Introduction: Surgical repairs of rotator cuff tears are associated with a high rate of complications; typically by full or partial re-nupture of the repair. In the clinical setting, several adjuvant therapies have been used to enhance healing of soft tissue injuries, including electrical stimulation\(^1\), laser photostimulation\(^2\) and ultrasound\(^3\). The ability of pulsed low intensity ultrasound (PLIUS) to stimulate bone growth was first reported in 1983\(^4\). Since this report, numerous studies have documented the efficacy of PLIUS in fracture repair. In addition to fracture healing, PLIUS has been shown to stimulate soft tissue healing. Recent studies have shown that treatment with PLIUS improved cartilage repair in a lapine model\(^5\) and enhanced the healing of MCL injuries in a preclinical murine model\(^6\). PLIUS has also been shown to improve the mechanical and histological properties of tendon-bone healing in an adult ovine stifle model of ACL reconstruction\(^7\). The study presented here evaluated the histological properties of rotator cuff repair healing when augmented with PLIUS in an ovine model that has been successfully used to study rotator cuff injuries in our laboratory\(^8\).

**Materials and Methods:** Eight sheep underwent rotator cuff repair using the infraspinatus tendon in a similar manner to that reported previously\(^9\). The tendon was isolated, sharply dissected and reattached to a bone trough created at the greater tuberosity of the humerus using a modified Mason-Allen suture pattern. The experimental group (US) received ultrasound treatment to the operated shoulder for 20 minutes/day. The control group (nonUS) received sham ultrasound for 20 minutes/day. After 12 weeks of healing animals were euthanized, the operated shoulders were harvested, and tissues prepared for histological analysis. One animal from the nonUS group developed an infection and the results are not included in this study. The tendon-bone complex was fixed in 10% neutral buffered formalin, decalcified and then embedded in paraffin. Tissue sections were stained with hematoxylin and eosin and graded according to a scale developed in our laboratory to stage histological sections. Grading of histological sections was made in 0.5 increments. The effects of ultrasound treatment on qualitative rankings (cellularity, vascularity, bone remodeling, and tendon integration beneath the suture) was found to be easily applicable, extremely specific to the features we were scoring system described in Figure 1.

**Results:** Overall results for histological evaluation of tendon healing are presented in Table 1. Well-organized fibrocartilage tissue was observed surrounding the suture material. Inflammatory cells were not frequently observed. Lamellar and woven bone were observed in all histological sections. Osteoclastic bone resorption and osteoblastic bone formation was predominantly present at the interface between the bone and cartilage. Collagen interdigitation between tendon and bone was observed in sections of both treatment groups, with more extensive integration in sections from the US treated group. Subchondral bone remodeling was significantly more active (lower score) in the US treated group compared to the nonUS group (Figure 2). Because suture material was evident in all 4 sections from the US treatment group and was not evident in any of the nonUS treatment group sections, bone-tendon interface was evaluated throughout the entire attachment site. A mixture of direct and indirect tendon insertion was found for both treatment groups. Significantly better bone-tendon integration was observed in sections from the US treated group compared to the nonUS treatment group (Figure 3). Overall, US treated sections showed better collagen interdigitation and more frequent direct fiber insertion compared to the nonUS treatment group.

**Discussion:** From this semi-quantitative analysis, US treatment improved bone-tendon integration while improving bone remodeling beneath the suture. Often histological results are purely observational without grading scales applied. To the authors’ knowledge, grading of histological integration of rotator cuff tendon in preclinical evaluation has not been used. The grading system developed for this study was found to be easily applicable, extremely specific to the features we were investigating. Observer variability was not investigated, yet only one investigator (TM) evaluated all sections with concurrence of a second investigator (DW). Sections from both treatments showed delineations of decalcified and calcified fibrocartilage zones, consistent with healed or normal tendon attachment. Ultrasound treated sections had better bone remodeling and superior bone-tendon integration compared to nonUS tendons. Also, PLIUS treated tendons frequently revealed more intense new bone formation at the bone trough compared to untreated tendons. Based on previous work completed in this laboratory, bone-tendon integration beneath the suture has been shown to be different from integration away from the suture. Less evidence of bone-tendon integration is usually noticed beneath suture, indicating that biomechanical forces, which stimulate tendon healing, are not as active at the attachment site close to the suture material. All sections from the untreated tendon lacked suture material therefore evaluation of the quality of attachments with respect to proximity to suture material could not be assessed in this study.

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