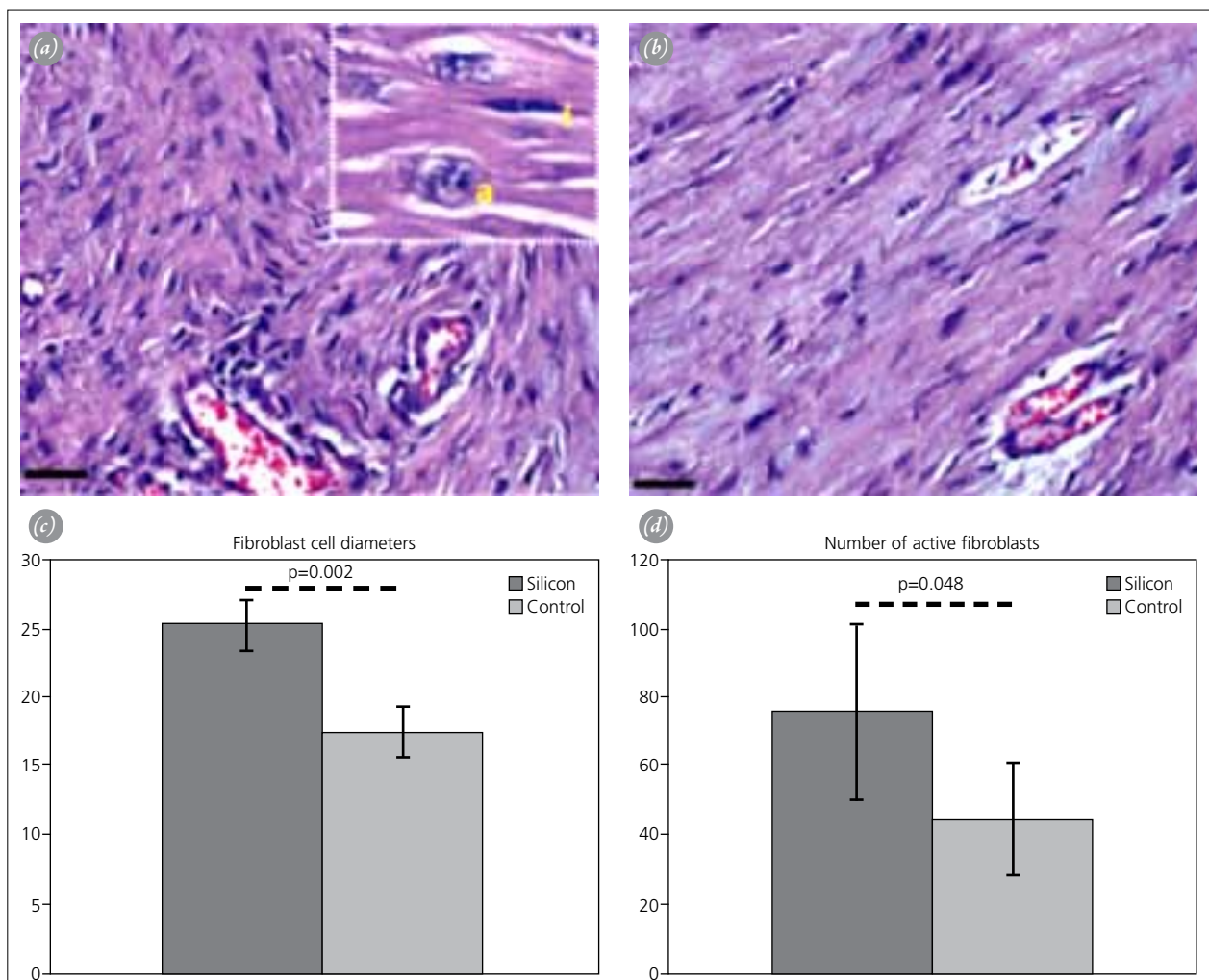

Fig. 1. An increased infiltration of active fibroblasts per area was observed with morphometric analysis of the tendon repair site in **(a)** the silicon group on Day 20 compared with **(b)** the control group (HE, x200). A magnified example of active (a) and inactive (i) fibroblasts is shown in Fig. 1a. (HE, x400). Scale bars showing the **(c)** fibroblast cell diameters and **(d)** number of active fibroblast data in both groups. Fibroblast cell diameter data: silicon: 25.3±1.6 Å, control: 17.49±1.8 Å. Number of active fibroblasts; silicon: 76.5±25.4, control: 45.5±16.1. The difference between groups for both measurements was significant. (Scale bars, 100 Å). [Color figure can be viewed in the online issue, which is available at www.aott.org.tr]

jacent to the repair/injection zone from each histological section were evaluated. Areas found to be hypervascular and partly containing a thin vessel at the periphery were selected in every sample for standardization of the immunohistochemistry working area. The streptavidin-biotin method was used for immunohistochemistry in the fully automatic BenchMarkXT (Ventana Medical Systems Inc., Tucson, AZ, USA) platform. Sections were incubated with smooth muscle actin primary antibody (Novocastra; 1:100) and bFGF (primary antibody: bFGF Abcam; 1:100) for 40 minutes at 24°C. Sections were again incubated with biotinylated secondary antibody (iVIEW DAB Detection Kit; Ventana Medical Systems Inc., Tucson, AZ, USA) for 4 minutes and streptavidin conjugated to horseradish peroxidase (iVIEW DAB Detection Kit) for 8 minutes. After incubating diaminobenzidine (iVIEW DAB Detection Kit)

for 8 minutes, copper (iVIEW DAB Detection Kit) was applied for mordanting. All sections were stained with Hematoxylin II (Ventana Medical Systems Inc., Tucson, AZ, USA) for 12 minutes. After washing with tap water, sections were dehydrated through a graded ethanol series, cleared in xylene, and mounted with the embedding agent Consul-Mount (Fisher Scientific UK Ltd., Leicester, UK). All slides were evaluated using a conventional light microscope (Olympus BX51; Olympus America Inc., Center Valley, PA, USA). bFGF immunoreactivity in the repair tissue was evaluated according to the distribution and graded as absent, few, moderate or strong. Evaluation was performed by two independent researchers blinded to the experimental groups.

For mechanical testing, tendons were harvested with the calcaneus on one end and a portion of muscle at the other. The diameter of the Achilles tendons were mea-
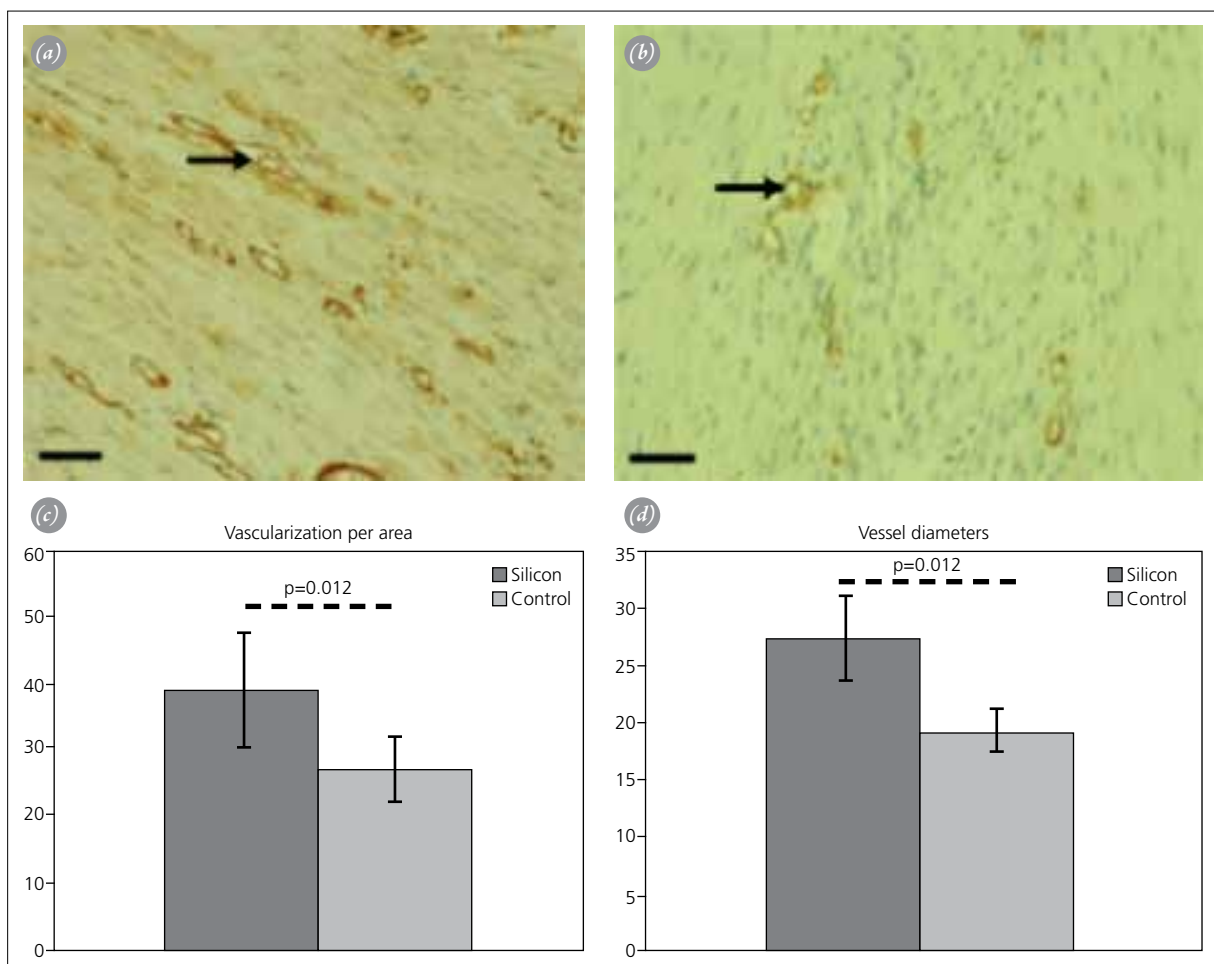

Fig. 2. Vascularization per area and diameter of vessels in **(a)** the silicon group were found to be significantly increased when compared to **(b)** the control group. Scale bars showing the **(c)** vascularization per area and **(d)** vessel diameter data in both groups (smooth muscle actin immunohistochemistry, x200). Vascularization per area; silicon: 39±8.7, control: 27.2±4.9. Vessel diameter; silicon: 27.3±3.7 Å, control: 19.4±1.8 Å. The difference between groups for both measurements was significant. (Scale bars, 100 Å). [Color figure can be viewed in the online issue, which is available at www.aott.org.tr]

sured with a digital sliding caliper and recorded. Tendons were wrapped in saline-soaked gauze, placed into a container, and stored at -10°C until testing. Tendons were completely thawed and all sutures were removed before testing. The proximal and distal ends of the tendons were secured between two strips of sandpaper by quick-setting superglue (UHU Super Glue; UHU GmbH & Co. KG, Bühl, Germany) and mounted on the crossheads of a material testing machine (LF Plus; Lloyd Materials Testing, West Sussex, UK). The machine was calibrated to secure equal tendon lengths in each session. Tendons were loaded at a strain rate of 10 mm/min. Load at failure and stiffness were recorded and statistically compared in both silicon and control groups.

Data was analyzed using the NCSS (Number Cruncher Statistical System) 2007 and PASS 2008 Statistical Software (Utah, USA) packages. The Mann-Whitney U-test and Student's t-test were used to evaluate the significance of differences. Differences of p<0.05 and 0.01 were considered statistically significant.

Results

An increased number of active fibroblasts per area was observed with morphometric analysis of the tendon repair site for the silicon group on Day 20 (silicon: 76.5±25.4, control: 45.5±16.1; p=0.048). Fibroblast cell diameters were statistically higher in the silicon group (silicon: 25.3±1.6 Å, control: 17.49±1.8 Å; p=0.002) (Fig. 1). Active fibroblasts reside in close association with collagen bundles, where they lie parallel to the long axis of the fiber. Inactive fibroblasts are smaller and elongated (Fig. 1a). Vascularization per area (silicon: 39±8.7, control: 27.2±4.9; p=0.012) and the diameter of the vessels (silicon: 27.3±3.7 Å, control:
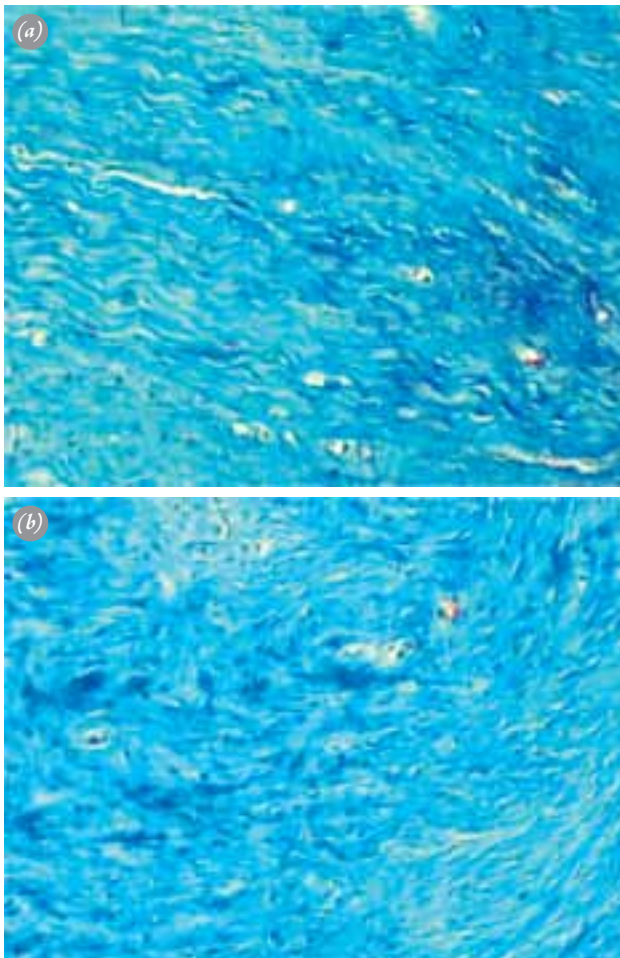

Fig. 3. The collagen fibrils around the active fibroblasts in the **(a)** silicon group exhibited better undulation and organization compared with **(b)** the control group. (Masson's trichrome, x100). [Color figure can be viewed in the online issue, which is available at www.aott.org.tr]
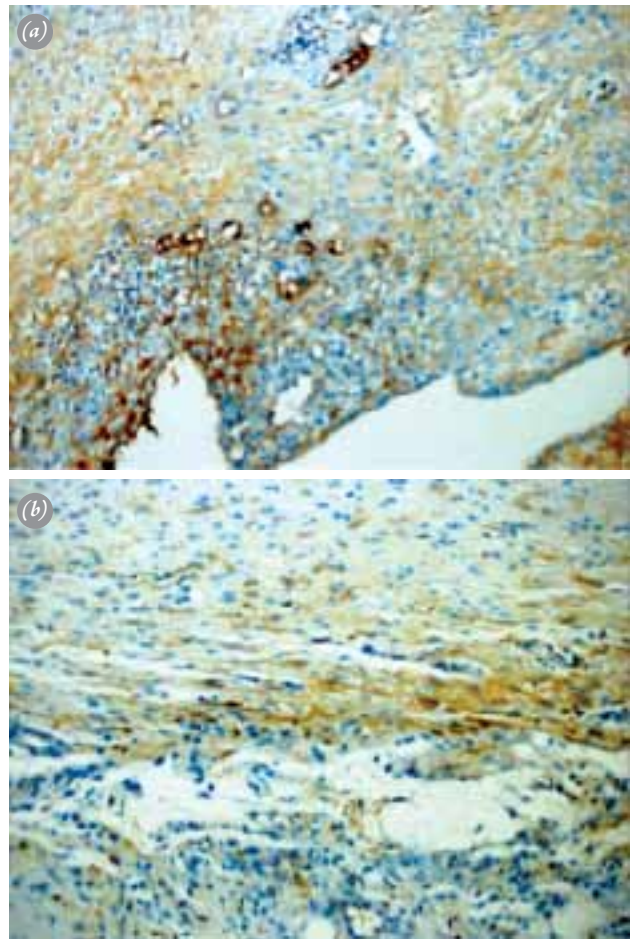

Fig. 4. Basic fibroblast growth factor (bFGF) immunoreactivity in the repair tissue was strong in **(a)** the silicon group when compared to **(b)** the controls that showed few immunoreactivity (primary antibody: bFGF Abcam; 1:100. HE, x200). [Color figure can be viewed in the online issue, which is available at www.aott.org.tr]

19.4±1.8 Å; p=0.002) were also significantly increased in the silicon group when compared to the control group (Fig. 2). Collagen fibrils around the active fibroblasts in the silicon group exhibited better organization (Fig. 3). Granuloma formation was not observed at the injection area of the healing tendons.

bFGF immunoreactivity in the repair tissue was strong in the silicon group in comparison to the control group which showed few immunoreactivity on Day 20 (Fig. 4).

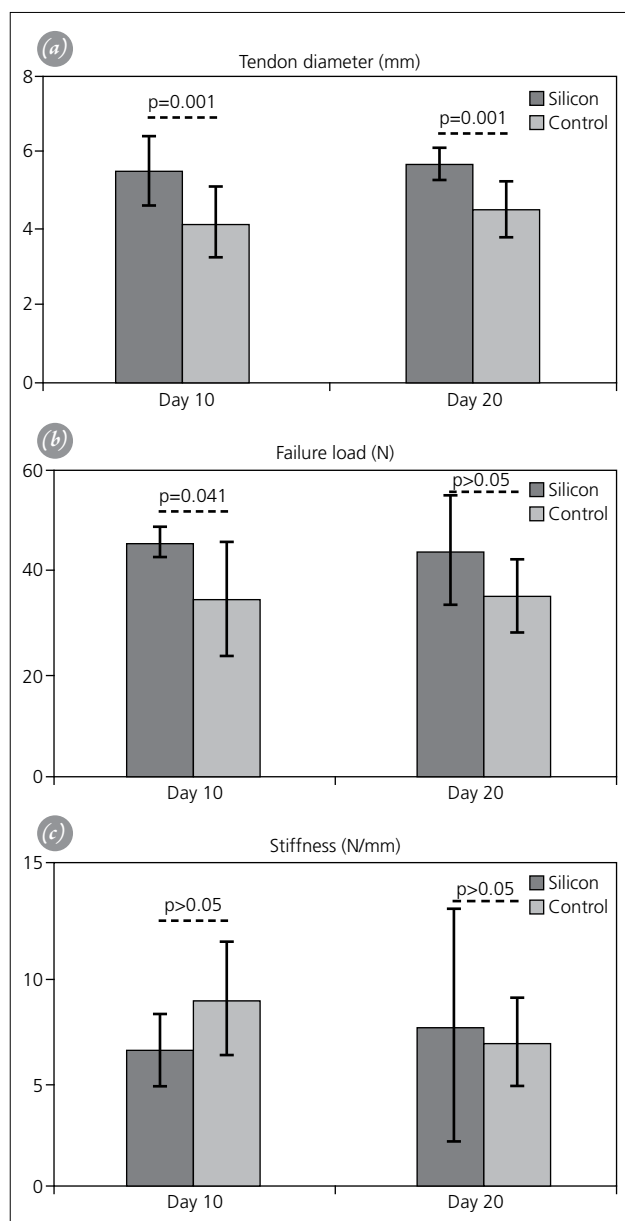The diameter of the healing tendon in the silicon group was statistically higher on Day 10 (silicon: 5.5±0.9

Şekil 6. Achilles tendons from the same rat. Silicon group tendons were thicker than control tendons. [Color figure can be viewed in the online issue, which is available at www.aott. org.tr]

mm, control: 4.2±0.9 mm; p=0.001) and Day 20 (silicon: 5.7±0.4 mm, control: 4.5±0.7 mm; p=0.001) (Figs. 5a and 6). The healing tendons in the silicon group had significantly better failure loads than the control group on Day 10 (silicon: 45.4±5.7 N, range: 33.6 N to 50.7 N and control: 34.7±10.8 N, range: 17.7 N to 47.6 N; p=0.041). The difference between the two groups was not significant on Day 20 (silicon: 44±22.9 N, range: 28.1 to 94.2 N) and control: 35.4±14.7 N, range: 13.9 N to 60.9 N; p=0.42) (Fig. 5b). The failure site in all samples was in the tendinous portion of the healing tendon. There was no statistically significant difference between the two groups in terms of stiffness on Day 10 (silicon: 6.4±1.6 N/mm, control: 8.7±2.6 N/mm; p>0.05) and Day 20 (silicon: 7.5±5.4 N/mm, control: 6.8±2 N/mm; p>0.05) (Fig. 5c).

Discussion

Silicon compounds are widely used for medical purposes. Due to their biocompatibility, they are appropriate for use in prosthetic replacement and scar treatment. Silicon (Si) atoms act as bridging elements for the constitutive elements of connective tissue via siloxanic (Si-O-Si) links.[12-14,29,30] Organic silicon compounds are in a different class than other silicon compounds, as they comprise silicon-carbon bonds and methyl elements. Due to their characteristics, organic silicon compounds are soluble and absorbable compounds, and they may join the blood flow and penetrate into the cells.[12,13,15,18] Silanols are one of the most studied compounds in this group. These compounds are biologically active and the absorption of silicon is considerably increased when provided as a silanol.[12-17]

Fig. 5. **(a-c)** Mechanical data at Days 10 and 20.

During the proliferative/repair stage of tendon healing, a noticeable increase in proliferation of fibroblasts takes place. These active cells both synthesize and reabsorb collagen.[26] In the present study, fibroblast number and size were significantly increased in the silicon group and the histological fibroblast appearance was active. In addition, increased blood supply enhances the repair process.[28] In this study, vascularization per area and diameter of vessels were significantly increased in the silicon group. These findings can be considered to be positive effects on the early period of the healing process.

The role of growth factors in tendon healing has been well described.[24,26] Studies have shown that bFGF is a powerful stimulator of angiogenesis and a regulator of cellular migration and proliferation.[22-24] Hanasono et al. suggested that silicon increases bFGF level in fibroblast cell culture.[16] In the present study, bFGF immunoreactivity in the repair tissue was strong in the silicon group, which may indicate stimulation of angiogenesis and cellular proliferation. In addition to increased and active status of fibroblasts, increased vascularity and increased immunoreactivity of bFGF along with the parallel organization of collagen bundles with parallel orientation of fibroblasts and vessels in the silicon group indicated an improved healing rather than a reactive fibrotic response (Fig. 3).[31] These findings may be considered indicators of the stimulation of the healing process. The silicon may influence the tissue repair process through a mechanism cell proliferation and angiogenesis with increased bFGF production.

In the present study, parameters were measured 10 and 20 days after injury as the early stimulation of tendon healing may increase the resistance against mechanical loading at earlier stages. In addition, because parameters had not yet reached the optimal level within 20 days, the effects of the silicon could therefore be studied. Organic silicon provided better failure load on Day 10 and reached the normal tendon values given in the literature.[25] On Day 20, failure load of silicon-injected tendons were still high, but the gap between the two groups started to decrease with the improvement of failure load values in the control group tendons. A possible explanation of this equalization may be that the advancing healing process provided better mechanical properties to the control group tendons. On the other hand, there were no statistically significant effects of organic silicon on the stiffness of healing tendons. Increased tendon diameter shall increase the failure load.[32,33] Findings suggested that the silicon injections improved tendon strength with increased diameter (Fig. 6). However, although failure load increased at both Day 10 and 20,

the improvement in the mechanical properties of healing tendon was not significant. With the progression of the healing process, alteration in collagen composition can affect the mechanical properties of a tendon.[33,34] There was no statistically significant difference between the two groups in terms of stiffness in either time point and the values were very close. It can be said that the organic silicon injections has no harmful effect on the stiffness of the healing tendon. The observations concerning the total changes in tendons throughout the experiment look promising. It may also be necessary to determine if these supportive effects of silicon are long-lasting.

Cross-sectional area measurement of tendons remains controversial. Precise measurements of a tendon cross-sectional area will provide a better understanding of tendon mechanical properties. Enhanced mechanical tendon properties may be related to increased collagen content. Collagen fibrils with large diameters may positively affect the biomechanics because of the greater possibility of cross-links. Fibril diameter measurement with electron microscopy is a valuable method for the evaluation of tendon fibril morphology.[33] In the light of this knowledge, further study of the effects of organic silicon on collagen fibrils using electron microscopy is necessary.

While organic silicon compounds are not carcinogenic, they are minimally antigenic and do not support bacterial growth. However, different types of silicon compounds may have different potentials for complications. A local inflammatory reaction and granulomas following the silicon injection are well known complications.[35] No granuloma formation was observed in our study. Small amounts of purified silicon, injected using the microdroplet technique, may prevent these complications.[35]

There were several limitations to the present study. Although organic silicon compounds have a positive effect on healing, the exact mechanism is unknown and the number of studies into the background of organic silicon's effect on tissue healing is limited. In this study, a single organic silicon application protocol was performed. The effect of different dose protocols on tendon healing is not clear and the application's long-term effects have not been studied. Due to the irregular shape of the tendon, the precise measurement of the cross-sectional area and typical parameters describing the tendon mechanical properties could not be obtained. An objective methodology for the evaluation of collagen organization may provide more precise results.

In conclusion, this was the first study in the literature addressing the effects of organic silicon compounds

on tendinous tissue healing response. Silicon's effect on bFGF levels has been shown in vitro and this study also suggested similar effects on the tendinous tissue in vivo. Organic silicon is suitable for further experimental studies on host healing response modification. The histological and biomechanical improvement in the repair process caused by organic silicon use appears to depend on the enhancement of bFGF production, fibroblast proliferation and angiogenesis of the repair process in the early period.

Conflicts of Interest: No conflicts declared.

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