Healing Tissue Response with ITU (Intense Therapy Ultrasound) in Musculoskeletal Tissue, Feasibility Study

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ABSTRACT

Background/ Objective: ITU effectively creates thermal injury zones inside soft tissue, initiating a tissue repair cascade in the skin, promoting collagen generation. It may be feasible to promote a robust healing response in musculoskeletal tissue accelerating healing from injury. The objective of the study is to establish feasibility of generating healing response via ITU thermal injuries in live rabbit Achilles tendon model.

Protocol/Methods: The rabbit studies were performed under protocol approved by IACUC, University of Arizona. Anesthetized animals were imaged with conventional ultrasound (Spark, Ardent Sound, AZ, USA). The Achilles tendon of one limb was exposed and partially transected, the other tendon exposed only and served as an operative control. 24 hours post-surgery Achilles tendons were treated with ITU (Gen 2, GTS, AZ, USA). One set of 2 rabbits, 4 tendons represented 4 groups (cut or not, treated or not). At time points of 4, 14 and 21 days post-treatment the tendons were explanted and subjected to PCR to examine growth factors, cytokine and collagen gene expression. At time points 14 and 21 days tendons were mechanically tested to measure stress-strain curves and rupture strength. Five sets of rabbits (20 tendons) were sacrificed: 1 at 4 days, 2 each at 14 days and 21 days. At all time points the limbs were ultrasonically imaged and recorded.

Results and Conclusions: Results of PCR showed significant increase of the growth factors (TGFβ1), inflammation related interleukin-1 beta (IL-1β) and expected time related reduction and increase Collagen 1A1 and Collagen 1A2. Ultrasound images showed complete tendons' recovery at time points of 21 days when treated with ITU. Mechanical testing for stress-strain and rupture showed no compromise in ITU treated tendons vs. control: uncut/untreated tendons. In conclusion, feasibility of initiating a healing cascade in musculoskeletal tissue in live animal model was demonstrated utilizing ITU.

I. TECHNOLOGY

Intense Therapy Ultrasound (ITU). Ultrasound is well established as a versatile, safe imaging modality that causes no discernable long-term effects on tissue. However, by using a highly directive source geometry with the source energy settings increased significant, ultrasound energy can be focused spatially in a tightly confined region (< 1mm³) to cause selective tissue thermal coagulation [1,2]. The size and location of the lesions can be precisely varied. The ultrasound waves induce a vibration in the tissue molecules during propagation, and the friction developed between intrinsic molecules is the source of the generated heat. ITU is similar to high intensity focused ultrasound (HIFU), however, here we specifically use ITU to refer to the treatment modality which creates multiple, small coagulative lesions, with the specific purpose of stimulating a reparative tissue response. The ITU device used in the study was developed by Guided Therapy Systems (GTS) AZ, USA. A series of selective thermal coagulation zones can be produced along a straight line at a given depth within the tissue (up to 25mm line of discrete lesions spaced 0.5 – 5.0 mm apart). For each series of exposures, the following source conditions can be varies: shot dose (J), shot time (ms), length of exposure line (mm), distance between shots (mm), and time delay after each shot (ms). An example of these lesions is shown in Figure 1.

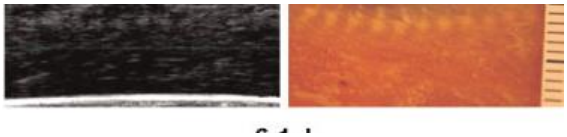

Figure 1. Ultrasound image (left) and sliced gross appearance of ITU lesions in porcine muscle tissue.

II. HYPOTHESIS

The long-term goal of the research was to establish Intense Therapeutic Ultrasound (ITU) as an effective strategy for promoting healing of tendon tissues. ITU can selectively and effectively create minute thermal injury zones deep inside tissues, while sparing overlaying structures [1,2]. Because these damage zones are small (0.1-1mm) they initiate a tissue repair cascade [8]. This effect has been best studied in the skin, where ITU has been shown to promote collagen generation [3,4,5] and is FDA approved for brow elevation and submental skin tightening.

Based on the proven effects in skin we believe that ITU has a promise to promote robust healing response in tendons, leading to an accelerated and/or improved healing from injury. We proposed to test this hypothesis in a rabbit model of tendon

injury (partial transection). The hypothesize is that ITU will do one or more of the following: 1) cause an earlier, more robust healing response (measured by cytokine, growth factors, and collagen production gene expression); 2) lead to an earlier return to normal mechanical properties (measured by ultimate load and maximum tangential modulus, and 3) lead to a healed tendon with collagen structure more similar to control tendon (measured by multiphoton microscopy (MPM), optical coherence tomography (OCT) images and atomic force microscopy (AFM)).

III. CLINICAL APPROACH

ITU creates small zones of thermal injury in the tissue at desired depth

- Reaches depths up to 20 mm below the skin
- Restarts and enhances the production of endogenous growth factors in connective tissue through three phases of activity:

  1). Inflammatory Phase- disruptive cells release "growth factors"

  2). Proliferative Phase – angiogenesis and fibroblasts migrate, deposit type II collagen

  3). Maturation and Remodeling Phase – type III collagen converts to type I and elastin fibers, along with formation of collagen fiber cross linkage

The proposed effort represents to our knowledge, the first attempt to use intense therapy ultrasound to modulate healing response in tendon tissue. One reference was found using high intensity focused ultrasound to successfully create lesions inside *ex vivo* bovine tendon [6], but the lesions formed were relatively large (~10 mm^3) and no *in vivo* study was performed. ITU is well established clinically for facial collagen rejuvenation [3,4,5,11,12], but very little is understood about the mechanisms of that treatment.

Some of the analysis procedures that were used are novel and are being applied to tendon treatment for the first time. Quantitative analysis of second harmonic generation, two-photon excited fluorescence, optical coherence tomography images and atomic force microscopy is relatively new and our study has provided greater insight into healing tendon. The understanding of gene expression in pathologic and treated tendons is incomplete and these studies will help provide a clearer picture of the healing process.

Following are the general procedures used in the study:

a).Rabbit surgery: The right hindlimb of New Zealand White rabbits were shaved and depilated. An incision was made in the skin, the Achilles tendon complex dissected free, an incision made in the paratenon, and the lateral 50% of the Achilles tendon divided 1.5 cm above the calcaneus. A 5-0 Prolene stitch was placed 8 mm from the injury site to aid post- surgical diagnostic ultrasound imaging. The left limb had undergone the same procedures except that the Achilles tendon was not divided (operative control). While a partial transection could introduce some variability, we have found in practice that a 50% transection is reproducible, and that the rabbits require no immobilization from this procedure. They were confined to their cages for the first 7 days, after which they were be allowed every-other-day exercise.

b).ITU treatment: A commercial system from Guided Therapy Systems was utilized for this study 24 hours after surgery. This device has interchangeable handpieces for frequencies from 4-10 MHz, and variable pulse energy (.01 – 3 J), and pulse spacing (0.5 to 5 mm). For this study, 6 lines were placed in the tissue model. For *in vivo* treatments with 7.5 MHz transducers, 3 mm nominal focal depth, 80 shots were placed into the tendon complex from the calcaneus to the muscle insertion points.

c).Ultrasound imaging: An additional commercial high frequency ultrasound system (Spark™) was used to visualize the entire rabbit Achilles tendon before surgery, before and after therapy, and at each subsequent time point.

d).Explant: After humane euthanasia, the Achilles was exposed and paratenon removed. For PCR, a segment of tissue 1.5 cm long, with the incision at the midpoint, was removed and immediately placed in the fixative RNA Later. For mechanical testing, the entire tendon from the calcaneus to the muscle was explanted, wrapped in saline-soaked gauze, and frozen at -20°C. For imaging, the tendon was removed between the calcaneus and muscle insertion points, and fixed in Histochoice.

IV. STUDY DESIGN

The study aimed to check expression of growth factors, cytokines, and collagen at an early stage, and mechanical properties/collagen structure at a late stage of healing, to assure that there was some basis for optimism about this technology in tendon. A total of 12 New Zealand White rabbits were utilized in a study to determine the effect of ITU in a model of partially cut Achilles tendon. The animals were anesthetized, and fur over the hindlimbs shaved and depilitated. The hindlimbs were imaged with ultrasound. The skin and peritenon of the right hind limb were opened and the Achilles tendon was carefully separated and the width was measured. A cross-sectional incision was made from the lateral side of the tendon to the midway point. Incisions were closed and the leg wrapped. In the left leg, incision of skin and peritenon only was performed as surgical control.

The next day, all animals received intense therapeutic ultrasound (ITU) therapy. The following settings were used: energy 1.5 J, spacing between lesions 1.3mm, total number of lesions 80. The hindlimbs were imaged with ultrasound before and after therapy. The leg to receive therapy was alternated between right and left. Therefore, two rabbits (4 tendons) equaled one full set of cut or not and treated or not. One set was used for PCR at 4 days post treatment), two sets for OCR 14 days post treatment, and two sets for mechanical testing/histology at 21 days post treatment.

V. TESTING METHODS AND RESULTS

Figure 2 shows a set of ultrasound data, displaying how the cut, ITU therapy coagula, and healing can be visualized *in vivo*. Ultrasound images show a progression of incision healing on day 1, 4, and 21, with completely healed tendon at day 21.

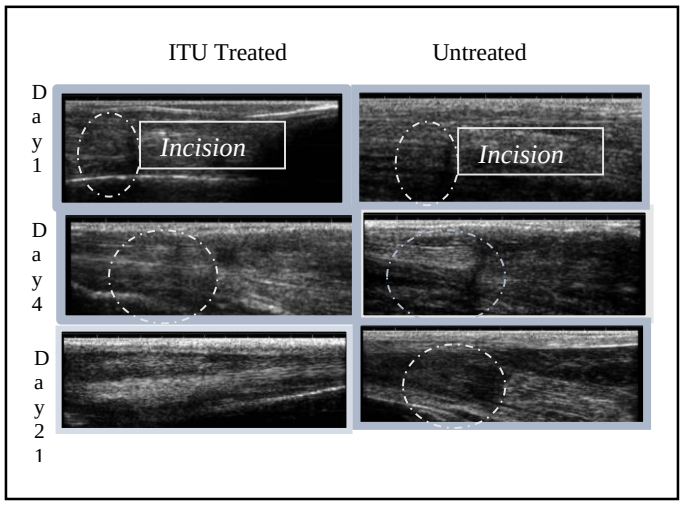

Figure 2. Tendons cut and treated vs. uncut and untreated

Figure 3.PCR Results

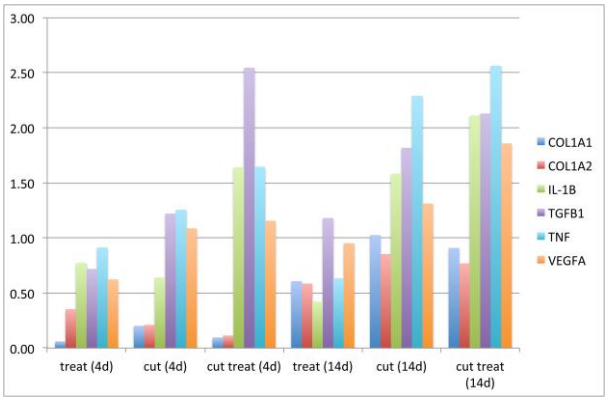

Figure 3. Day 4 (4d) results are from a single tendon each bar, Day 14 (14d) are average of two samples. Normalized to control (uncut/untreated) tendon.

While the sample size is small, results appear to indicate that collagen 1 expression is downregulated early but returns to near normal at 14 days post treatment. At early stages of healing, these results are reasonable and consistent with other findings of cut tendon [7,9]. The results also show that growth factors and cytokines (IL-1β, TGFβ1, TNFa, VEGFA) are upregulated in the cut and treated category at both time points and are uniquely upregulated at the early timepoint (IL-1β, TGFβ1 higher than cut tendon). These results could indicate that healing will be more rapid and/or robust in the treated tendon. Inspection, imaging, and mechanical testing of the tendons at 21 days post treatment provides assurance that treated tendons healed at least as well as untreated tendons with no excessive scar formation (as might occur with excessively high levels of TGFβ1) [10] or more adhesions than the untreated tendon . Photographs and OCT images of the tendons at the explant are given in figure 4. There is no obvious gross evidence of injury.

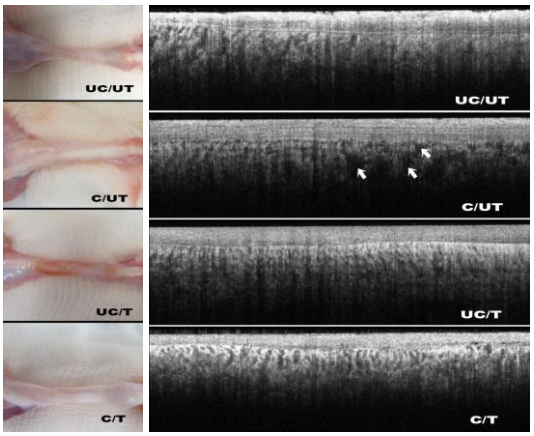

Figure 4. Gross photographs (left) and OCT images (right) of explanted tendons at 21 days. Arrows: fluid pockets seen in untreated tendon. C:cut, T:Treated, U:un. OCT 4 mm x 1.4 mm.

OCT provides cross sectional images of tissue in a manner analogous to ultrasound, except that near-infrared light is used. The result is high (~8 µm) resolution images of tissue microstructure. Shown in Figure 4 are longitudinal OCT images, 4mm long by 1.4 mm deep, over the location of the cut (or comparable location on uncut tendons). Image appearance from animal to animal is similar, although the cut and untreated tendon appears to show some hypointense fluid pockets. All tendons show the overlying sheath, the characteristic crimp pattern (vertical banding) and birefringence pattern (horizontal banding). The banding periods can be quantified are a robust measure of collagen organization.

The tested tendons were fixed, paraffin embedded, and sectioned. Sections were rehydrated and imaged with MPM.

An example pair of images from the MPM, simultaneously obtained second harmonic generation (SHG) and two photon excited fluorescence (TPEF) images of the cut/treated tendon are given in Figure 5, showing fine, parallel collagen fibrils, as would be expected in normal tendon.

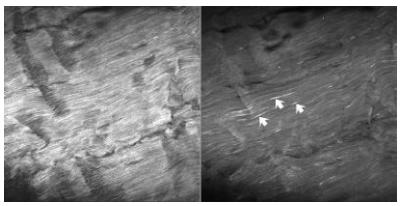

Figure 5. Example SHG and TPEF images of the cut and treated tendon section. SHG images show fine detail of collagen structure. TPEF images show collagen and bright fibroblasts (arrows). Images are 400 µm x 400

AFM was performed on the section of tendons adjacent to the surgical cut with FOV of 35 micron, 9 micron and 3 micron and resolution <1 nanometer. Figure 7 below demonstrated very well organized fibrils of tendon typical for healthy tissue for cut and treated tendon at 21 days.

Atomic Force Microscopy (AFM)

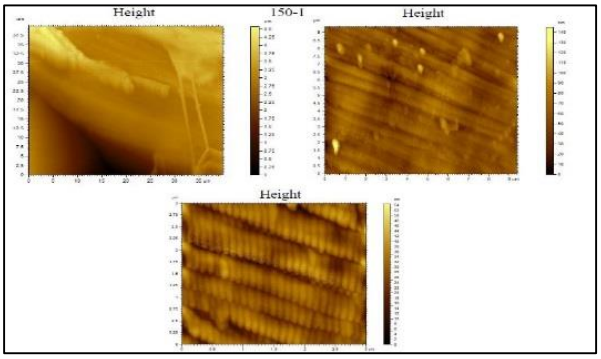

Figure 7. Cut/Treated tendon (21 days)

During mechanical testing the tendons were clamped at the proximal end near the insertion point into the muscle, and the distal end directly above the calcaneus. They were pulled at 1mm/s to failure. Ultimate load (a measure tissue strength) and maximum tangential modulus (MTM) are shown in figure 6.

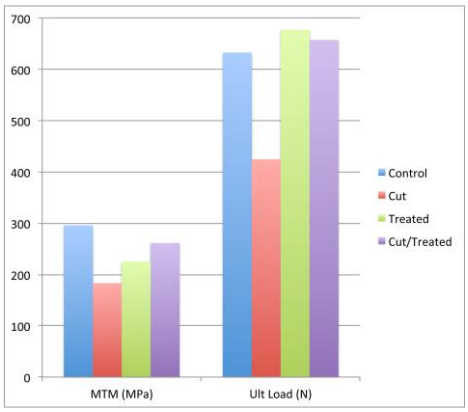

Figure 6. Mechanical properties of tendon in feasibility study (MTM: maximum tangential modulus, Ult: ultimate. Average of two samples each category.

The cut and treated tendons appear to have mechanical characteristics closer to control than cut tendons, suggesting that the treatment may speed or improve healing.

VI. CONCLUSIONS

- The rabbit Achilles tendon model was used for a feasibility study. It appears to be workable and robust for the purpose.
- PCR results show the significant increase in growth factors and collagen following ITU treatment.
- OCT and AFM confirm the recovery of the tendon and formation of new collagen.
- Mechanical testing results show unremarkable differences in strength between controls and treated tendons.
- A comparison between ultrasound imaging of the treated and untreated may show an accelerated healing of the tendon.
- It is feasible to modulate the healing tissue response by ITU in musculoskeletal tissue.

VII. REFERENCES

[1] T.D. Mast, I. R. Makin, W. Fadi, M. Runk, P. Barthe and M. Slayton, "Bulk Ablation of Soft Tissue with Intense Ultrasound: Modeling and Experiments" J. Acoust. Soc. Am. 118 (4). October 2005.

[2] W. M. White, I. R. Makin, P. Barthe, M. Slayton, and R. Gliklichl, "Selective Creation of Thermal Injury Zones in the Superficial Musculoaponeurotic System Using Intense Ultrasound Therapy" Arch. Facial Plast. Surg. Vol. 9, Jan/Feb 2007.

[3] S. Suh, M. Shin, S. Lee, J. Rho, M. Lee, N. Kim, and K. Song "Intense Focused Ultrasound Tightening in Asian Skin: Clinical and Pathologic Results," Dermatol Surg 2011;37:1-8.

[4] H.J. Laubach et al., "Intense Focused Ultrasound Evaluation of a New Treatment Modality for Precise Microcoagulation Within the Skin", Dermatol Surg, 2008. 34(5):p. 727-34.

[5] M. Alam, L. White, N. Martin, J. Witherspoon, S. Yoo, and D. West, "Ultrasound Tightening of Facial and Neck Skin: A Rater-Blinded Prospective Cohort Study", Meeting of the American Sociaty for Dermatologic Surgery, Chicago, IL, Oct. 11-14, 2007 and the American Societ for Laser Medicine and Surgery, Kissimmee, FL., April 2-6, 2008.

[6] R. Muratore, T. Akabas, and I. Muratore, "High-Intensity Focused Ultrasound Ablation of *Ex Vivo* Bovine Achilles Tendon", Ultrasound in Medicine and Biology, Volume 34, Issue 12, Pages 2043-2050, Dec. 2008.

[7] M. Berglund, D.A. Hart, and M. Wiig, The Inflammatory Response and Hyaluronan Synthases in the Rabbit Flexor Tendon and Tendon Sheath Following Injury", J Hand Surg Eor Vol October 2007 vol. 32 no. 5 581-587.

[8] D. Manstein, G.S. Herron, R.K. Sink, H. Tanner, and R. R. Anderson, "Fractional Photothermolysis: a New Concept for Cutaneouse Remodeling Using Microscopic Patterns of Thermal Injury", Lasers Surg Med 2004.

[9] S. Sun, Y. Ding, B. Ma and Y. Zhou, "Two-stage Flexor Tendon Reconstruction in Zone II Using Hunter's Technique, Orthopedics, Dec. 2010, Volume 33, Issue 12

[10] J. Chang, R. Thunder, D. Most. M.T. Lonhaker and W.C. Lineaweaver, "Studies in flexor tendon wound healing: neutralizing antibody to TGF-beta1 increases postoperative range of motion. Plast Reconstr Surg 2000, Jan; 105(1):148-55.

[11] J.L. MacGregor and E.L. Tanzi, "Microfocused Ultrasound for Skin Tightening", Semin Cutan Med Surg 32:18-25, 2013.

[12] M. Slayton, R. Sharpe, "Handheld Intense Therapy Ultrasound: Prospective Clinical Study of Lower Facial and Submental Skin Tightening" ASLMS 33rd Annual Conference, April 2013.