

# Ultrasound-Guided Dry Needling of the Healthy Rat Supraspinatus Tendon Elicits Early Healing Without Causing Permanent Damage

Corinne N. Riggin<sup>1</sup>, Mengcun Chen,<sup>1,2</sup> Joshua A. Gordon,<sup>1</sup> Susan M. Schultz,<sup>3</sup> Louis J. Soslowsky,<sup>1</sup> Viviane Khoury<sup>3</sup>

<sup>1</sup>McKay Orthopaedic Research Laboratory, Perelman School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania, <sup>2</sup>Department of Orthopaedics, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China, <sup>3</sup>Department of Radiology, University of Pennsylvania, Philadelphia, Pennsylvania

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**ABSTRACT:** Overuse-induced tendinopathy is highly prevalent in the general population. Percutaneous fenestration, or dry needling, techniques have been increasing in popularity, but despite their current use, there are no controlled laboratory studies to provide fundamental support for this practice. The objective of this study was to establish a model for percutaneous needling of the rat supraspinatus tendon using ultrasound guidance and to evaluate the biological response of needling healthy tendon. A total of 44 male Sprague–Dawley rats ( $477 \pm 39$  g) were used to evaluate the effect of dry needling on healthy supraspinatus tendon properties. Ten rats were reserved as un-neededled control animals, and the remaining animals underwent either mild or moderate bilateral needling protocols and were sacrificed at 1 or 6 weeks post-needling ( $n = 8–10/\text{group}$ ). Color Doppler ultrasound imaging was performed to analyze blood flow within the tendon. Histological and immunohistochemical analyses were used to determine cellular, inflammatory, and extracellular matrix properties of the tissue. Finally, quasi-static tensile mechanical analysis was performed to obtain viscoelastic, structural, and material properties to evaluate the tendon healing outcome. Data were tested for normality, and then two-way analysis of variance tests were performed followed by post hoc tests for multiple comparisons. Both the mild and moderate needling groups caused a transient healing response at early time points as shown by a statistically significant ( $p < 0.05$ ) reduction in mechanical properties, and increase in blood flow, inflammation, and production of collagen III and glycosaminoglycans as compared to the control. Furthermore, mild needling properties returned to or exceeded pre-needling values at the 6-week time point. Clinical significance: Needling the rat supraspinatus tendon is a feasible technique that causes a transient healing response followed by a return to, or improvement of, normal tendon properties, indicating potential applicability in understanding the effects of current practices utilizing dry needling of tendons in humans. © 2019 Orthopaedic Research Society. Published by Wiley Periodicals, Inc. *J Orthop Res* 37:2035–2042, 2019

**Keywords:** ultrasound; dry needling; fenestration; tendon; tendinosis

Overuse injuries that lead to tendon degeneration, termed tendinosis, are prevalent in recreational and professional athletes and manual laborers.<sup>1–5</sup> Tendinosis is characterized by pain, swelling, and impaired performance<sup>6,7</sup> and may result in tendon rupture.<sup>8–11</sup> Originally thought to be an inflammatory condition, tendinosis is now understood to be a degenerative process, characterized by hypercellularity, vascular hyperplasia, and collagen disorganization.<sup>6–8</sup> Common sites of tendinosis include the rotator cuff, common extensor, patellar, and Achilles tendons.

Tendinosis treatment begins with conservative measures including rest, activity modification, bracing, physical therapy, and non-steroidal anti-inflammatory medications. Although often effective, these methods sometimes have limited success in treating tendinopathy symptoms.<sup>12</sup> Recently percutaneous and minimally invasive treatments of chronic tendinosis have increased in popularity.<sup>13–18</sup> Percutaneous dry needling involves repeat fenestration of diseased tissues using a

needle, typically performed under ultrasound (US) guidance to provide real-time imaging and needle guidance to target the precise area of disease.<sup>19–21</sup> Evidence suggests that micro-trauma to the diseased tendon may lead to disruption of pathological tissues, induction of bleeding, and release of factors to stimulate healing.<sup>13,22</sup> The theoretical aim of dry needling is to convert a chronic, quiescent lesion into an acute, activated lesion, which may have greater healing potential. This US-guided dry needling technique has been described alone and in combination with an injection of platelet-rich plasma,<sup>23</sup> autologous blood,<sup>24</sup> or glucose,<sup>25</sup> among other substances.<sup>25</sup> These treatments are thought to promote the influx of growth factors involved in the inflammatory phase of healing.<sup>25</sup> Several clinical studies have suggested this can be an effective treatment.<sup>13,14,26,27</sup> However, no controlled laboratory studies have been performed to provide a rigorous scientific evaluation of this commonly used clinical practice. With widespread clinical use, lack of such studies demonstrates a significant gap in the supportive medical literature.

The objective of this study was to investigate the feasibility of dry needling the supraspinatus tendon using US guidance in an established rat model for studying rotator cuff injury and disease and to determine its effects on tendon vascular, compositional, and mechanical properties in healthy tendon samples. The rat rotator cuff model provides a unique advantage because it is the only tendinosis animal model similar

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Correspondence to: Louis J. Soslowsky (T: 215-898-8653; F: 215-573-2133; E-mail: soslowsk@upenn.edu)

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to the etiology of overuse in humans.<sup>28</sup> While this study aims to evaluate the dry needling method in healthy tendons, future studies will evaluate dry needling on the rat tendinosis model. We hypothesize that the needling procedure will cause consistent micro-injuries initiating a transient healing response, but without permanent damage to the tendon tissue. The results will provide a model system for evaluating the effect of dry needling.

## METHODS

This prospective study was performed in accordance with the University of Pennsylvania Institutional Animal Care and Use Committee.

### Validation of Needling Technique

Thirteen male Sprague–Dawley rats were used for four sets of studies to determine the most consistent needling procedure of the rat supraspinatus tendon. Live animals were not necessary for this, so procedures were performed within 30 min post-sacrifice to ensure that rigor mortis would not interfere with tissue properties. The procedure included injecting a small amount of dye during different dry needling approaches, followed by immediate dissection and evaluation of the location of the injected dye to confirm needle placement in the tissue. A musculoskeletal radiologist with experience in the diagnostic US and US-guided injection procedures performed all studies. A high-frequency transducer and a clinical scanner were investigated. The animal's shoulder was placed in external rotation and adduction to expose more of the supraspinatus tendon from under the acromion.<sup>29–31</sup> The shoulder was imaged and needled either in the long axis of the supraspinatus tendon from lateral to medial or in the transverse plane from posterior to anterior. We investigated three needle sizes, 23, 25, and 27 gauge, and two needle lengths, 1" and 0.5". To identify needle placement, Verhoeff stain (~1 µl) was injected using US guidance into the tendon. The shoulder was immediately dissected to confirm the location of the dye within the tendon.

### Study Design

A live animal study was then performed to determine the effect of dry needling on healthy rat supraspinatus tendons. Forty-four male Sprague–Dawley rats (477 ± 39 g) were obtained from Charles River Laboratories (Wilmington, MA), housed in standard cages with two rats/cage, and provided food and water ad libitum. A subset ( $n = 10$ ) were reserved as un-needled control animals, and the remaining animals were randomized into mild or moderate bilateral needling protocols and were sacrificed at 1 or 6 weeks post-needling ( $n = 8–10$ /group). Animals sacrificed at 6 weeks post-needling also underwent color Doppler US imaging prior to needling, 5 and 24 h post-needling, and 6 weeks post-needling prior to sacrifice. After sacrifice, one shoulder was used for histological and the other for mechanical evaluation. A power analysis was performed to determine sample sizes.

### Dry Needling Procedure

Dry needling was performed based on the results of the validation procedures listed in the methods section above. Animals were anesthetized with isoflurane, and the shoulder was prepared by removing hair locally. Using the 14–5 MHz linear array transducer (Zonare, Mountain View, CA), the

supraspinatus tendon and the surrounding landmarks (acromion and humeral head) were identified in the transverse plane. Under US guidance, a 27 G 1/2" needle was introduced posteriorly and guided into the supraspinatus tendon in the superior aspect of the subacromial space. For the mild protocol, the needle was inserted three times, and for the moderate protocol the needle was inserted an additional 3 times fanned proximally and three times distally (nine times total). All animals were provided with Buprenorphine for pain management and recovered from anesthesia under a heat lamp until they became fully responsive and demonstrated a return to normal behavior.

### Doppler US Imaging

Color Doppler US was performed using a 40 MHz center frequency transducer (MS550D; VisualSonics, Toronto, ON) in the same positioning. Eight to 10 images were acquired for each shoulder and blood flow within the tendon was analyzed using an IDL program (Harris Geospatial Solutions, Herndon, VA). Mean color level (average blood flow velocity), fractional area (% area of Doppler signal), and color weighted fractional area (weighted average of blood flow velocity/unit area) were quantified. Measures for each image within a specimen were averaged.

### Histological Evaluation

Tendon samples for pre-needling control and 1 and 6 weeks post-needling ( $n = 6–8$ /group) were dissected, processed, sectioned, and stained with hematoxylin–eosin (H&E), Safranin-O and fast green, and immunohistochemical staining for type III collagen (Col III, C7805, 1:500; Sigma-Aldrich, St. Louis, MO), interleukin 1 $\beta$  (IL-1 $\beta$ , AB1832, 1:400; Chemicon, Burlington, MA), and tumor necrosis factor  $\alpha$  (TNF- $\alpha$ , NBP1-19532, 1:750; Novus Biologicals, Centennial, CO). Approximately 2–3 sections along the width of each specimen were blinded and imaged at 2 different locations along the tendon at 200 $\times$ . H&E and Safranin-O stains were semi-quantitatively evaluated by three blinded investigators and graded for cell shape (1 = spindle to 3 = round), cellularity (1 = less cells to 4 = more cells), and amount of Safranin-O stain (1 = less stain to 4 = more stain). Immunohistochemical stains were quantitatively analyzed for percent area of positive stain using a MATLAB program (Mathworks, Natick, MA).<sup>32</sup>

### Mechanical Evaluation

Tendons from pre-needling and 1 and 6 weeks post-needling ( $n = 8–10$ /group) were blinded and dissected, leaving the bone insertion intact. Verhoeff stain lines were applied at the insertion and in 2, 4, and 8 mm increments to define the insertion and midsubstance regions. Cross-sectional area was measured using a laser-based device from the insertion up to the 8 mm stain line.<sup>33</sup> The proximal end of the tendon was fixed with sandpaper and adhesive at the 8 mm stain line. The humerus and humeral head were secured in polymethylmethacrylate without obstructing the tendon motion. The specimen was attached between two fixtures and submerged in a 37°C phosphate-buffered saline bath. The protocol consisted of (i) preloading (0.08 N), (ii) preconditioning (10 cycles from 0.1 to 0.5 N), (iii) stress-relaxation (5% strain for 10 min), and (iv) ramp to failure (0.3% strain/s). The strain was measured optically.<sup>34</sup> Percent relaxation was calculated from the stress-relaxation test and structural (max load, stiffness) and material (max stress, modulus) properties were determined from the ramp to failure test.

### Statistics

Normally distributed data (Doppler and mechanics) was analyzed using a two-way analysis of variance (ANOVA) followed by Bonferroni tests. For parameters with significance over time, one-way ANOVAs were performed within each group with Bonferroni tests across time point. Non-parametric data (histology) was analyzed using Kruskal–Wallis and Dunn's tests. Significance was set at  $p < 0.05$ .

## RESULTS

### Dry Needling Validation

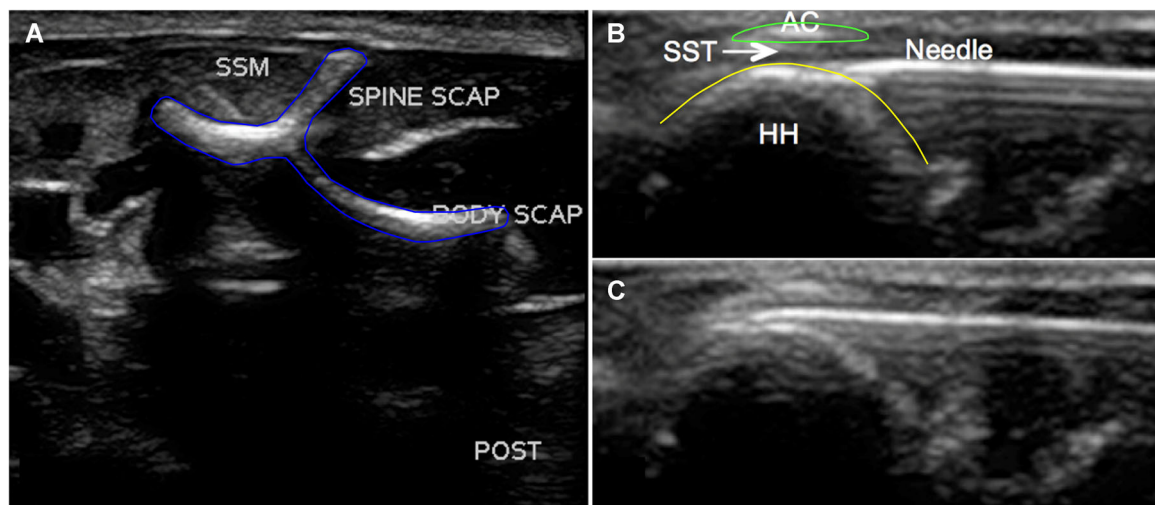
In our initial investigations, sacrificed animals were used to visualize the rat rotator cuff anatomy, learn how to best position the shoulder for the optimal view and access for US-guided injection, and determine the smallest needle size that can accurately be navigated to the location of the tendon. We determined that a 14–5 MHz transducer was the most appropriate for this application since it provided both good resolution and penetration for imaging the rotator cuff tendons, and the hockey-stick probe shape allowed for ease of needle positioning.

When comparing the longitudinal and transverse approaches, it was determined that the transverse approach was the most consistent. With the longitudinal approach, where the subacromial space was visualized in the long axis of the supraspinatus tendon, only one out of six shoulders had proper placement of the needle with dye injected in the tendon. For the transverse approach, the US transducer was positioned transverse to the shoulder with the needle entering the shoulder posteriorly and directed anteriorly into the supraspinatus tendon. This proper anatomical location was identified by imaging medial to the joint and in the sagittal plane of the scapula and locating the “Y” of the scapula (which made up of the scapular body, coracoid process, and

scapular spine) (Fig. 1A). By sliding the transducer laterally, the scapular spine can be followed to the acromion to where it is in the same plane as the humeral head. The hypoechoic region (space) between these two osseous structures is where the supraspinatus tendon lies (Fig. 1B and C). In these testing sessions, 12 out of the 14 shoulders that were injected with dye showed proper positioning of the needle, and the remaining two shoulders were inconclusive due to failure to visualize any dye injected in the tendon or surrounding area. It was also determined that the 27-gauge needle of 0.5" length was the smallest possible size while still being able to accurately guide the needle into the correct anatomical location.

### Color Doppler US

Color Doppler US parameters were evaluated in the supraspinatus tendon prior to needling (Fig. 2A), 5 and 24 h following needling (Fig. 2B and C), and 6 weeks post-needling (Fig. 2D). All three parameters showed significant interactions between treatment and time point. The mean color level, representing blood flow velocity, was not significantly altered over time, but there was a significant increase in the mild group compared to the moderate group at the 24 h time point (Fig. 2E). However, both fractional area and color weighted fractional area significantly increased 5 and 24 h after needling in both groups compared to the pre-needling values. Only the mild group showed a significant decrease in the 6-week post-needling compared to these earlier time points. However, neither group showed differences between pre-needling and 6 weeks post-needling (Fig. 2F and G). Finally, color weighted fractional area was significantly increased in the mild group compared to the moderate group at 24 h post needling (Fig. 2G).



**Figure 1.** Ultrasound images of the rat shoulder in the sagittal plane (transverse approach). (A) Image medial to the shoulder joint depicting the “Y” of the scapula and the supraspinatus muscle (SSM). The acromion was identified by sliding the transducer laterally and following the scapular spine until the humeral head is in view. (B) Image of the humeral head (HH) and the acromion (AC), with the needle (27 gauge) entering from posterior to anterior, and (C) the needle entering between the acromion and the humeral head into the supraspinatus tendon (SST) [Color figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

**Histological Evaluation**

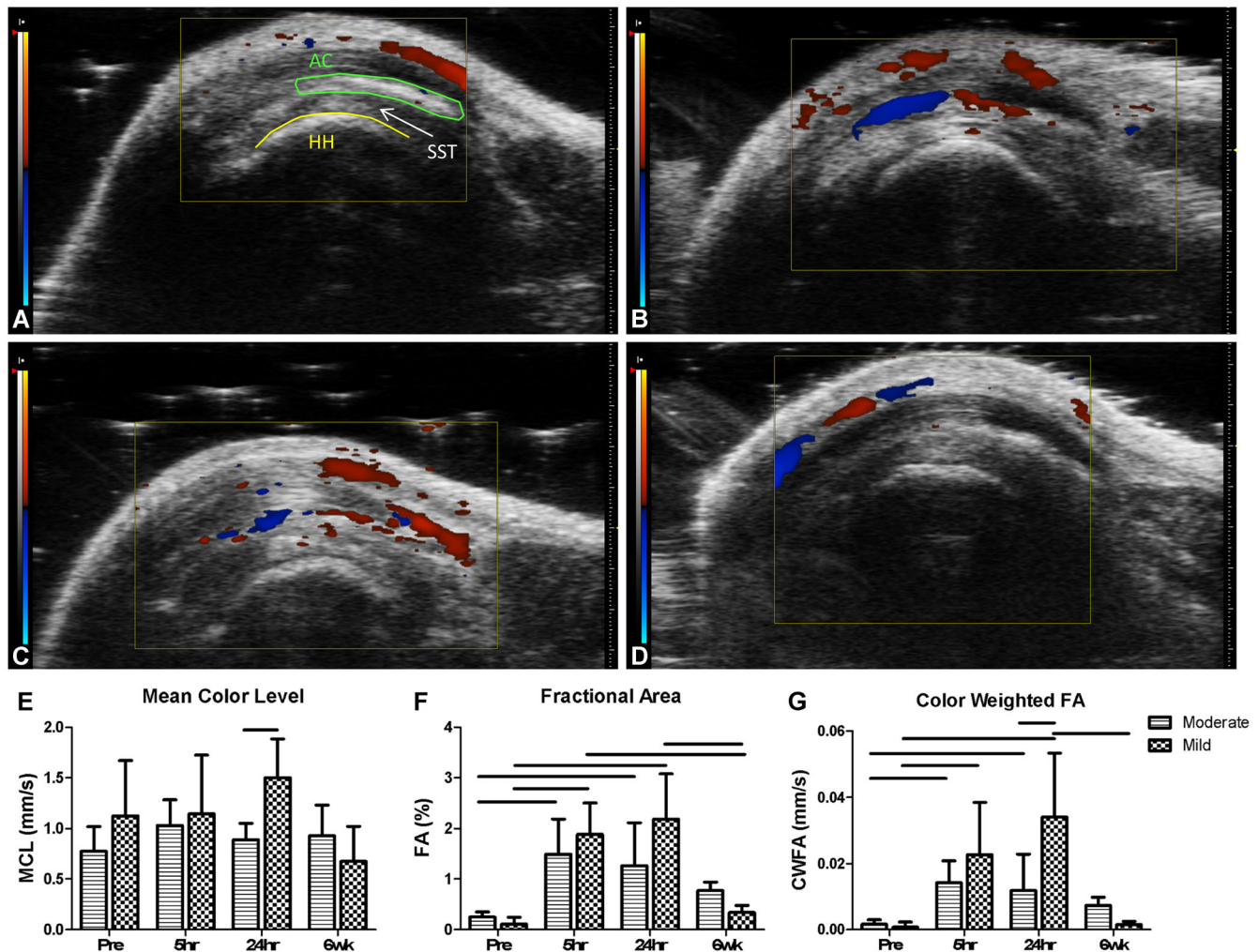
The H&E cellular evaluation (representative images are shown in Fig. 3A) demonstrated that there was a significant increase in cellularity and more rounded cell shape in the moderate needling group at week 1 compared to control. While the mild group qualitatively demonstrated increases in these properties as well, there were no statistically significant changes over time (Fig. 3B and C). Additionally, for the Safranin-O staining (representative images shown in Fig. 3A), the moderate group showed a significant increase from control at week 1, and a significant increase compared to the mild group at week 6 (Fig. 3D).

The immunohistochemical evaluation (representative images are shown in Fig. 4A) demonstrated that 1-week post-needling there were significant increases in all three targets Col III, TNF $\alpha$ , and IL-1 $\beta$  (Fig. 4B–D). Both groups were also significantly decreased at 6 weeks compared to 1 week (except for the TNF- $\alpha$

moderate group), and statistically not different from control values (Fig. 4B–D).

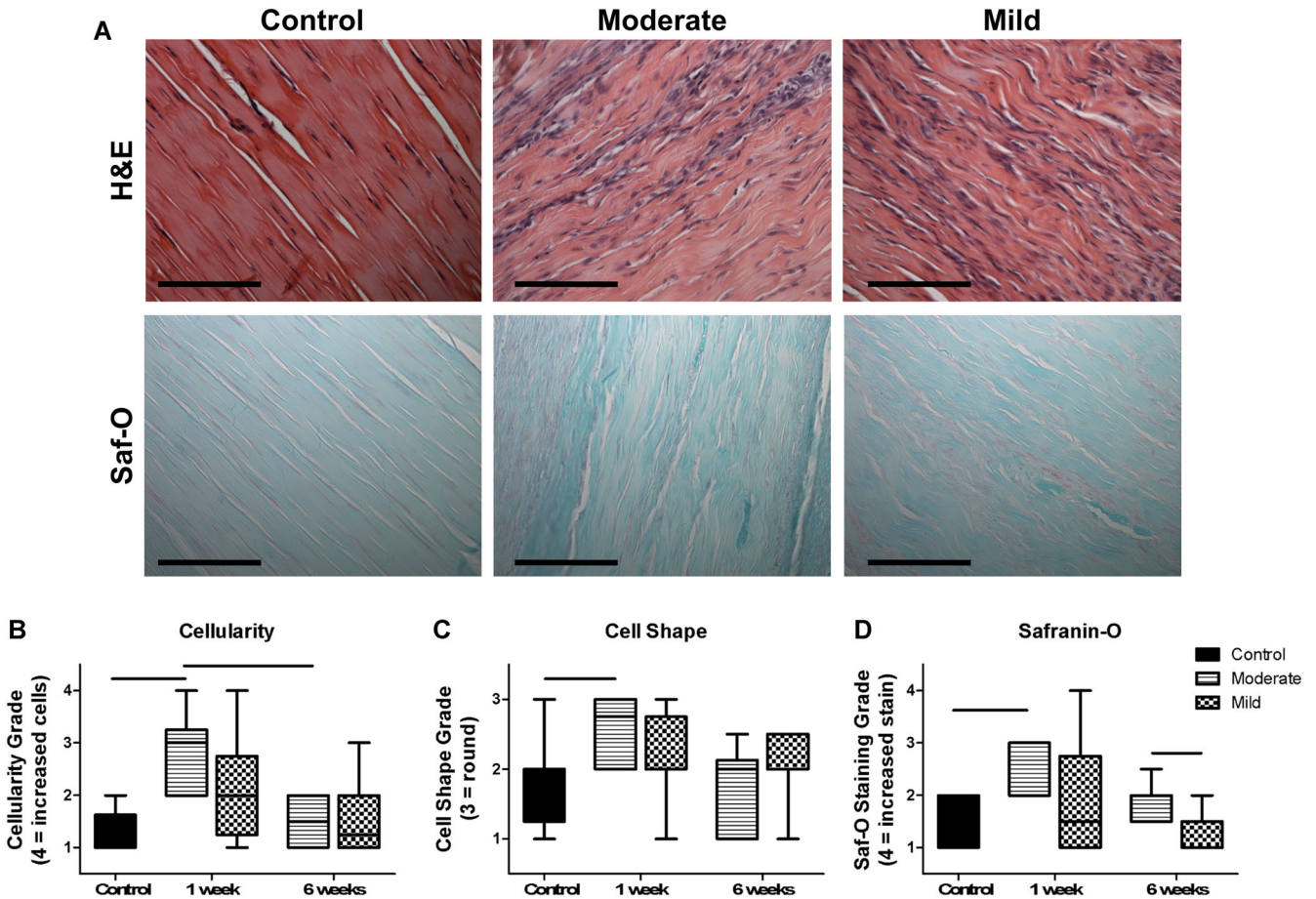
**Mechanical Evaluation**

There was a significant increase in cross-sectional area in the moderate needling group by 1 week that returned to baseline by 6 weeks, whereas there were no significant changes in the mild group (Fig. 5A). Both needling groups caused a reduction in percent relaxation that continued out to 6 weeks post-needling (Fig. 5B). There was a statistically significant interaction between needling group and time point for both cross-sectional area and percent relaxation. Both groups also demonstrated a significant increase in max load at 6 weeks compared to both the un-needled control and the 1-week post-needling groups (Fig. 5C). The moderate group showed a decrease in max stress at 1 week that recovered to pre-needling values by 6 weeks (Fig. 5D). The tendon stiffness and modulus were reduced in both



**Figure 2.** Color Doppler ultrasound representative images from the mild group showing (A) pre-needling, and (B) 5, (C) 24 h, and (D) 6 weeks post-needling. The pre-needling image has the humeral head (HH), acromion (AC) and supraspinatus tendon (SST, located between the HH and AC) labeled. Quantification of (E) mean color level (MCL), (F) fractional area (FA), and (G) color weighted fractional area (CWFA). Both the moderate and mild needling groups showed a large increase in fractional area measures at 5 and 24 h post-needling, with a return to baseline values by week 6. Data are displayed as average and standard deviation and all significance lines indicate  $p < 0.05$  between groups [Color figure can be viewed at wileyonlinelibrary.com]





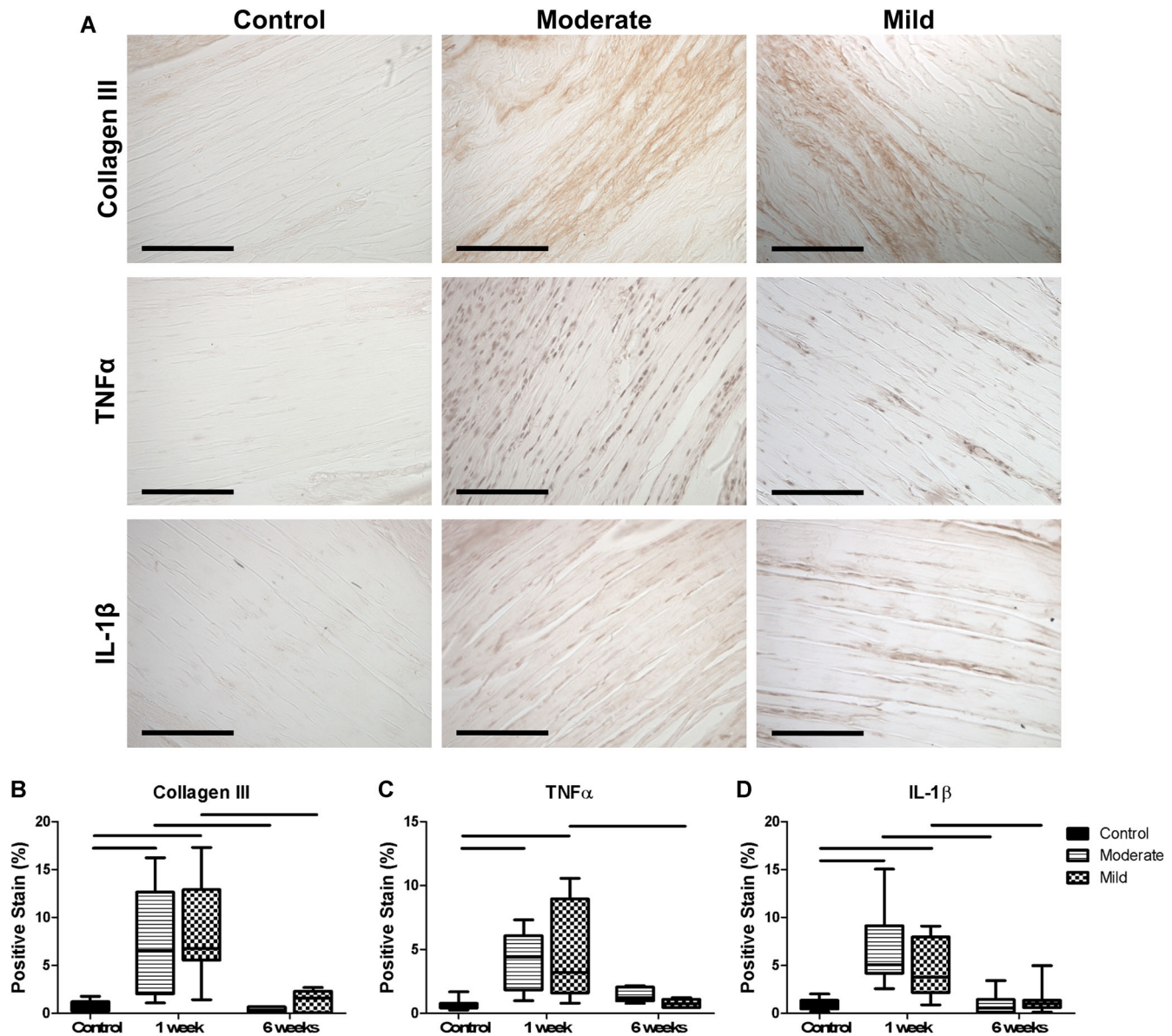
**Figure 3.** (A) Representative histological images of control (un-neededled) and moderate and mild samples at 1-week post-needling (scale bar = 100  $\mu$ m). Purple hematoxylin staining in H&E indicates the presence of cell nuclei and pink/red staining in Safranin-O images indicates the presence of proteoglycans. The plots display semi-quantitative histological grading of (B) H&E cellularity (1 = less cells to 4 = more cells) and (C) cell shape (1 = spindle to 3 = round), and (D) area of Safranin-O staining (1 = less stain to 4 = more stain). The moderate group showed increases in all three parameters at 1-week post-needling. Data are displayed as median and interquartile range, and all significance lines indicate  $p < 0.05$  between groups. H&E, hematoxylin–eosin [Color figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

the moderate and mild groups at 1 week (Fig. 5E and F). While stiffness recovered to pre-needling values for both groups, only the mild group recovered pre-needling tendon modulus by week 6 (Fig. 5E and F). Additionally, the mild group modulus was significantly increased compared to the moderate group at week 6 post-needling (Fig. 5F).

**DISCUSSION**

While several clinical studies suggest that dry needling, with or without injection of various substances, provides effective pain relief for tendinosis,<sup>13,14,26,27</sup> the effect on the tendon tissue health and predisposition to rupture remains unknown. Additionally, there have been no controlled laboratory studies to provide scientific support for this increasingly common clinical practice. Additionally, it is also unknown how the tendon responds to varying amounts of dry needling and if it may cause permanent damage to tendon tissue despite potential improvements in pain.

This study presents the development of a consistent method for US-guided needling of a rat supraspinatus tendon in order to determine the response that tendons have to dry needling, and to validate this method for future use in tendinopathy models. Validation procedures confirmed the sonographic anatomy of the rat rotator cuff, determined positioning of the animal and the probe, and established the optimal needling parameters and approach. It was determined that dry needling caused a transient increase in vascularity and an increase in inflammation, deposition of type III collagen, and in the case of the moderate needling group an increase in cellularity and glycosaminoglycan content. Additionally, qualitative observation of both the moderate and mild needling groups demonstrated more disorganized tissue structure at 1-week post-needling. The compositional and vascular changes returned to baseline values 6 weeks after treatment in both needling groups. Additionally, both needling procedures induced a minor injury response, with the moderate group showing more significant

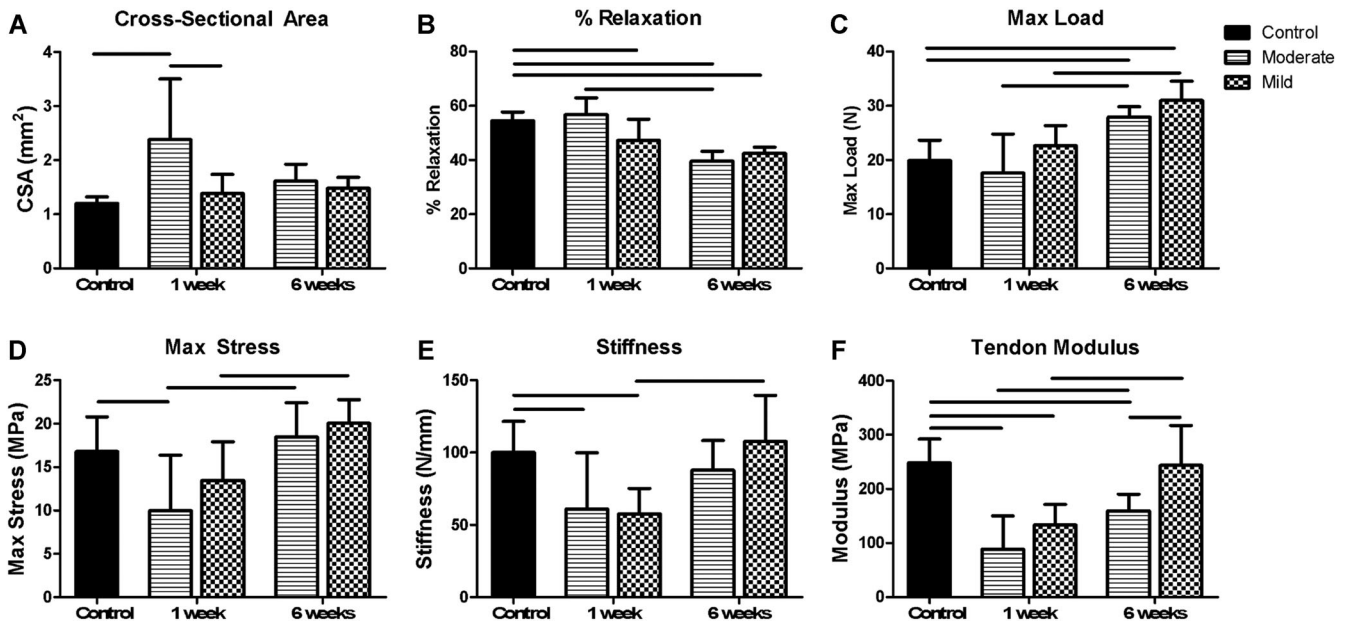


**Figure 4.** (A) Representative immunohistochemical images of control (un-needed) and moderate and mild samples at 1-week post-needling (scale bar = 100 μm). Brown staining indicates the presence of the protein of interest, as indicated by the row labels. The plots display quantitative evaluation of the percent of positive (brown) staining of (B) collagen III, (C) TNF-α, and (D) IL-1β within the images. All groups showed significant increases in these proteins at 1-week post-needling. Data are displayed as median and interquartile range, and all significance lines indicate *p* < 0.05 between groups. IL-1β, interleukin 1β; TNF-α, tumor necrosis factor α [Color figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

alterations. Specifically, only the moderate group demonstrated an increase in cross-sectional area and a decrease in max stress. However, both groups caused reductions in stiffness and modulus at 1 week. While the mild group recovered properties, the moderate group did not. Interestingly, both groups had reduced percent relaxation and increased max load at 6-weeks, indicating a more permanent beneficial change. Results suggest that both mild and moderate needling induced microdamage that elicited a healing response at early time points, and that the mild group completely recovered properties back to or better than pre-needling values. Since these results were performed in healthy tendon, this

indicates that a mild amount of dry needling of the tendon could be considered safe in this model, with the potential for improvement in a tendinopathic tendon.

Currently, the only supraspinatus tendinosis animal model similar to the etiology of overuse in humans is in the rat.<sup>28</sup> While it was necessary to first establish a non-detrimental dry needling method in healthy tendons, future studies will evaluate dry needling on a tendinosis model which is the primary pathology treated by needling techniques clinically. The rat is a well-established model for the rotator cuff and has the similar anatomical architecture to the human.<sup>35</sup> Additionally, the repetitive motion of the supraspinatus tendon



**Figure 5.** Mechanical evaluation of moderate and mild dry needling. (A) Only the moderate group showed an increase in cross-sectional area at 1 week. (B) Both groups decreased in percent relaxation by 6 weeks. (C) Max load was increased in both groups by 6 weeks. (D) Max stress, (E) stiffness, and (F) modulus all show reductions at 1 week. Both Max stress and stiffness recovered to baseline values by 6 weeks, whereas tendon modulus only recovered to baseline for the mild group. Data are displayed as average and standard deviation and all significance lines indicate  $p < 0.05$  between groups.

passing under the acromion is similar to that which occurs in the human shoulder during overhead activities, which can be a cause of rotator cuff tendinosis.<sup>35</sup> The rat model similarly creates degenerative tendinosis following an overuse treadmill running protocol.<sup>28</sup> This model is well accepted as there are now over 200 full-length peer-reviewed publications in the literature using the rat supraspinatus model. Other animal models of tendinosis include injections (collagenase, prostaglandins, fluoroquinolones, and cytokines), but these chemical methods do not mimic naturally occurring initiation of degeneration. We, therefore, chose the rat supraspinatus tendon for our dry needling procedure using US guidance so that we will be able to implement this procedure in a well-established tendinosis model that mimics the clinical condition.

This study has several limitations. First, the use of an animal model does not directly parallel the human condition. However, the rat supraspinatus tendon model mimics the human condition well. Additionally, the same size scales of the tendon and needle are not easily achieved in an animal model. While we used the smallest needle possible while maintaining the capability to guide the needle through tissue, it is still large relative to the small rat tendon compared to human tendons. We compensated for this by reducing the number of times that the tendon is needled (3–9× in the rat vs. ~30× in a human). Finally, this current study was performed on healthy tissue. This was an important first step to determine if this dry needling procedure is not detrimental to healthy tendons. Additionally, this study on healthy tendons will provide historical control data for future experiments on pathologic tendons. Despite these limitations, we have demonstrated that a rat

model allows for controlled, repeatable needling that elicits a healing response but does not cause long-term tissue damage. Unlike clinical studies where the outcomes are limited to functional measures, animal models allow the study of biological and mechanical mechanisms behind tendon healing after treatment. The comprehensive analysis offered by an animal model is necessary to fully understand the implications of current dry needling treatment techniques. This motivation for an animal model outweighs the small limitations and differences in procedure.

This study shows that US-guided dry needling of the rat supraspinatus tendon is a feasible technique allowing the specific targeting of a normal animal tendon in vivo. We found that dry needling causes a significant mechanical and biological response initially after needling, but that this response is transient and recovers over time. While dry needling is frequently performed clinically and has been shown to reduce pain, there is no research evaluating either beneficial or harmful tissue-level changes occurring after dry needling and how that may affect the tendon over time. This work supports future studies evaluating the effects of dry needling on a tendinosis condition, providing evidence to better inform the underlying mechanism and utility of this already common procedure.

#### AUTHORS' CONTRIBUTION

C.N.R. contributed to the research design, data acquisition, data analysis and interpretation, as well as drafting, revising, and approval of the manuscript. M.C. contributed to the data analysis and manuscript revision and approval. J.A.G. contributed to the research design, data



acquisition, and manuscript revision and approval. S.M.S. contributed to data acquisition and manuscript revision and approval. L.J.S. contributed to the research design, data interpretation, and manuscript drafting, revision, and approval. V.K. contributed to the research design, data acquisition, data interpretation, and manuscript drafting revising, and approval. All authors have read and approved the final submitted manuscript.

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