The Effects of Low-Intensity Pulsed Ultrasound on Tendon-Bone Healing in a Transosseous-Equivalent Sheep Rotator Cuff Model

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INTRODUCTION:

Rotator cuff surgery has improved through use of suture anchors and understanding mechanical issues related to fixation. Biological improvements in tendon-bone healing in the shoulder represent an area of new focus to augment patient outcome. Low-Intensity Pulsed Ultrasound (LIPUS) has been shown to have positive biological effects on different stages of bone healing, including angiogenesis, chondrogenesis and osteogenesis [1]. LIPUS increases many aspects of cell proliferation, protein synthesis, and cytokine production which can have an in vivo effect [2]. In an intra-articular sheep ACL model LIPUS was shown to increase mechanical strength and improve healing by a notable increase in vascularity and cellular activity at the tendon-bone interface [3].

This study examined the effects LIPUS has on tendon-bone healing in a clinically relevant extra-articular transosseous-equivalent ovine rotator cuff model. We hypothesized that LIPUS would enhance and hasten tendon-bone healing.

METHODS:

8 adult cross-bred wethers (18 months of age) were randomly allocated to either the control or LIPUS group following ethics approval. The infraspinatus tendon was detached from its insertion into the greater tuberosity to expose the footprint and repaired with a double row suture bridge construct using 4.5 mm PEEK Corkscrew Suture Anchors (Arthrex, Karlsfeld, Germany). LIPUS treatment was applied daily for the duration of 20 minutes at the repair site until sacrifice at 4 weeks.

Tendon-bone constructs were harvested and processed for histology, immunohistochemistry and microcomputed tomography (micro-CT). Histological sections were cut at 5 microns and stained with H&E for tissue morphology and cellular constituents. Histology was qualitatively graded for new bone formation, cellular activity, Sharpey's fibres and collagen fibre allignment. Immunohistochemistry staining for protein expression of VEGF and appropriate controls was performed using routine techniques [4]. Protein expression was graded according to both staining intensity and cellular distribution.

Intact humeral head – infraspinatus tendon complex was scanned using a Siemens Inveon micro-CT system (Siemens Medical Solutions, USA), with a resulting effective pixel size of 50.88μm. Scans were examined using MIMICS software (Mimics 12.0, Materialise, Belgium). The trans-axial midline of the tendon footprint was identified visually by aid of suture anchors in each sample. Five evenly spaced axial slices were evaluated for BMD measurement within five circular regions of interest of each slice. Average BMD was taken across 5 slices.

BMD data was analyzed using SPSS version 17.0 for Windows (SPSS Inc., USA). T-Test was performed ($p \leq 0.05$) to examine the effect of LIPUS.

RESULTS:

Micro-CT analysis of volumetric bone density at the tendon-bone interface demonstrated an overall increase in BMD in the LIPUS group *(Figure 1)*.

Histological results showed a thicker region of newly formed bone at the tendon-bone interface with increased osteoblast activity along the bone surface in the LIPUS-treated group compared to the controls. The interface in the LIPUS-treated group revealed a continuum between the tendon and bone in an interdigitated fashion in contrast to the control group where discontinuous contact between the tendon and bone was observed. Structurally, more mature collagen fibre allignment and less disorganization was noted in the LIPUS group compared to the controls.

VEGF expression was strongest in the LIPUS-treated group with a broader distribution and a higher intensity of positively stained cells at the tendon bone interface *(Figure 2)*.

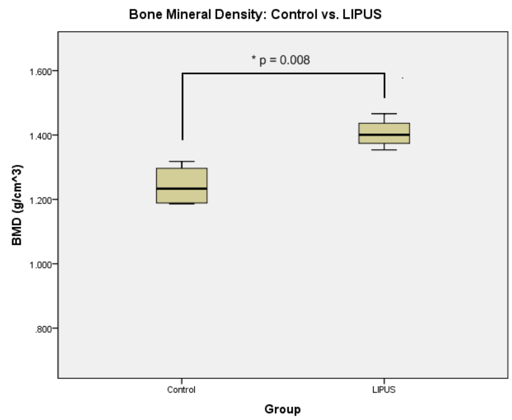

Figure 1. *Increase in Bone Mineral Density (p = 0.008) at the tendon-bone interface was noted between the LIPUS-treated group and control group at 4 weeks.*

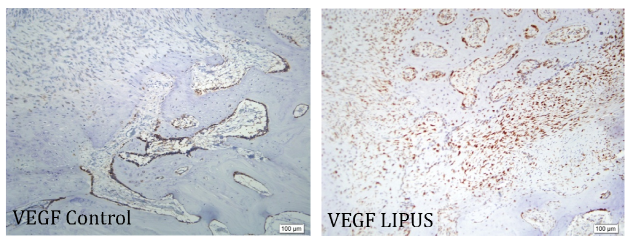

Figure 2 *VEGF expression at the tendon-bone interface at 4 weeks (10x objective).*

DISCUSSION:

The current study explored the effects of LIPUS application following an ovine rotator cuff repair using a transosseous-equivalent technique which optimizes tendon to bone contact dimensions, while providing initial strength sufficient to withstand immediate weight-bearing by the sheep postoperatively. A biological effect was noted following only a 4 week treatment with LIPUS compared to controls. Micro-CT BMD measurements as well as histological grading revealed greater woven bone formation in the LIPUS-treated group.

LIPUS group also expressed stronger VEGF positive signals, including osteoblasts within woven bone and vascular endothelial cells, then the control group. This was confirmed by increased cellular distribution as well as staining intensity of the positively stained cells.

This study is limited by small animal numbers and only one time point however it confirms results from previous in vitro[5] [6] and in vivo [7] [8] studies suggesting that LIPUS improves tendon-bone healing by up-regulating angiogenic and osteogenic pathways. Furthermore this study broadens this research into the context of tendon-bone healing in a clinically relevant extra-articular rotator cuff model.

LIPUS is a simple and non-invasive treatment that has the potential to increase the quality of repair following rotator cuff surgery.

REFERENCES:

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