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Elasticity of the Supraspinatus Tendon-muscle Unit is Preserved after Acute Tendon Tearing in the Rabbit

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Supraspinatus tendon tearing is one of the most common causes of the shoulder pain and dysfunction, which often requires a surgical repair. In this situation, proximal tendon stump is usually retracted medially from its original insertion. For successful reduction of the retracted tendon stump to its original insertion, the elasticity of the tendon-muscle unit should be preserved by the time of surgery. The purpose of the present study was to clarify the chronological changes in the elasticity of the supraspinatus tendon-muscle unit after acute tendon tearing to determine the optimal timing for the surgery. Right supraspinatus tendon was detached (detached side) in 40 male Japanese white rabbits, with left shoulders served as controls (control side). Eight animals were euthanized at 3 days and 1, 2, 4, or 8 weeks after surgery. Tissue sound speed that closely correlates to its elasticity was measured with a scanning acoustic microscope. In the supraspinatus tendon, tissue sound speed at 3 days after surgery was 1691.1 m/s, compared to 1714.3 m/s at the control side, but the difference was not statistically significant at any postoperative time period up to 8 weeks. In the supraspinatus muscle, tissue sound speed was not affected at all by the detachment of the tendon. The present study indicated that the elasticity of the supraspinatus tendon-muscle unit was well preserved for 8 weeks after the detachment. In the clinical practice, the retracted supraspinatus tendon stump could be repaired without excessive tension by 8 weeks from the acute tendon tearing. ——— elasticity; tissue sound speed; scanning acoustic microscope; supraspinatus tendon; tendon tearing.
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Rotator cuff tendon consists of supraspinatus, infraspinatus, subscaplaris and teres minor, and plays an important role both in the stabilization and the active motion of the shoulder joint. It is known that the tearing of rotator cuff tendons is one of the most common causes of the shoulder pain and dysfunction. In the clinical practice, tear is seen most frequently in the supraspinatus tendon, especially close to its insertion (Matsen et al. 1998). In the treatment of rotator cuff tears, surgical repair is usually considered when non-operative therapies fail (McLaughlin 1944; Gartsman 2001). One of the key issues for successful rotator cuff repair is whether the retracted tendon stump can be advanced laterally to its original insertion at the greater tuberosity. It has been

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reported that the tension after repair directly correlate with the surgical outcome (Davidson and Rivenburgh 2000). Generally speaking, the tension after repair is mainly determined by the elasticity of the tendon-muscle unit and the size of tear. Although the relationship between the size of tear and the surgical outcome was well described (Cofield et al. 2001), little has been known concerning the chronological changes of the elasticity of the tendon-muscle unit after tendon tearing. In 2004, Gimbel et al. reported that the elasticity of the tendon first decreased after detachment, then gradually increased with time (Gimbel et al. 2004a, 2004b). Safran et al. (2005) reported that a detached infraspinatus muscle showed a significant increase in its elasticity at 12 weeks. Unfortunately, however, these authors tested either bone-tendon or bone-tendon-muscle complex as one unit (Gimbel et al. 2004a, 2004b; Safran et al. 2005). As a result, they failed to determine which part of the unit was the most responsible for such chronological changes of the tissue elasticity.

Scanning acoustic microscopy (SAM) was first developed by Lemons and Quate (1975) to measure the tissue acoustic properties at a microscopic level. Using SAM, tissue acoustic properties including the sound speed and the intensity can be measured and displayed as two-dimensional images (Saijo et al. 2005). Another advantage of SAM is that it can be applied for formalin-fixed and paraffin-embedded glass slides. This makes it possible to compare the two-dimensional distribution of the tissue sound speed to its histologic structure (Sano et al. 2006).

In a homogenous isotropic medium, the tissue sound speed is given by the following equation.

$$c = \sqrt{\frac{E(1-\sigma)}{\rho(1+\sigma)(1-2\sigma)}} \quad \cdots\cdots\cdots\cdots \text{ Equation 1}$$

, where c is the sound speed in the specimen, E is the Young's modulus, ρ. is the density, and s. is the Poisson's ratio (Elmore and Heald 1985; Saarakkala et al. 2004). Among these parameters, it is known that the density does not vary significantly in the living tissues (Hikichi 1982; Otubo et al. 1982; Saijo et al. 1997). Moreover, it is technically difficult to measure the real Young's modulus and Poisson's ratio of the living tissue at a microscopic level (Patil et al. 2004). Equation 1 indicates that the Young's modulus of the tissue is directly proportional to the square value of the sound speed measured by SAM.

Based on these facts, we attempted to measure the tissue sound speed of the rabbit supraspinatus tendon and its muscle after surgical detachment from its humeral insertion. Especially, we aimed to describe the chronological changes of the tissue elasticity of the tendon-muscle unit after acute tendon tearing.

Materials and Methods

The present study was carried out under the approval of the Ethics Committee of Animal Experimentation, Tohoku University School of Medicine.

Operative procedure

Forty male Japanese white rabbits (age: 17 weeks old) were used in the present study. Their average body weight was 2.7 kg (with the range of 2.3 to 3.1 kg). The operative procedure was performed under general anesthesia. Ketamine hydrochloride (Ketalar®, Daiichi-Sankyo, Tokyo, Japan) was injected into the back muscle at a dose of 25 mg/kg. Then, 10 mg/kg of ketamine and 3 mg/kg of Xylazine hydrochloride (Sedeluck®, Zenoaq, Koriyama, Japan) were administered intravenously for each rabbit. Right omovertebral and deltoid muscles were retracted to expose the supraspinatus tendon insertion. With a sharp scalpel, supraspinatus tendon was surgically detached just proximal to its insertion. Then, the proximal stump of the supraspinatus tendon was wrapped with a polyvinylidine fluoride membrane (Durapore® SVLP02500, Millipore Bedford, MA, USA, thickness: 125 μm, pore size: 5 μm) to prevent spontaneous reattachment (detached side) (Matsumoto et al. 2002). As a sham operation, left supraspinatus tendon was only exposed without cutting. The wound was closed immediately after exposure, which served as controls (control side).

Preparation of specimens for SAM measurements

To describe the chronological changes of the elasticity after cutting the supraspinatus tendon, eight rabbits

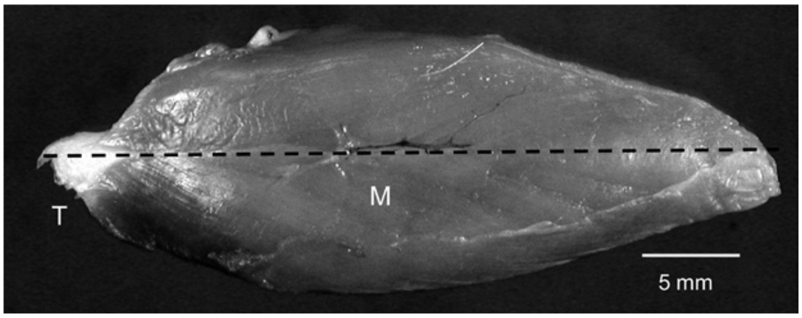

Fig. 1. Cutting line on the harvested supraspinatus tendon-muscle unit (viewing from the cranial side).
A whole supraspinatus tendon-muscle unit is harvested. To standardize the plane both for SAM measurement and the histologic assessment, supraspinatus tendon and its muscle are cut longitudinally at its center along their fibers (dash line).
T: supraspinatus tendon, M: supraspinatus muscle

were euthanized with overdosed (100 mg/kg) pentobarbital sodium (Nenbutal®, Dainippon Sumitomo pharma, Osaka, Japan) at 3 days, 1, 2, 4, and 8 weeks after surgery. Then, whole supraspinatus tendon-muscle unit was harvested (Fig. 1), which was fixed with 10% neutralized formalin thereafter.

Supraspinatus tendon with its muscle was cut at its center longitudinally along the fibers, to standardize the plane both for SAM measurement and the histologic assessment (Fig. 1). After embedding in paraffin, the specimens were sliced at 10-μm thickness using a microtome (TU213, Yamatokoki Industrial, Asagiri, Japan).

SAM measurements

SAM system specially developed in Tohoku University was used for the present study (Saijo et al. 2005). Specimens were mounted on glass slides without a cover slip and the paraffin was removed from the sections by the gradient alcohol method just before the ultrasonic measurement (Saijo et al. 1997). Distilled water was used as the coupling medium between the transducer and specimen, which was maintained at 20 degrees centigrade during the measurement procedure.

Ultrasound pulses at a pulse width of 5 ns were emitted and received by the same transducer above the specimen. The aperture diameter of the transducer was 1.2 mm, and the focal length was 1.5 mm. The central frequency was 80 MHz, and the pulse repetition rate was 10 kHz. The diameter of the focal spot was estimated to be 20 μm at 80 MHz by taking into account the focal distance and sectional area of the transducer. The reflections from the tissue surface and those from the interface between the tissue and glass were received by the transducer and were introduced into a digital oscilloscope (TDS 5052, Tektronix, USA). The frequency range was 300 MHz, and the sampling rate was 2.5 GS/s. Four values of the time taken for a pulse response at the same point were averaged in the oscilloscope in order to reduce random noise (Saijo et al. 2005). The reflections from the tissue surface and the interface between the tissue and glass cannot be separated in the time domain. Thus, the two reflections were separated by frequency domain analysis (Hozumi et al. 2004; Saijo et al. 2005) (Fig. 2).

The transducer was mounted on an X-Y stage with a microcomputer board that was driven by the computer installed in the digital oscilloscope. The X-scan was driven by a liner servomotor, and the Y-scan was driven by a stepping motor. Finally, the two-dimensional distri-

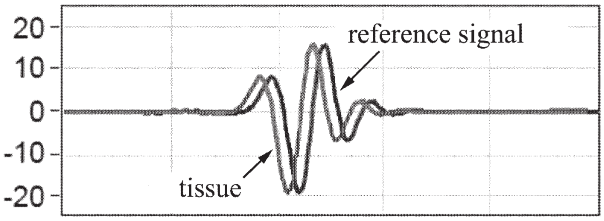

Fig. 2. Typical reflection waves from the tissue mounted on the grass slide in SAM measurement.
The reflections from the tissue surface (tissue) and those from the interface between the tissue and glass (reference signal) are received by the transducer, which are then introduced into a digital oscilloscope (time-domain analysis). The difference between two reflections is precisely calculated in the frequency domain for further analyses.

bution of the ultrasonic intensity, sound speed, and thickness of the specimen in an area of 2.4 × 2.4 mm were visualized using 300 × 300 pixels (Saijo et al. 2005). A gray-scale image of the 2-dimensional tissue sound speed distribution was also saved as an image file for further quantification. In the present study, we focused on the tissue sound speed, which closely correlates to its elasticity (Saijo et al. 2005; Sano et al. 2006; Hattori et al. 2007).

Region of interest (ROI)

In SAM measurements, each ROI should include an area without tissue as a reference, where ultrasound directly reflected from the glass surface. Thus, we decided to measure both the bursal and articular surfaces of the supraspinatus tendon/muscle as ROIs in the present study. For tendon tissue, the ROI was set at the musculo-tendinous junction to exclude artifacts caused by surgical transection (Fig. 3). For muscle tissue, the ROI

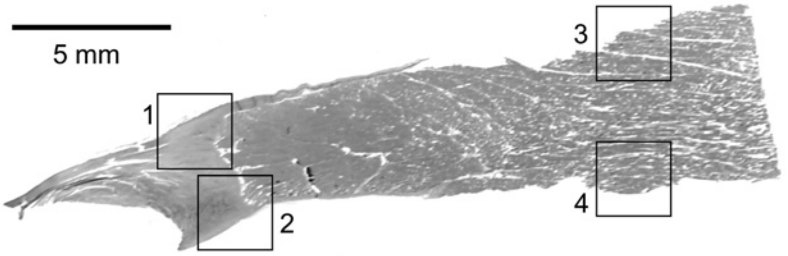

Fig. 3. Regions of interest (ROI) in the tendon-muscle unit for SAM measurement.
Both the bursal and articular surfaces of the supraspinatus tendon/muscle are chosen as ROIs using the histologic section, which is created by cutting the supraspinatus tendon-muscle unit along the line showing in Fig. 1. The ROI is set at the musculo-tendinous junction for tendon tissue and at the mid-part of the muscle belly for muscle tissue, respectively.
ROI 1, bursal side tendon; ROI 2, articular side tendon; ROI 3, bursal side muscle; ROI 4, articular side muscle. (Elastica-Masson trichrome staining, original magnification: × 1)

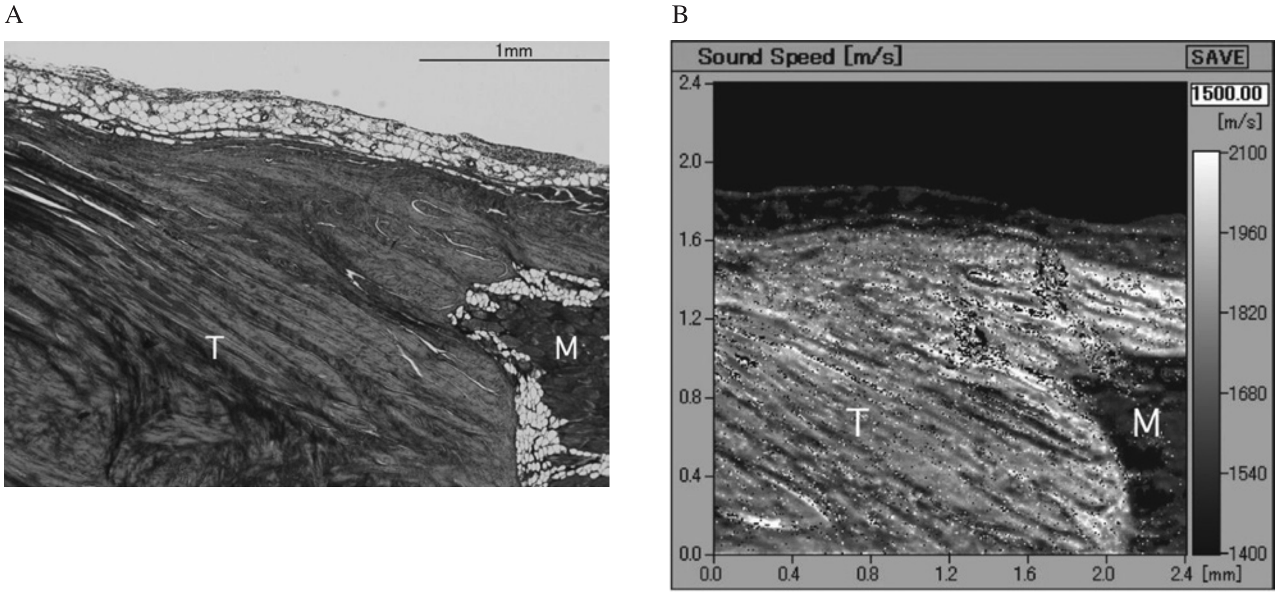

Fig. 4. Histologic staining and the sound speed distribution in the representative specimen.
The average value of the tissue density is measured using a gray-scale image of two-dimensional distribution of the tissue sound speed, which is then converted again to the tissue sound speed (ROI 1 shown in Fig. 3, T: tendon tissue, M: Muscle).
A: Elastica-Masson trichrome staining. B: The two-dimensional distribution of the tissue sound speed is displayed with the gray-scale.

was set at the mid-part of the muscle belly for the consistency of the measurements (Fig. 3). Then, the ROIs for the SAM measurements were determined using a neighboring section stained with Elastica-Masson trichrome (Fig. 4A).

Quantification of the tissue sound speed

A gray-scale image representing the distribution of the tissue sound speed (Fig. 4B) was imported to the commercial software, Photoshop Elements 2.0 (Adobe systems incorporated, San Jose, CA, USA). With this image, the average value of the color density (between 0 and 255) in each ROI was calculated using the analysis option, “histogram”. The average value of the color density was converted again to the sound speed for each ROI. Then, the mean value of the tissue sound speed between articular side and bursal side was calculated for each tendon or muscle, which was determined as the tissue sound speed of the whole supraspinatus tendon or muscle.

Data assessments

To clarify the chronological changes of the elasticity after detachment of the supraspinatus tendon, tissue sound speed in the whole supraspinatus tendon/muscle was compared between the detached side and the control side at each postoperative time period.

Statistical analyses

Commercial software, JMP (5.01J, SAS Institute Inc., Cary, NC, USA), was used for the statistical analyses in the present study. Tukey-Kramer multiple comparison test was used to determine the difference of the tissue sound speed among the specimens. The difference was considered statistically significant when the *p*-value was less than 0.05.

Results

Surgeries were successfully performed in all animals. However, displacement of the polyvinylidine fluoride membrane was seen in 1 rabbit (4-weeks group). Another 1 rabbit represented postoperative infection of the shoulder joint. Thus, these 2 animals were excluded from further analyses. In the rest of 38 animals, no tendon stumps represented spontaneous reattachment to the humeral head.

In the supraspinatus tendon, detached side generally showed lower tissue sound speed than control side (Fig. 5). At 3 days after surgery, mean tissue sound speed in the detached side was 1691.1 m/s, compared to 1714.3 m/s at the control side. The lowest tissue sound speed was seen at 4 weeks after the detachment (1671.2 m/s). However, the difference between the detached side and the control side was not statistically significant at any postoperative time period.

Furthermore, tissue sound speed of the supraspinatus muscle was not affected at all by the detachment of the supraspinatus tendon (Fig. 6).

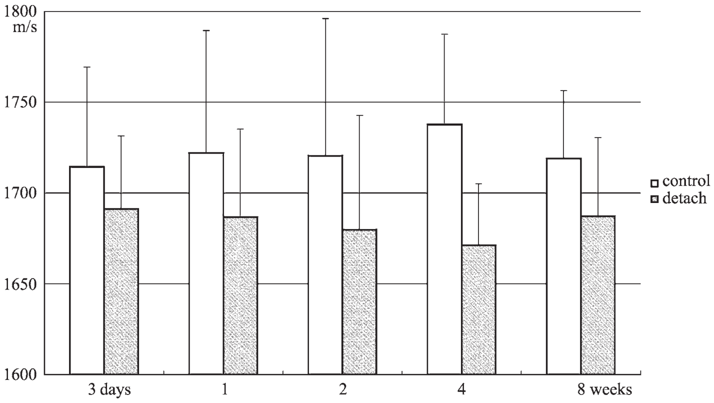

Fig. 5. Tissue sound speed of the whole supraspinatus tendon.
In the supraspinatus tendon, detached side generally shows lower tissue sound speed than control side. However, the difference between the detached side and the control side is not statistically significant at any postoperative time period.

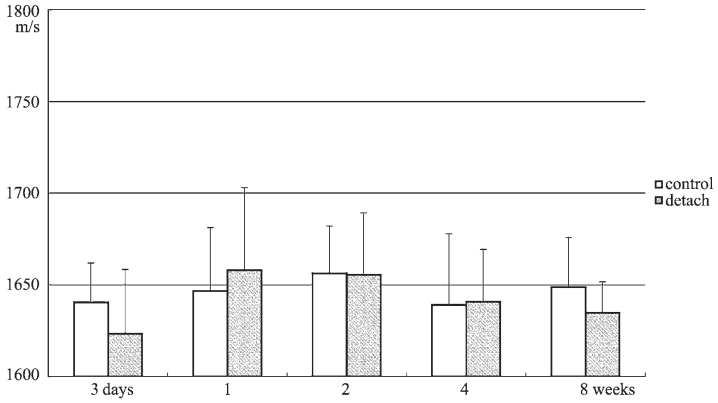

Fig. 6. Tissue sound speed of the whole supraspinatus muscle.
The tissue sound speed of the supraspinatus muscle is not affected by the detachment of the supraspinatus tendon.

DISCUSSION

In the measurement of the material properties of living tissues with SAM, one should take their anisotropic characteristics into consideration. In other words, the material properties of living tissue might vary according to the direction of the measurement. Several authors had already investigated this issue in SAM measurement. Topp and O'Brien (2000) measured the sound speed of excised rat semimembranosus and soleus muscles with their fibers oriented at 90° and 45° to the incident sound beam. They reported that the anisotropy of these muscles was quite small (Topp and O'Brien 2000). As for tendon tissue, Kuo et al. (2001) measured the sound speed of fresh bovine Achilles tendon in various directions of propagation. They found that the maximal speed occurred when the propagation was parallel to the longitudinal fiber axis. The speed decreased as the angle relative to the fiber axis increased (Kuo et al. 2001). Based on these previous reports, we believed that the anisotropy of the tissue did not affect the measurement results significantly in the present study, since we standardized the cutting direction of the specimens and used identical cutting plane for SAM measurements.

Our results revealed that the sound speed of the detached supraspinatus tendon was slightly decreased by 4 weeks and increased at 8 weeks. In the tendon tissue, it has been reported that the fiber arrangement and the mechanical properties were well correlated (Gimbel et al. 2004b). Marffuli, et al. (2000) reported that various degrees of loss of ordered arrangement were seen in the ruptured tendon. In the present study, the sound speed of the tendon stump might reflect the histologic changes including such disorganization of the fiber arrangement followed by the scar formation.

Relationship between the tissue elasticity and the tension of rotator cuff repair

The optimal timing for rotator cuff repair was still controversial. From the biomechanical point of view, one of the key issues for successful cuff repair was the tension of the repair site. In the simple stretching of elastic bodies, it was known that the stress-strain curve in an elastic region is defined as that the stress equals the modulus times strain. The modulus both for tensile and compressive stresses was called as Young's modulus (E). Since the stress was defined as force (F) divided by the area (A), the stress- strain equation could be shown as following equation using ΔL (change in the length) and L (the original length) (Rensik et al. 1992).

$$\frac{F}{A} = E\frac{\Delta L}{L}$$

or

$$F = EA \frac{\Delta L}{L} \quad \cdots\cdots\cdots\cdots\cdots\cdots\text{Equation 2}$$

When this theory was applied to the surgical repair of torn supraspinatus tendons, the total length of the muscle-tendon unit could be regarded as the original length (L), the advancement of the retracted tendon stump during surgery as the change in the length (ΔL), and the repair tension as the force (F). According to Equation 1, the Young's modulus was directory proportional to the square value of the tissue sound speed measured with SAM. Therefore, the repair tension was proportional to both the square value of the tissue sound speed and the amount of advancement of the tendon stump (Equation 2). In other words, the tissue elasticity strongly influenced the tension of the repair at the time of surgery.

Clinical relevance

Davidson and Rivenburgh (2000) investigated the relationship between the repair tension and the clinical outcome in 67 rotator cuff tear patients. They found that high repair tension measured intraoperatively was associated with poor subjective and objective outcomes (Davidson and Rivenburgh 2000). Bassett and Cofield (1983) reported that the postoperative pain relief was acceptable when the surgery was performed within 3-months from the injury. More recently, Lähteenmäki, et al. (2006) also reported that acute rotator cuff tears could always be successfully repaired to its original insertion within 3weeks from the injury (Lähteenmäki et al. 2006). The results of the present study indicated that there were no significant changes in the tissue sound speed after the detachment of the supraspinatus tendon. We assumed that the elasticity of the tendon-muscle unit was well preserved for 8 weeks after the acute tendon tearing.

Although the results of the present study provide important information for determining the timing of surgery, further studies including longer duration models would be necessary to clarify the optimal timing for surgical repair of the torn supraspinatus tendons.

Limitations

There were several limitations in the present study. First, the anatomy of the rabbit shoulder was different from that of humans (Soslowsky et al. 1996). Moreover, the model did not represent any degenerative changes prior to the surgical detachment. Second, the Young's modulus was not directly measured in the present study. As Equation 1 indicated, both the Poisson's ratio and the density of the tissue should be measured at a microscopic level to determine the exact tissue Young's modulus. Third, foreign body reaction due to polyvinylidine fluoride membrane might affect the results of SAM measurements. The circumstance in which the tendon stump was wrapped with polyvinylidine fluoride membrane might differ from that of the torn human rotator cuff tendons.

CONCLUSIONS

Tissue sound speed of the supraspinatus tendon and its muscle was well preserved for 8 weeks after the detachment of the tendon. These results suggested that the retracted supraspinatus tendon stump could be repaired without excessive tension by 8 weeks from the acute tendon tearing.

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