In Vivo Evaluation of a Biomimetic Biphasic Scaffold in Sheep

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Introduction: Injuries to the rotator cuff often occur at the supraspinatus tendon-to-bone insertion[1,2], thus biological fixation of the tendon is crucial for long term stability and repair. The native tendon-to-bone junction consists of a continuous transition progressing from the tendon proper to non-calcified and calcified fibrocartilage, and to bone[3-5]. However, this matrix heterogeneity is not regenerated following rotator cuff repair; thus significant demand exists for grafting systems that enable functional tendon-bone integration through insertion site regeneration. To this end, a biomimetic biphasic scaffold with contiguous nonmineralized (Phase A) and mineralized (Phase B) regions designed to facilitate the regeneration of the tendon-bone insertion has been developed. Specifically, Phase A is composed of nanofibers of Polylactide-co-glycolide (PLGA) and Phase B consists of composite PLGA nanofibers and hydroxyapatite (HA) nanoparticles (PLGA-HA). Previous studies have demonstrated the biphasic scaffolds promoted the regeneration of the native tendon-bone insertion both in vitro and in vivo in a rat model[6,7]. However, due to the small size, surgical procedures performed on rats are not representative of surgeries in clinical settings. Therefore, before clinical evaluation, it is necessary to evaluate the scaffold in a large animal model to assess surgical feasibility and efficacy. To this end, the objective of this study is to test the biphasic scaffold using a sheep rotator cuff model, due to the similarity in size of the sheep infraspinatus tendon to that of the human tendon [8]. It is hypothesized that the commonly practiced double row suture bridge technique can be used to repair the tendon graft with the scaffold, and the biphasic scaffold will be biocompatible and enable the regeneration of fibrocartilage interface between repaired tendon and bone.

Methods: Scaffold Fabrication and Characterization: Biphasic nanofiber scaffolds composed of aligned fibers of PLGA (85:15,Lakeshore) and PLGA-HA (15% HA, 100-150nm, Nanocerox) were produced via electrospinning[9, 10]. A trial surgery was performed on a sheep cadaver to determine the size of scaffold that is required to cover the whole sheep infraspinatus tendon-to-bone footprint. The final scaffolds used were 20mm x 15 mm x 0.4 mm. Scaffolds were sterilized with ultraviolet radiation and kept sterile until use. In Vivo Model: In skeletally mature female sheep (n=18, 200±19 lb, > 4 years), a unilateral surgery was performed on the right shoulder with or without the biphasic scaffold. During surgery, the infraspinatus tendon was identified and detached at the tendon-to-bone junction followed by removal of fibrocartilage at the insertion site. Four anchor tunnels were drilled on the greater tuberosity of the humerus for suture anchor placement. For the experimental group, the nanofiber scaffold was inserted between cancellous bone and the distal end of the detached tendon in a specific orientation such that the PLGA phase contacted tendon and the PLGA-HA phase contacted the bone, and the fiber alignment of the scaffold matched that of the native tendon. The scaffold was placed underneath the tendon and fixed to bone via the double row suture bridge method. In the control group, the tendon was reattached to the humeral head using the same technique, without the scaffold. Animals were sacrificed at 4 and 16 weeks, and ultrasound (n=9), MRI (n=2), serology (n=9), muscle fat content (n=9), and histological analyses (n=9) of the samples were performed. End-Point Analysis: Prior to surgery, the ultrasound images of the right shoulder of each sheep and a blood sample were obtained as “native” for the purpose of comparison. At each time point, prior to sacrifice, blood was collected. After sacrifice, ultrasound images of the repaired shoulder were obtained. In addition, for each time point, a MRI scan of one sheep from each group was obtained. The humerus of each sheep with only infraspinatus tendon attached was harvested and fixed in 10% NBF for one month and stored in 35% ethanol for long term preservation. Samples were cut longitudinally into 3-5 mm pieces and scanned with microCT for mineral content at the interface. In addition, collagen, GAG, general matrix formation as well as mineral distribution were determined through histology with paraffin and PMMA embedding. Statistical analysis of blood parameters and mineral density results was performed using ANOVA and the Tukey-Kramer post-hoc test for all pair-wise comparisons (p<0.05).

Results: All sheep survived through the week 4 time point, and two sheep died from unrelated causes on week 15. Ultrasound images revealed that in the short term (week 4), the void space on the humeral head created by suture anchors are visible. By week 16, the void space is filled by newly formed tissue in both groups (Fig. 1). MRI images showed similar findings. Interestingly, MRI also revealed the formation of ectopic bone between tendon and bone in the control shoulder, which was confirmed visually by sample dissection. This was not observed for any of the scaffold repaired samples. Blood parameters, such as calcium, phosphate, neutrophil count, and protein content remained the same between groups up to week 16. The mineral content of the scaffold group at week 4 was higher than that of the control group; mineral content was maintained at week 16 and found to be comparable between groups (Fig 2). Histological staining revealed that at weeks 4 and 16, an extracellular...
matrix rich in collagen was formed at the tendon-bone junction in both groups. However, polarized images revealed that the newly formed tissue in the scaffold group was well organized, with parallel fibers inserting from tendon to bone. In contrast, collagen organization was absent in the control group at both tendon and interface regions. In addition to positive proteoglycan staining at the tendon-bone junction in the scaffold group, a well-organized mineralized neointerface region was observed at week 16 (Fig. 3).

Figure 1. Ultrasound images of un-operared, shoulder, control repaired shoulder and biphasic scaffold repaired shoulder at weeks 4 and 16 post surgery. Images show that the gap between tendon and bone visible on week 4 images (arrows) is filled by regenerated tissue by week 16.

Discussion: The biphasic scaffold was successfully used to repair the infraspinatus tendon to bone using the double row suture bridge technique in the sheep model. Results showed that the biphasic scaffold is biocompatible, as blood parameters were
comparable to controls, and no excess neutrophil or blood calcium and phosphate presence were detected. The mineral density of the region of interest of the scaffold repaired group was higher than that of the control group at week 4, which maybe important in the early stages of repair and mineralization of the tendon-bone junction. Histology results showed that although a collagen-rich extracellular matrix was formed at the tendon-bone junction in the control group, the matrix is less organized and more scar tissue-like. Therefore, the aligned nanofiber organization of the scaffold acts as a guide for the host cells to infiltrate and deposit matrix. Most importantly, a fibrocartilage-like matrix with a well-organized mineralization front was observed with biphasic scaffold repair. These results collectively demonstrate the clinical translation potential of the biphasic scaffold for integrative rotator cuff repair.

**Significance:** Rotator cuff tears are the most common shoulder condition, with over 300,000 cuff repair surgeries performed in the United States annually. Clinical success is hindered by an unmet demand for grafting systems that could promote tendon-bone integration.

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