INTRODUCTION: Over 300,000 rotator cuff repair surgeries are performed annually[1]. Injuries to the rotator cuff often occur at the tendon-to-bone insertion[2], thus tendon integration is critical for long-term stability and functionality of clinical repair. The native tendon-to-bone junction consists of a continuous transition from the tendon proper to non-calcified and calcified fibrocartilage, and then to bone[3-5]. This controlled matrix heterogeneity, however, is not regenerated following rotator cuff repair; thus significant demand exists for integrative grafting systems that enable functional tendon-to-bone healing.

To promote biological fixation, we have developed a biomimetic biphasic scaffold with contiguous non-mineralized (Phase A) and mineralized (Phase B) regions that are designed to facilitate the regeneration of the tendon-bone insertion. Specifically, Phase A is composed of nanofibers of poly(lactide-co-glycolide) (PLGA) and Phase B consists of composite nanofibers of PLGA and hydroxyapatite (HA) nanoparticles (PLGA-HA) (PLGA-HA). The objectives of this study are two-fold: 1) to evaluate the formation of non-mineralized and mineralized fibrocartilage on the biphasic scaffold using a subcutaneous rat model, and 2) to determine the osteointegration strength of the mineralized region (Phase B) of the biphasic scaffold. It is hypothesized that Phase B of the biphasic scaffold will integrate with bone and distinct yet continuous regions of non-calcified and calcified interface-like tissue will form on the biphasic scaffold in vivo.

METHODS: Scaffold Fabrication/Characterization: Aligned, biphasic nanofiber scaffolds (1x0.5x0.028 cm) composed of PLGA (85:15, Lakeshore) and PLGA-HA (15% HA 100-150nm, Nanocerox) were produced via electrospinning[6,7]. Mineral distribution and scaffold mechanical properties were determined. Cells/Cell Culture: Chondrocytes were obtained by enzymatic digestion of full-thickness cartilage isolated from the tibial plateaus of neonatal calves. Cells were seeded on biphasic scaffold (3.5x10^6 cells/scaffold) and cultured in fully supplemented DMEM (10% FBS) for two days prior to implantation. Scaffolds were pre-coated with fibronectin (10µg/mL) before seeding. For in vivo tracking, cells were pre-labeled with DiO (green, Invitrogen). In Vivo Model/Study Design: Athymic male rats (n=20, NIH-RNU, 220±19g) were used in this study. Following isoflurane anesthesia, four individual subcutaneous pouches (1.5cm) were formed via single 220±19g) were used in this study. Following isoflurane anesthesia, four individual subcutaneous pouches (1.5cm) were formed via single

significant differences observed between acellular and cellular groups at week 8 (p<0.05). The implanted cells remain viable in vivo and are identified throughout the biphasic scaffold at both weeks 3 and 8 (Fig. 3D, DiO-green). Micro-CT and histology of the scaffold-bone interface also demonstrate extensive osteointegration (Fig. 3B,C, week 3).

DISCUSSION: The long-term goal of this research is to design a scaffold system that will promote the integration of tendon graft and bone and enable insertion site regeneration. Specifically, in this study, we evaluate scaffold osteointegration potential in vivo and examine tissue formation within each scaffold region. The biphasic scaffold design provides region-dependent mineral content and allows for an increase in compressive mechanical properties across the scaffold from Phase A to Phase B which mimic those of native direct insertion sites[5,10]. Our in vivo results demonstrate that the biphasic scaffold supports the formation of a collagen- and GAG-rich matrix, and this effect is enhanced when pre-seeded with chondrocytes. Furthermore, similar to the native interface, distinct yet continuous phases of non-calcified and calcified regions of fibrocartilage-like tissue were observed on the biphasic scaffold. It is evident in this study that Phase B is indeed osteointegrative, and moreover, the strength of integration increases with time and is enhanced by cell seeding. These results collectively demonstrate that the biphasic scaffold is a promising grafting system for integrative rotator cuff repair, and future studies will focus on evaluating its efficacy in a rotator cuff repair model.