Shock Wave-Enhanced Neovascularization at the Tendon–Bone Junction: An Experiment in Dogs

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The purpose of the research was to study the phenomenon of neovascularization at the Achilles tendon–bone junction after low-energy shock wave application. The study was performed on eight mongrel dogs. The control specimens were obtained from the medial one-third of the right Achilles tendon–bone unit before shock wave application. Low-energy shock waves of 1000 impulses at 14 kV (equivalent to 0.18 mJ/mm^2 energy flux density) were applied to the right Achilles bone–tendon junction. Biopsies were taken from the middle one-third of the Achilles tendon–bone junction at 4 weeks and from the lateral one-third at 8 weeks, respectively, after shock wave application. The features of microscopic examination included the number of new capillaries and muscularized vessels, the presence and arrangements of myofibroblasts, and the changes in bone. New capillary and muscularized vessels were seen in the study specimens which were obtained in 4 weeks and in 8 weeks after shock wave application, but none were seen in the control specimens before shock wave application. There was a considerable geographic variation in the number of new vessels within the same specimen. Myofibroblasts were not seen in the control specimens. Myofibroblasts with haphazard appearance and intermediate orientation fibers were seen in all study specimens obtained at 4 weeks and predominantly intermediate orientation myofibroblast fibers at 8 weeks. There were no changes in bone matrix, osteocyte activity, and vascularization within the bone. Two pathologists reviewed each specimen and concurrence was achieved in all cases. The results of the study suggested that low-energy shock wave enhanced the phenomenon of neovascularization at the bone–tendon junction in dogs. (The Journal of Foot & Ankle Surgery 41(1):16–22, 2002)

Key words: neovascularization, shock wave, tendon–bone junction

The value of shock wave has been proven clinically in the treatment of fracture nonunions with a 75% success rate (1–3). There is also a positive effect in the treatment of tendinopathies including tennis elbow, painful heel syndrome, and calcifying tendinopathy of the shoulder (1, 4–7). The mechanism of shock wave is not yet known. It has been postulated that shock waves produce microtrauma or microfracture and hematoma formation, which in turn stimulate neovascularization and osteoblast formation and enhance bone healing at the fracture site. It is thought to provoke painful levels of hyperstimulation analgesia at the tendon–bone junction which in turn relieves pain (1, 8, 9). However, the phenomenon of neovascularization after shock wave application has not yet been confirmed. We hypothesize that shock waves enhance bone healing and relieve pain at the tendon–bone junction by increasing neovascularization. To our knowledge there has been no report on the phenomenon of neovascularization enhanced by shock wave application. The purpose of this study was to investigate the effect of shock wave on the phenomenon of neovascularization at the Achilles tendon–bone junction in dogs.

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Materials and Methods

This study was approved by the Institutional Review Board and was performed under the guidelines regarding the care and use of animals in research. This study included eight mongrel dogs weighing between 9 and 12 kg. The dogs were housed in individual cages and were cared for by a veterinarian.

Three separate biopsies of the Achilles tendon–bone unit were taken (Fig. 1). The first biopsy (A) was performed from the medial one-third of the right Achilles tendon–bone unit of the right heel before shock wave application and was used as control. The second biopsy (B) was obtained from the middle one-third of the right

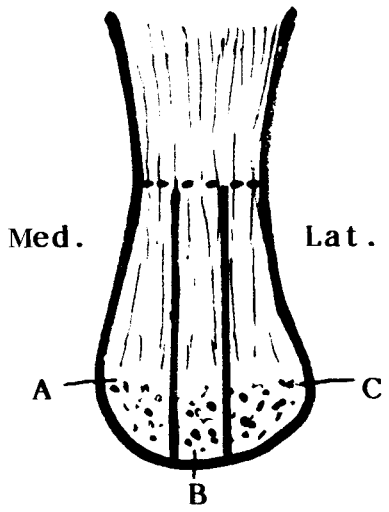

FIGURE 1 The biopsy sketch of the Achilles tendon–bone unit. A indicates biopsy of the medial one-third of the Achilles tendon–bone unit before shock wave application; B indicates biopsy of the middle one-third of the Achilles tendon–bone unit 4 weeks after shock wave application; C indicates biopsy of the lateral one-third of the Achilles tendon–bone unit 8 weeks after shock wave application.

Achilles tendon–bone unit 4 weeks after shock wave application, and the third biopsy (C) from the lateral one-third of the right Achilles tendon–bone unit 8 weeks after shock wave application.

Each dog was anesthetized with intramuscular ketamine (15 mg/kg) and intravenous pentobarbital (30 mg/kg). The right hindlimb was scrubbed and prepared in a standard fashion. A 2.0-cm-long skin incision was made along the Achilles tendon near its insertion into bone. The skin and subcutaneous tissues were carefully reflected and the Achilles tendon was exposed. A 2.0-cm-long specimen that included the medial one-third the Achilles tendon and heel bone was removed. The wound was closed in routine fashion after hemostasis and irrigation.

With the dog still under anesthesia, low-energy shock waves of 1000 impulses at 14 kV (equivalent to 0.18 mJ/mm^2 energy flux density) from the OssaTron orthopedic lithotripter[3] were applied to the Achilles tendon–bone junction of the right hindlimb. The shock wave tube was centered at the Achilles tendon–bone junction and the depth was confirmed by the control guide of the shock wave machine. Surgical lubricant was placed on the skin at the point of contact with the shock wave tube. The frequency of shock wave application was adjusted by the control panel. Immediately after shock wave application, the right heel was examined for redness, edema, swelling, ecchymosis, or hematoma. The hindlimb was covered with bulky dressing. Postoperatively, prophylactic ampicillin (50 mg/kg/day) was given intramuscularly for 5 days.

[3] High Medial Technology, Kreuzlinger, Switzerland.

Four weeks after shock wave application, a biopsy from the middle one-third of the Achilles tendon–bone junction was taken using the same perioperative technique. The same skin incision was utilized and the skin and subcutaneous tissues were dissected to expose the Achilles tendon. A 2.0-cm-long specimen that included the middle one-third of the Achilles tendon and bone was removed. The third 2.0-cm biopsy was performed 8 weeks after shock wave application that included the lateral one-third of the Achilles tendon–bone junction.

The biopsy specimens were kept in formaldehyde solution until microscopic sections were performed. They were later stained with hematoxylin-eosin. The presence of myofibroblasts was confirmed with smooth muscle actin stain. The features of microscopic examination included the number of capillaries and muscularized vessels if present, the presence and arrangement of myofibroblasts, and the changes in bone. The muscularized vessels were distinguished from capillaries by thicker and compact walls. The smooth muscle in the media of these vessels showed positive smooth muscle actin immunostain.

The presence of new capillaries was reviewed in the entire specimen and the number of capillaries in three most representative areas under high power field (200×) was calculated. The average was chosen as the reference number of new capillaries of that particular specimen. Similarly, the number of muscularized vessels was determined from the entire specimen and the average number of the three most representative areas under high power field (200×) was used as the reference number of that particular specimen. The number of new capillaries and muscularized vessels was examined for the control specimens and the study specimens obtained in 4 weeks and in 8 weeks, respectively.

The presence of myofibroblasts was further confirmed with smooth muscle actin stain. The arrangement and orientation of myofibroblast fibers were assessed in a semi-quantitative manner, and were graded as absent, haphazardly arranged, intermediately oriented pattern, or well-oriented fibers. Well-oriented fibers denoted that 75% or more of proliferative myofibroblasts arising from the peritendon were parallel to the direction of constituent collagen fibers in the tendon proper. A haphazard appearance was assigned if the majority of the proliferative myofibroblasts grew in randomly intersecting bundles. Usually they presented with 25% or fewer of myofibroblasts being parallel in orientation to the direction of tendon proper. Intermediately oriented fibers showed an arrangement of myofibroblasts lies between "haphazard" and "well-oriented" fibers. The microscopic features of bone included new bone formation, osteoblast activity, and vascularization. All of the designations were confirmed separately by two pathologists. One of them was one of the investigators of this study (H.Y.H.).

Results

New Capillaries

None of the control specimens showed any evidence of new capillary formation within the collagen fibers and in the peritendineum. The parallel and homogenous collagen fibers with normal fibrocyte distribution within the fibers were noted. There was no extravasation or inflammatory tissue reaction.

New capillaries were noted in all study specimens which were obtained in 4 weeks and in 8 weeks, respectively, after shock wave application. The average number of new capillaries in the study specimens obtained at 4 weeks was 17.3 (6.7–37.3) per high power field (200×). There was a considerable geographic variation in the number of new capillaries within the same specimen as well as an interspecimen variation as high as six times. Most of the new capillaries were located adjacent to the peritendineum. The collagen fibers were mildly thickened and wavy in appearance, but they remained in homogenous and parallel orientation. The fibrocyte distributions appeared normal in most areas. There was no tissue necrosis, inflammatory changes, or fibrinoid degeneration within the collagen fibers or in the peritendineum.

The average number of new capillaries in the study specimens obtained 8 weeks after shock wave application was 16.1 (5–33) per high power field (200×). The size and shape of new capillary seen at 8 weeks were similar to those seen in 4 weeks after shock wave application. The distribution of new capillary varied considerably from location to location in the same specimen as well as an interspecimen variation as high as six times. Most of the new capillaries were noted adjacent to the peritendineum and the adjacent tendon tissues. The collagen fibers were thinner and less wavy and had the appearance of normal tendon fibers. The fibrocyte distribution was normal and there was no tissue necrosis or inflammatory tissue reaction within the collagen fibers and the peritendineum. The details of new capillary formation at the tendon–bone junction after shock wave application are summarized in Table 1. A representative case of new capillary formation after shock wave is shown in Figure 2. It was noted that the phenomenon of enhanced neovascularization was observed as early as 4 weeks after shock wave application and the number of new capillaries remained unchanged up to 8 weeks. The exact time when the neovascularization first occurred and the fate of new capillaries beyond 8 weeks were unknown.

TABLE 1 New capillary formation after shock wave application

Specimens	Number of capillaries[a]	Average (range)
Control	0, 0, 0, 0, 0, 0, 0, 0	0
Study (4 wks)	17.7, 6.7, 7.7, 37.3, 24.3, 10.3, 20.3, 14.3	17.33 (6.7–37.3)
Study (8 wks)	33, 19.3, 10, 31, 6.7, 10.3, 5, 13.7	16.13 (5–33)

[a]200× high power field.

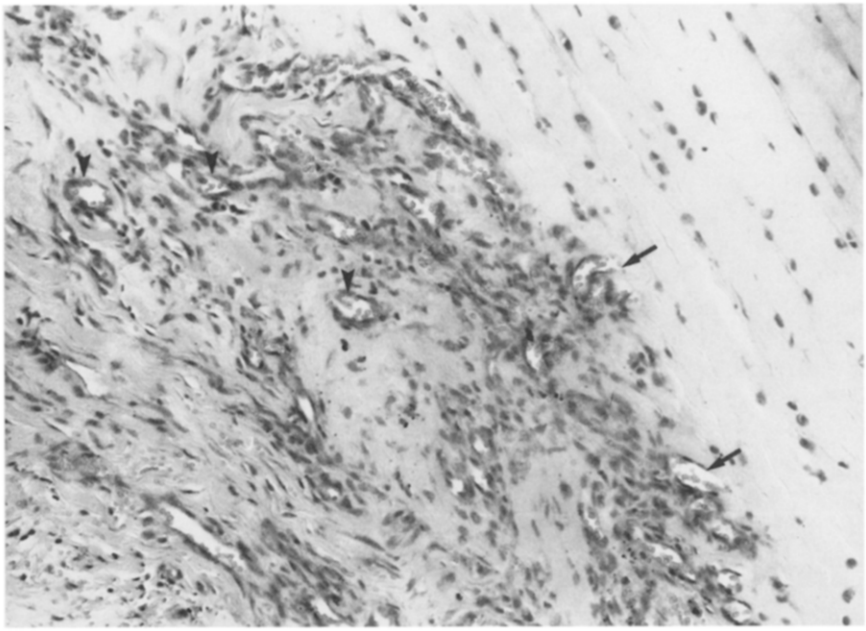

FIGURE 2 The biopsy specimen of the tendon–bone unit 4 weeks after shock wave application demonstrating new capillary within the tendon proper (*arrows*) and the peritendineum (*arrowheads*) (H & E stain ×200).

Muscularized Vessels

None of the control specimens showed any evidence of muscularized vessels but were seen in all study specimens obtained at 4 weeks. The average number of muscularized vessels was 2.75 (1–6) per high power field (200×). There was a considerable geographic variation in the number of muscularized vessel in the same specimen as well as an interspecimen variation. The number of muscularized vessel appeared proportional to the number of new capillaries in the same specimen.

Six of eight study specimens that were obtained at 8 weeks after shock wave application showed muscularized vessels. The average number of muscularized vessels was 4.63 (0–8). There was a considerable geographic variation in the same specimen as well as an interspecimen variation as high as 6-fold. The details of muscularized vessels after shock wave application are summarized in Table 2. A representative case of muscularized vessels after shock wave is shown in Figure 3. It appeared that shock wave application induced young muscularized vessels as early as 4 weeks and most of them persisted up to 8 weeks. However, the exact time when the muscularized vessels first occurred and the fate beyond 8 weeks were unknown. The muscularized vessels were most noticeable in the peritendineum and the adjacent tendon.

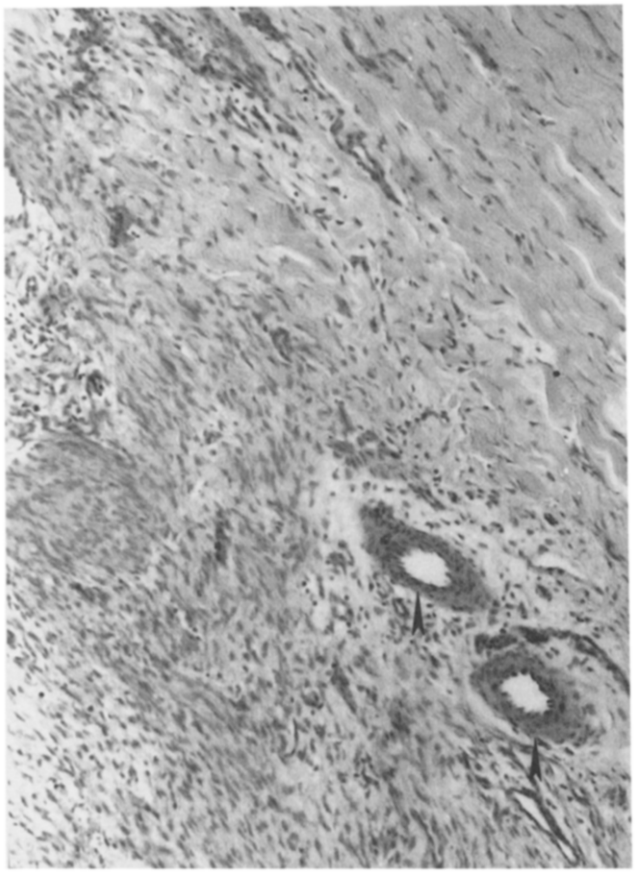

FIGURE 3 The biopsy specimens of the tendon–bone junction 8 weeks after shock wave application demonstrating two muscularized vessels (*arrowheads*) in the interface of tendon proper and the peritendineum (H & E stain ×200).

Arrangement of Myofibroblasts

Myofibroblasts were not seen in any of the control specimens. The control specimens showed mature fibrocytes evenly distributed within the collagen fibers. Myofibroblasts were noted in all study specimens that were obtained in 4 weeks after the shock wave application and the arrangements of myofibroblasts were calculated. Four were demonstrated to be haphazard and four demonstrated an intermediate orientation. These fibers were thicker, heavier, and slightly wavy in appearance, but they remained in parallel and homogenous fashion. None of the specimens showed well-oriented mature collagen fibers. There was no tissue necrosis, fibrinoid degeneration, or inflammatory reaction in the tendon or peritendineum. A representative case of myofibroblast formation after shock wave is shown in Figures 4A and 4B. Myofibroblasts were present in five and absent in three of the eight study specimens that were obtained in 8 weeks after shock wave application. The arrangements of myofibroblasts were haphazard in one and had an intermediate orientation in four specimens. The myofibroblast fibers were lighter and less wavy and they remained in homogenous and parallel fashion similar in appearance to normal collagen fibers. None of the specimens showed well-oriented mature fibers. The details of myofibroblast formation and their arrangements are summarized in Table 3.

TABLE 2 Muscularized vessels after shock wave application

Specimens	Number of vessels[a]	Average (range)
Control	0, 0, 0, 0, 0, 0,0, 0	0
Study (4 wks)	2, 6, 1, 2, 4, 2, 1, 4.	2.75 (1–6)
Study (8 wks)	4, 5, 0, 8, 3, 4, 0, 5.	4.63 (0–8)

[a]200× high power field.

TABLE 3 Arrangements of myofibroblast after shock wave application

	Control (N)	Study 4 wks (N)	Study 8 wks (N)
No myofibroblasts	8	0	3
Haphazard appearance	0	4	1
Intermediate orientation	0	4	4
Well oriented	0	0	0

N = number of specimens.

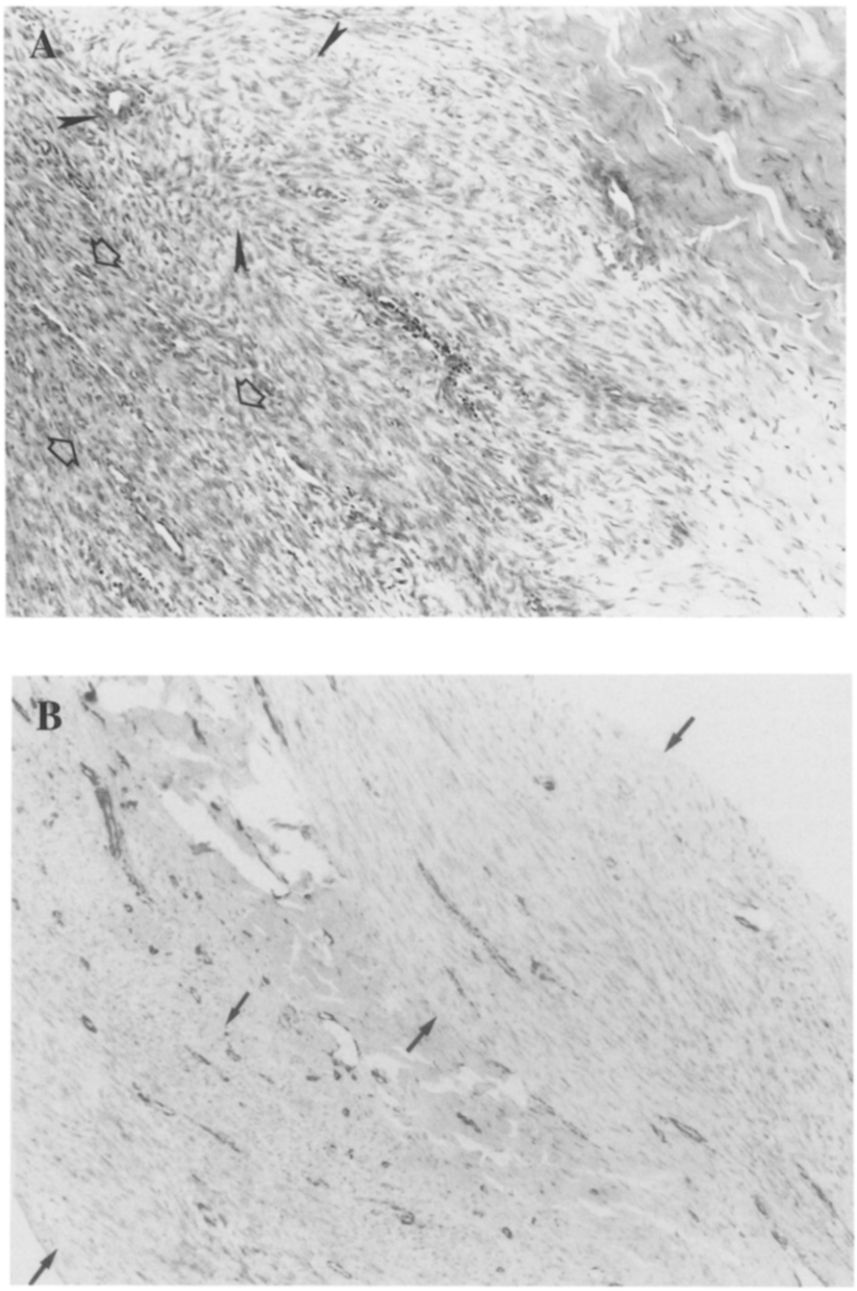

FIGURE 4 *A*, The biopsy specimen of the tendon–bone unit 4 weeks after shock wave application demonstrating the presence of myofibroblasts in intermediate orientation parallel with the tendon proper (*open arrows*), and the haphazard arrangement of myofibroblasts (*arrowheads*) (H & E stain ×100). *B*, Immunohistochemical stain of the same specimen showing diffuse strong reactivity of smooth muscle actin in proliferative myofibroblasts in the peritendineum (*arrows*). The tendon proper in the center, however, was only weakly positive for smooth muscle actin stain (×100).

Bone Tissue

All specimens including the control and the study groups showed normal cancellous bone architecture. There was no evidence of increased osteoblastic activity, new bone formation, or new capillaries within the bone. It appeared that low-energy shock wave had no effect on bone tissue.

Discussion

The etiologies of tendinopathy at the tendon–bone junction, including lateral epicondylitis of the elbow, painful heel syndrome, and tendinopathy of the shoulder, are multifactorial and the exact cause is unknown (6, 10, 11). However, it is generally believed that a degenerative process with an inflammatory reaction may

play an important role. The vascular changes and focal hyaline degeneration in surgical biopsy specimens support the theory of a degenerative, rather than an inflammatory process (1, 7, 10–12). Gould and Korson demonstrated that pathological changes of tenosynovitis of the tendo Achillis included myxomatous degeneration of collagenous tissue, fibrosis, round cell inflammatory infiltrate, and proliferation of fibrovascular connective tissues (13). As a result, there was insufficient evidence to support any method as the best method of treatment for tendinopathy. Recently, shock wave has been used for treatment of certain orthopedic diseases, including nonunion of long bone fractures, refractory lateral epicondylitis, persistent painful heel syndrome, and recalcitrant calcifying tendinopathy of the shoulder, with good clinical results (1–8). The results of shock wave treatment in certain tendinopathies, including lateral epicondylitis, painful heel syndrome, and tendinopathy of the shoulder were equal to or better than the results from the currently available methods of treatment (1, 4–7, 11, 13, 14). In addition, surgical treatment can still be performed after shock wave therapy if necessary, making shock wave treatment an attractive alternative.

It is our opinion that pain arising from tendinopathy is due to hypovascular change with a degenerative process with or without trauma. Surgery can cause an increase of vascularization in the local area; however, it is usually associated with scar tissue formation and may not relieve pain. Some authors make a number of vertical slits in the tendon to "vent" the tendon and encourage in-growth of new blood vessels (13–15). Shock wave relieves the pain of tendinopathy at the tendon–bone junction by inducing neovascularization and improving tissue regeneration (1, 7, 9, 15). The results of this study showed that the phenomenon of neovascularization occurred in all study specimens as early as 4 weeks after shock wave application. There was a considerable interspecimen variation in the number of new capillaries and muscularized vessels. This probably explained the reason why only certain good or excellent clinical results are seen, and why some cases respond clinically better than others. It was speculated that better clinical results were associated with improved neovascularization at the tendon–bone junction.

Despite good clinical results, the mechanism of shock wave treatment remains unknown. It has been postulated that shock wave application may induce or enhance neovascularization, which can improve bone healing at the fracture site and relieve pain at the tendon–bone junction. Rompe et al. have demonstrated dose-related effects of shock waves on rabbit tendo Achillis and suggested that energy flux density of more than 0.28 mJ/mm^2 should not be used clinically in the treatment of tendon disorders (9). In their study, a statistically significant increase of capillary formation was noted with higher energy shock wave (0.60 mJ/mm^2), which also caused more tissue reaction and potential damage to the tendon tissue. It was postulated that the mechanism of shock wave may involve molecular changes such as increased signal transduction of nitric oxide and heat shock protein, which in turn enhance neovascularization (12, 16, 17). The presence of new capillary and muscularized blood vessel is indicative of neovascularization. The muscularized vessel differs from the capillary by the presence of contractile smooth muscle in the vessel wall during the early phase of new vessel formation (12, 15). The results of this study showed that new capillaries and young muscularized vessels were present in all study specimens obtained in 4 weeks and most of these new vessels persisted and proliferated up to 8 weeks after shock wave application. The presence of muscularized vessels appeared to be proportional to the number of new capillaries. The fate of new capillary and muscularized vessels beyond 8 weeks was unknown. However, we speculated that these new vessels would proliferate and mature with time. In addition, the presence of myofibroblasts is further evidence that this cell is the pivotal cellular element for fundamental wound healing and tissue regeneration (15, 16). Myofibroblasts were noted as early as 4 weeks in the study group and the haphazard fibers gradually decreased from 4 to 8 weeks. These findings suggest that shock wave application enhanced the phenomenon of neovascularization at the tendon–bone junction in dogs. However, we cannot say conclusively if the changes seen in our specimens were caused by the shock wave application or the fact that there was surgical intervention to obtain the controls.

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