A Biomechanical Assessment of Tendon Repair After Radiofrequency Treatment

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Abstract

After acute tendon injury, rapid mobilization prevents adhesion and improves the ultimate strength of the repair. Radiofrequency (RF) ablation is proposed to enhance angiogenesis in the early stages of healing.

The mechanism and effect of RF have not yet been described in an animal model of tendon injury. To investigate the biomechanical effect of bipolar RF on acute injury in a rabbit model of partial Achilles tendon transection and suture repair, RF-treated tendon repairs were compared to untreated tendons. Cross-sectional area, Young's modulus, and ultimate tensile strength were determined.

At 6 and 12 weeks after repair, RF-treated tendons had significant increases in cross-sectional area ($P<.0001$, $P<.0001$) and ultimate tensile strength ($P<.0001$, $P = .01$). Young modulus of RF-treated tendons was increased at 6 weeks ($P<.01$) but not at 12 weeks. Compared with untreated tendons, RF-treated tendons showed faster return to mechanical integrity. This may allow earlier rehabilitation.

Tendon injury is a frequent clinical problem. It can result from chronic degeneration (ie, rotator cuff rupture, Achilles tendon rupture), acute trauma (ie, flexor or extensor tendon laceration), and, occasionally, after tendon transfer. Whatever the setting, early surgical repair is important for optimal resting muscle length and function.[1] Despite advances in surgical repair, weakness, or even gap formation can still occur, decreasing resting muscle tension with concomitant atrophy and denervation. Postoperative immobilization also can be associated with adhesions and weaker ultimate tendon strength.[1,2] Thus, there is interest in biological augmentation to improve tendon healing (ie, hand, rotator cuff, lateral elbow, and Achilles) at the time of repair. Improved strength or faster progression through the early phases of tendon healing could shorten the duration of postoperative immobilization and allow patients to start rehabilitation earlier. This would result in less overall disability and potentially better biomechanical functioning of the tendon.[3]

Radiofrequency (RF) ablation is one proposed method of augmenting tendon healing. Plasma "coblation" is a form of RF ablation in which, in an ionic solution and under appropriate voltage and current conditions, localized plasma forms at the tip of the instrument.[4-7] Studies in animal models of tendon repair have shown controlled inflammation and new vessel formation in RF-treated tendons.[8,9] Collagen shrinkage with RF using multiple models, both with and without limb immobilization, shows variable effects on the biomechanical properties of tendon, ligament, and joint capsule.[9-12] In a rat model of supraspinatus healing, there was no statistical difference between RF-treated tendons and controls.[13] In RF microtenotomy clinical studies for lateral epicondylitis, investigators found sustained improvement in symptoms up to 2 years after surgery.[14,15] RF is currently being used with some caution, given reports of chondrolysis after thermal capsulorrhaphy[16-19] and of osteonecrosis after RF during knee arthroscopy.[20]

We conducted a study to investigate the effect of RF on the biomechanics of acute tendon repair in a nonimmobilized rabbit Achilles tendon. The null hypothesis was that there would be no difference between RF-treated and untreated suture repairs for both gross and biomechanical assessments 6 and 12 weeks after surgery.

Materials And Methods

Surgical Methods

Before initiating this project, we obtained approval from the Institutional Animal Care and Use Committee (IACUC) at the University of California San Diego for all procedures and protocols. Forty mature (>1 year old) New Zealand White rabbits were used, 10 in each group of RF-treated and untreated tendon repairs, with analysis at 6 and 12 weeks. Each rabbit received ketamine and xylazine as induction anesthetics, preoperative buprenorphine for analgesia, and enrofloxacin.

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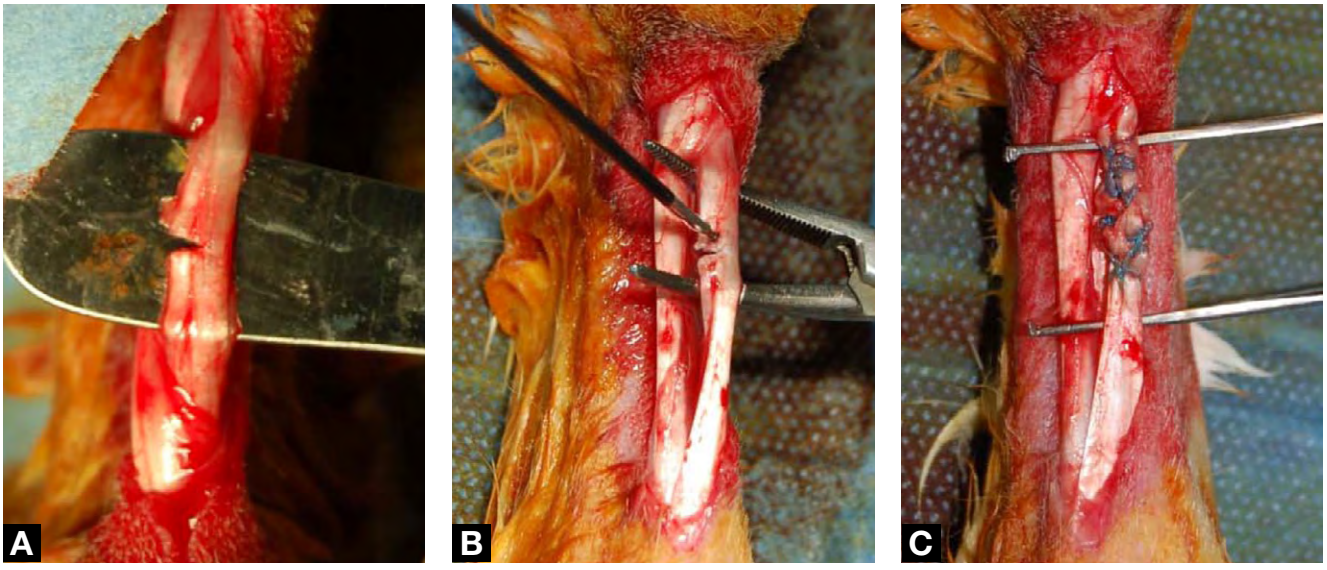

Figure 1. Partial-thickness tenotomy, radiofrequency treatment, and surgical repair of rabbit Achilles tendon. (A) Fifty percent tenotomy is created through middle bundle of rabbit Achilles. (B) Radiofrequency pulse is applied to ends of tenotomy. (C) Tendon after repair with nonabsorbable braided polyethylene suture.

For each surgery, sterile technique was used, the rabbit was positioned prone, and the surgical leg was prepared and draped. To minimize bias from the surgical approach, the surgical leg was alternated between left and right for each successive surgery. A longitudinal incision was made over the Achilles tendon, and the paratenon was identified and incised longitudinally as a separate layer. The 3 bundles of Achilles tendon were identified, and the central bundle was separated bluntly from the medial and lateral bundles. A partial-thickness tenotomy (approximately 50% of tendon bundle width) was created, beginning at the medial aspect of the bundle, 2 cm proximal to the calcaneus (Figure 1A). Each tendon was measured, and photographed using a high-resolution digital camera (Nikon D50), before and after the tenotomy. Postsurgical assessment by image analysis software (ImageJ, National Institutes of Health, Bethesda, Maryland) provided a more accurate ratio of the actual intact-to-transected tendon cross-section. A partial tenotomy of a single tendon bundle allows the rest of the tendon to act as an internal splint for the nonimmobilized repair. In pilot studies, both a complete tenotomy of the central bundle and a nonlocking suture repair resulted in mechanical tendon failure, with suture shredding through the tendon 2 weeks after surgery.

A bipolar RF wand (Topaz; ArthroCare, Sunnyvale, California) set at 500 ms per pulse on setting 4 (180 mV ± 10%), with sterile saline irrigation at 1 mL/s, was used to apply a single RF pulse to each end of the tenotomy, such that each RF-treated rabbit received 2 pulses total (Figure 1B). It was not possible to clinically standardize the applied pulse, so the quality and pressure of each pulse were subjectively graded by the surgeon on a 1-to-5 scale, 1 being a weak pulse with weak pressure and 5 being a strong pulse with stronger pressure and associated tissue color changes. The most commonly recorded value for the pulse was 3. Control rabbits received no RF treatment (untreated). The repair was performed using No. 0 nonabsorbable ultra-high-molecular-weight braided polyethylene suture (Ultrabraid; Smith & Nephew Endoscopy, Mansfield, Massachusetts) in a locking, running Krakow configuration on both ends of the tenotomy. The suture was then tied with the foot in plantar flexion to oppose the ends of the tenotomy (Figure 1C). The paratenon was closed with simple interrupted 3-0 Vicryl sutures, and the skin was closed with running subcuticular 3-0 Vicryl suture.

Postsurgical Care and Tendon Harvest

After surgery, each rabbit was allowed to recover from the anesthesia in a heated recovery chamber under continuous observation. Respiratory rate and heart rate were recorded every 15 minutes until sternal recumbence was achieved. Once recovered, each rabbit was returned to its cage and allowed unrestricted food and cage activity for the duration of the study.

Each rabbit was observed daily the first 2 weeks after surgery, with attention paid to food intake, urine and stool output, incisional healing, and signs of pain. For the rest of the study, each rabbit was cared for daily by the institutional animal care staff and observed weekly by laboratory personnel. All animals were euthanized for tendon harvesting at either 6 or 12 weeks after surgery. The 6-week period was chosen to determine the biomechanical state of the repair during soft-tissue remodeling, and the 12-week period was chosen for biomechanical assessment after expected restoration of tendon architecture. Hind limbs were frozen immediately after harvest and were kept frozen until ready to be prepared for biomechanical testing.

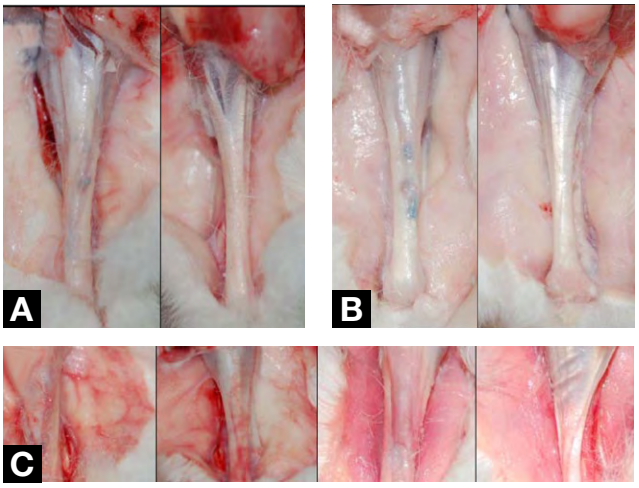

Figure 2. Twelve-week radiofrequency-treated and untreated tendons with contralateral control tendon. At 12 weeks, treated tendon (A) appeared thicker than untreated tendon (B) at repair site. There was no visible difference in peritenon vascularity among treated, untreated, and contralateral control (C) tendons.

Biomechanical Methods

Biomechanical testing was performed in a blinded manner. After the limb was thawed, the central bundle of Achilles tendon was harvested from both the experimental and the contralateral unoperated hind limbs. Whenever possible, the tendon attachment to the calcaneus and the proximal centimeter of the calcaneus were preserved. Fine-line markings were applied axially to the tendon surface above and below the repair with Verhoeff's elastic stain. Cross-sectional areas of the tendons were measured at these locations first by placing the tendon into a custom-built unconfined compression device using a linear displacement transducer (Series 240; Trans-Tek, Ellington, Connecticut) connected to a balanced weight platform with a ball probe. The height of the probe was measured as the probe rested on the tendon in a groove of known width providing the cross-sectional area to an accuracy of 0.1 μm^2. Tendon fixation into the testing device (Model 1122; Instron, Norwood, Massachusetts) was achieved by locking the calcaneus to a fixation jig with a pin. If the calcaneus was damaged during dissection or drilling, the distal portion of the tendon bundle was securely attached to a clamp fixed to the load frame. After the distal portion of the bundle was mounted, a proximal clamp attached to the load cell was lowered to grasp the proximal end of the bundle, which was splayed and reinforced with gauze to reduce crushing stresses at the clamp. The load cell was then raised to a prestress tension of 5 N. An environmental chamber around the fixed tendon was filled with phosphate-buffered saline for complete submersion of the tendon. Changes in tendon length were recorded by a digital video camera with a fixed view of the fine-line markings on the mounted tendon bundle. A video dimension analyzer was connected to the camera and calibrated to the measured cross-sectional area at the fine-line markers. Each tendon bundle was precycled 10 times at 10 mm/min with extension to 5% of the total prestressed length to reduce the effect of hysteresis. Average precycle frequency was 1.59 cycles per minute. The tendon was then stretched at 5 mm/min to the same 5% extension, with video monitoring enabled to establish the stress–strain curve. Nonlinear elastic moduli and viscoelastic properties were calculated from the stress–strain curves. Creep rates were established by reproducing the resultant curve over 5 cycles, stopping for 5 minutes, then repeating for another 5 cycles. After the viscoelastic properties were established, tendon bundles were loaded to failure at 10 mm/min cross-head speed. Ultimate tensile strength, percentage strain, and failure mode were recorded for each tendon. The cross-sectional area at point of failure was then calculated by identifying the exact point of failure on the recorded video and rewinding to the original dimensions at that location before loading, then measuring with the video dimension analyzer. This resulted in an accuracy of 0.05 mm^2 because of the resolution of the video image.

During the load-to-failure assessment, the stress–strain curve was obtained from the recorded force, cross-sectional area, tendon length, and change in tendon length. The prestressed tissue was assumed to be pseudoelastic, as the stress–strain curves gave similar elasticity in tension testing. Young's modulus was then determined for each tendon as the slope of the linear region of the stress–strain curve. Average Young's moduli were compared between the unoperated contralateral controls and the 2 experimental groups at each time point. Average maximum load to failure for experimental limbs was also compared with that for the unoperated contralateral controls and between the 2 experimental groups at each time point.

Statistical Analysis

A priori calculations for sample size were based on a study of RF in a rat model of supraspinatus repair.[13] The investigators in that study found decreased maximum stress for the standard repair at 4 weeks (4.99 N/mm^2) and 12 weeks (3.95 N/mm^2) in comparison with sham operated controls (7.89 N/mm^2 at both time points). For the RF-augmented repair, maximum stress values were 7.05 N/mm^2 (4 weeks) and 8.48 N/mm^2 (12 weeks), with SDs of 0.39 and 0.11 between standard and augmented repairs. Power analysis based on these data determined that 9 rabbits were needed in each group at each time point to achieve 90% power. The number of rabbits per group was increased to 10 to ensure adequate power and to account for potential attrition over the course of the study. With no rabbits being lost to attrition, statistical power of 93.5% would be recorded.

To determine the effects of time and tenotomy, we compared Young's modulus and ultimate tensile strength values between repairs and contralateral controls with 2-way factorial analysis of variance. The

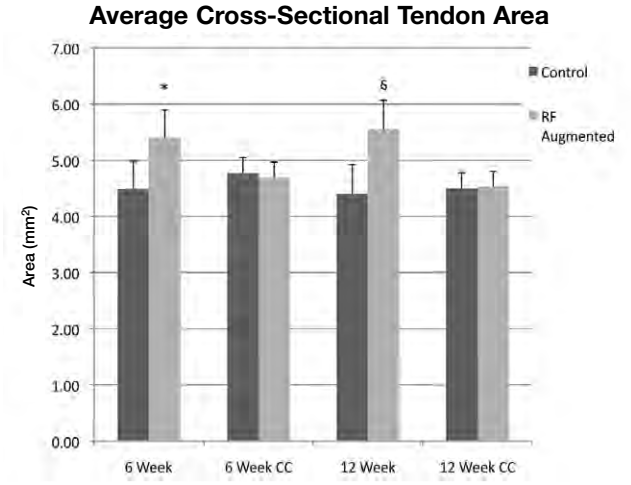

Figure 3. Average cross-sectional tendon area at 6 and 12 weeks. CC indicates contralateral control leg. Bars show mean values; error bars show SDs. Significant difference between radiofrequency-treated and untreated (control) animals at 6 and 12 weeks. *P = .0001, §P<.0001.

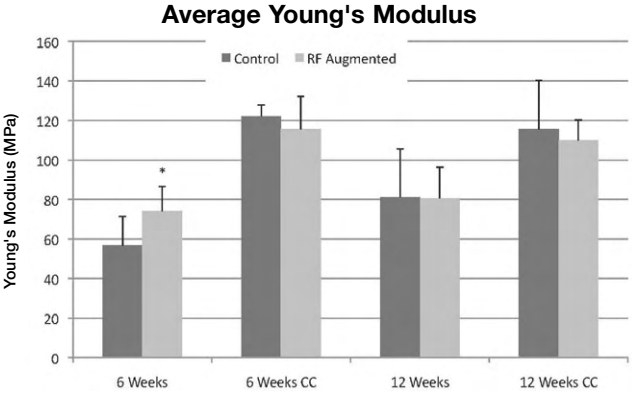

Figure 4. Average Young modulus for radiofrequency-augmented and unaugmented tendons. CC indicates contralateral control leg. Bars show mean values; error bars show SDs. Significant difference between treated and untreated (control) animals at 6 weeks; no difference at 12 weeks. *P = .01.

Fisher protected least significant difference test was used post hoc to assess the difference between repairs at 6 and 12 weeks and differences between RF-augmented and unaugmented groups. For all analyses, P<.05 was considered significant.

Results

Gross Morphology

There was no noticeable difference in gross morphology between the RF-treated tendons and the untreated control tendons at 6 weeks. By 12 weeks, the RF-treated bundles appeared thicker at the repair sites, and paratenon vascularity was similar for all groups and time points (Figure 2A and 2B). This finding was confirmed by cross-sectional area measurements for both the 6- and 12-week specimens (Figure 3). Peritenon vascularity appeared the same for all groups and all time points (Figure 2C).

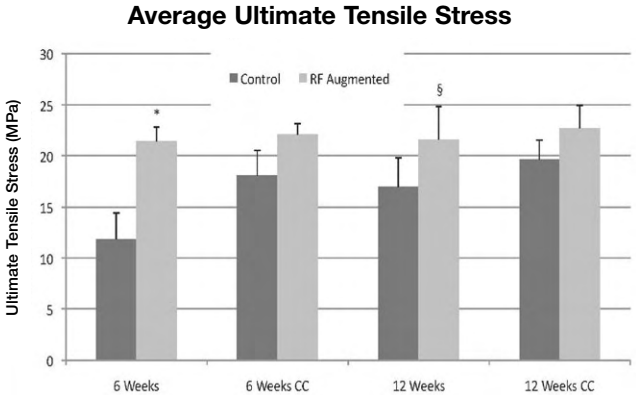

Figure 5. Average tendon ultimate tensile stress at 6 and 12 weeks. CC indicates contralateral control leg. Bars show mean values; error bars show SDs. Significant difference between radiofrequency-treated and untreated (control) animals at 6 and 12 weeks. *P<.0001, §P = .01.

Biomechanical Results

The results of the biomechanical testing are presented in the Table. At 6 weeks, there was a statistically significant increase in Young's modulus for the RF-treated tendon bundles as compared with the untreated control tendon bundles (Figure 4); at 12 weeks, there was no difference in Young modulus. There was also a statistically significant difference between RF-treated and untreated tendons in ultimate tensile strength at 6 weeks that persisted to 12 weeks (Figure 5). The most common site of tendon failure during ultimate tensile stress testing was at the tenotomy site (29/40 failures). The rest of the tendons failed through the Krakow suture outside the tenotomy (8/40) or at the testing clamp (3/40).

Twelve-week results for ultimate tensile strength were similar to those in the rat study conducted by Lin and colleagues,[13] from which our sample sizes were calculated. The apparent statistical power achieved in our study was 99.2%, which was less than that achieved in the study by Lin and colleagues.

Discussion

We found a statistically significant increase in Young's modulus for the RF-treated tendon repairs compared with the untreated control tendons at 6 weeks, the period indicative of a state of tendon repair during soft-tissue healing. This difference was not found at 12 weeks, when tendon architecture is expected to be restored. We also found a relative increase in ultimate tensile strength for the RF-treated tendons at both 6 and 12 weeks. Therefore, we rejected the null hypothesis that there would be no difference in strength, based on biomechanical assessments between RF-treated and untreated tendons. As the tendons function in tension to transmit force from muscle to bone, the biomechanical parameters derived from tensile testing characterized the material properties of the tendons.[21] The increase in Young's modulus 6 weeks after surgery indicates that the RF-treated tendons were stiffer than the untreated ten-

Table. Biomechanical Properties of Radiofrequency-Augmented Tendon Repair

	Cross-Sectional Area (mm^2)	Young's Modulus (MPa)	Ultimate Tensile Strength (MPa)	% Strain
RF-Augmented Repair				
6 Week Experimental	5.40 ± 0.49	74.24 ± 12.37	21.42 ± 1.37	28.9 ± 2.1
6 Week Contralateral Control	4.69 ± 0.28	115.49 ± 16.56	22.08 ± 1.05	19.11 ± 1.6
12 Week Experimental	5.55 ± 0.52	80.74 ± 15.59	21.60 ± 3.20	26.8 ± 2.4
12 Week Contralateral Control	4.53 ± 0.27	110.13 ± 10.18	22.68 ± 2.27	20.6 ± 2.3
Unaugmented Repair				
6 Week Experimental	4.49 ± 0.34	56.86 ± 14.60	11.84 ± 2.55	20.8 ± 2.2
6 Week Contralateral Control	4.77 ± 0.27	121.95 ± 5.98	18.05 ± 2.5	14.8 ± 2.3
12 Week Experimental	4.40 ± 0.24	81.09 ± 24.31	16.92 ± 2.85	20.8 ± 2.7
12 Week Contralateral Control	4.50 ± 0.24	115.67 ± 24.64	19.64 ± 1.88	17.0 ± 1.8

Note: Values are expressed as mean ± standard deviation.

dons at the repair site, which may indicate an increased rate of repair over the first 6 weeks. For both groups, the ultimate tensile strength of the tendon bundle reached levels similar to those of the contralateral control limbs by 12 weeks, confirming restoration of tendon architecture and restoration of tendon biomechanical properties.

This study is noteworthy because it used a new in vivo model to study tendon repair. This model differs from models used in other studies of Achilles tendon repair in that our animals were not immobilized after surgery.

Other investigators have studied the effect of combined use of immobilization and RF on the biomechanical properties of soft-tissue structures. Schlegel and colleagues[9] studied RF shrinkage in a rabbit patellar tendon model in which RF was applied to the entire patellar tendon. At 8 weeks, the resultant stiffness was least in nonimmobilized animals compared with 2 other groups of animals that underwent varying amounts of postoperative immobilization. No group had tendon stiffness levels comparable to those of the contralateral controls by 8 weeks. The investigators did note increased cross-sectional areas in treated tendons compared with contralateral control tendons—a finding similar to ours for RF-treated repairs. Pötzl and colleagues[11] also assessed the effects of RF shrinkage and immobilization in a rabbit patellar tendon model. RF was applied in multiple passes over the width of the tendon. At 6 weeks, patellar tendon stiffness was significantly higher for the immobilized group than for a group that had been immobilized for 3 weeks and then had 3 additional weeks of no immobilization. Neither group had regained stiffness levels comparable to those of the contralateral controls by 6 weeks. In their RF-shrinkage studies, Schlegel and colleagues[9] and Pötzl and colleagues[11] both stimulated a much larger tendon surface area than we did, though neither of those groups mentioned how long RF was applied to tendons. Longer RF application may have been more damaging to tendons or their collagen ultrastructure than the limited RF stimulation used in our study. Clinically, there has been a trend toward minimizing postoperative immobilization for most tendon and ligament repairs, with the goal being to preserve joint and tendon range of motion.[2,22-24]

Why RF improves tendon biomechanics in vivo is not entirely evident, as the effects are not uniformly consistent. At certain higher voltages, the plasma at the tip of the RF device has enough energy to cut or coagulate without conducting significant amounts of heat to the rest of the saline or to surrounding tissue.[4-7] In a rat model of chronic supraspinatus tears, no difference was found in the strength or stiffness of the repaired tendons with and without RF treatment.[13] That study was limited by its testing methods, as several tendon specimens slipped at the clamp attachment, limiting the study numbers. Although their results approached statistical significance, the investigators noted the study was underpowered to detect a 30% difference in tendon strength. In addition, during ultimate failure testing, several repairs failed at the humeral neck rather than at the repair site, indicating that tendon repair strength exceeded that of bone and that true tendon strength may have been underestimated. Finally, there was no discussion of the rats' age; however, healing capacity may be greater for younger rats than for more mature rats, which could also minimize the difference in RF effects on tendon healing. Our model differs from the rat model in that it is an acute model for studying the effect of RF on tendon healing. A strength of our study is that it was sufficiently powered to detect a 10% difference in the biomechanical properties of tendons. We also used a different method of testing tendons, such that 3 of every 4 tendons failed directly at the repair site. The rest of the tendons failed at the suture site or at the testing clamp. As such, our method of tendon testing may be more reflective of the true strength of the repair.

Increased angiogenesis has been proposed as a mechanism of increased tendon healing after RF treatment.[8] A study of the effect of RF ablation on liver metastases found increased hepatic sinusoidal vessel formation as well as increased growth of micrometastases in an RF-treated area.[25] Further immunohistochemical analyses determined that the increased tumor growth was stimulated by localized hypoxia and the hypoxia

inducible factor signaling cascade. Angiogenesis was also stimulated by relative hypoxia after RF ablation, albeit by a different pathway. In a rabbit model of meniscal RF repair, monopolar RF treatment produced an area of fibrochondrocyte death at 2 weeks, with subsequent repopulation by fibroblast proliferation by 12 weeks.[26] The investigators also found an increase in autocrine motility factor in the RF-treated menisci and proposed that the relative hypoxia induced by RF stimulated fibrochondrocyte motility. In the musculoskeletal system, RF clearly changes the healing milieu, but increased angiogenesis may not be the primary mechanism of increased stiffness and improvement in the material properties of the tendon.

Clinically, RF microtenotomy has shown benefit in managing chronic lateral elbow epicondylitis,[8,14,15] with improvements in pain and functional scores up to 2 years after surgery. The mechanism of pain relief is unclear but may be initially related to the effect of RF on nerves rather than on the tendon. Increased neurogenesis is known to occur in the setting of chronic tendinopathy,[27] and RF has been shown to induce acute sensory nerve fiber degeneration with subsequent regeneration by 90 days after treatment.[28,29] In the setting of rotator cuff tendinopathy, RF microtenotomy provided pain relief and functional improvement equivalent to those obtained with arthroscopic subacromial decompression.[30] We know of no clinical study in which RF was used with the specific aim of augmenting tendon repair. Given the incidence of chondrolysis after thermal capsulorrhaphy in the shoulder[16-19] and reports of osteonecrosis after RF use in knee arthroscopy,[20] further elucidation of the mechanism for the effect of RF on tendon healing is warranted.

This study is limited in that no histologic or immunopathologic tests were performed on the repaired tendon bundles. Such studies would have helped elucidate the mechanism leading to increases in tendon strength and stiffness. Furthermore, though our model appears to be useful for studying acute tendon repair, it is not a model of tendinopathy. Consequently, the effect seen in the acute setting may not translate to tendon repairs made in managing a chronic degenerative process. Additional studies of RF-treated tendons in animal models of tendinopathy are also needed to confirm or refute the proposed effects of RF in the clinical setting.

Conclusion

In this study, we found an increase in Young's modulus in RF-treated tendon repairs 6 weeks after surgery that did not persist to 12 weeks. We also found increased ultimate tensile strength of RF-treated repairs at 6 and 12 weeks, with strength near that of contralateral control limbs. The faster return of mechanical integrity with RF-treated repairs may allow for less postoperative immobilization and earlier rehabilitation and ultimately for less clinical disability after acute tendon repair.

Authors' Disclosure Statement and Acknowledgment

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