Osteointegrative Biphasic Nanofiber Scaffold for Functional Rotator Cuff Repair

[1]Moffat, KL; [1]Cassilly, RT; [1]Dargis, BR; [1]Zhang, X; [1]Liu, XS; [1]Guo, XE; [2]Doty, SB; [1]Levine, WN; and [1]+Lu, HH
[1]Columbia University, Department of Biomedical Engineering, [2]Hospital for Special Surgery, New York, NY; +hl2052@columbia.edu

Introduction: Rotator cuff tendon tears are the most common shoulder injury[1] with the majority of ruptures occurring at the tendon-bone interface[2]. Thus, integrative tendon repair poses a significant clinical challenge. To address this critical problem we have designed a biomimetic biphasic scaffold with non-mineralized (*Phase A*) and mineralized (*Phase B*) regions for the regeneration of the tendon-bone interface. *Phase A* is composed of nanofibers of poly(lactide-*co*-glycolide) (PLGA) and *Phase B* consists of PLGA and hydroxyapatite (HA) composite nanofibers (PLGA-HA). The study **objectives** are: **1)** to evaluate the formation of distinct yet contiguous non-calcified and calcified fibrocartilage interface-like regions on the biphasic scaffold *in vivo*, and **2)** to determine the osteointegration potential of the mineralized phase (*Phase B*) of the biphasic scaffold. It is **hypothesized** that the novel biphasic scaffold will be osteointegrative and support the formation of a multi-tissue fibrocartilage interface *in vivo*.

Methods: ***Scaffold Fabrication***: Aligned biphasic nanofiber scaffolds (1x0.5x0.028cm) composed of PLGA (85:15, Lakeshore) and PLGA-HA (15% HA 100-150nm, Nanocerox) were produced via electrospinning[3,4]. Mineral distribution and scaffold mechanical properties were determined. ***Cells/Cell Culture***: Chondrocytes were enzymatically digested from articular cartilage of neonatal calves, and seeded on biphasic scaffolds ($3.5x10^6$ cells/scaffold), cultured in fully supplemented DMEM (10% FBS) for two days prior to implantation. ***In Vivo Model/Study Design***: Athymic male rats (n=20, NIH-RNU, 220±19g) were used. Four subcutaneous pouches (1.5cm) were formed on the rat dorsum of each rat. The *experimental group* included chondrocyte-seeded biphasic scaffolds, while acellular biphasic scaffolds and sham served as *controls*. The animals were sacrificed at 3 and 8 weeks, and the samples were evaluated for matrix deposition (n=4, *picrosirius red, alcian blue, von Kossa*). ***Osteointegration***: Biphasic scaffolds (0.75x21x0.028 cm) were wrapped around cylindrical bone cores (Ø0.5x1.5 cm) isolated from the tibial plateau of neonatal bovine (Fig. 3A), and implanted for 3 and 8 weeks as described above. Cell-seeded scaffolds were compared to acellular controls. Bone core push-out strength (n=8) was measured (Instron, 5 mm/min) and mineral distribution was quantified by micro-CT (n=4). Two-way ANOVA plus Tukey-HSD post-hoc test were performed ($p<0.05$).

Results: The biphasic scaffold consists of distinct, yet continuous regions of PLGA and PLGA-HA (Fig. 1), as confirmed by EDAX line scan analysis, with a sharp increase in Ca and P from *Phase A* to *Phase B*. This spatial mineral distribution is also maintained *in vivo* (Fig. 2) on both cellular and acellular scaffolds. Mechanical properties of the biphasic scaffold are phase-specific (Table 1, *$p<0.05$) and tensile properties approach that of native rotator cuff tendons[5]. ***Matrix Distribution***: Both collagen and GAG are abundant and well distributed throughout both phases of the cell-seeded scaffolds (Fig. 2). Matrix deposition and in-growth, however, is less extensive in the acellular control. ***Osteointegration***: Push-out strength for both groups increases over time (Fig. 3B, ^$p<0.05$), with significant differences observed between acellular and cellular groups at week 8. Micro-CT analysis of the scaffold-bone interface reveals significant increase in mineral content over time (Fig. 3D).

Discussion/Conclusions: In this study, we evaluated scaffold osteointegration potential *in vivo* and examined tissue formation within each scaffold region. Our results demonstrate that the biphasic scaffold supports the production of a collagen- and GAG-rich matrix, and this effect is enhanced when scaffolds are 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 formed on the biphasic scaffold. It is evident that *Phase B* is osteointegrative, and moreover, the strength of integration increases with time and is enhanced by cell pre-seeding. These results collectively demonstrate that the biphasic scaffold is a promising grafting system for integrative rotator cuff repair, and future studies will evaluate its efficacy in a rotator cuff repair model.

References:[1]Vitale *et al*., 2007; [2]Iannotti *et al*., 1994; [3]Reneker and Chun, 1996; [4]Moffat *et al*., 2009; [5]Itoi *et al*., 1995.
Acknowledgements:Technical assistance from S. Greco, S. Subramony, C. Erisken; Funding: NIH/NIAMS (AR056459-02), NYSTEM.

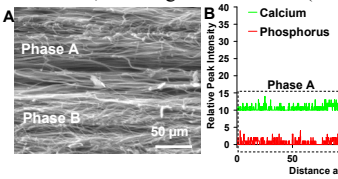

Fig. 1*: Biphasic Scaffold **A)** Contiguous Phase A and Phase B (SEM, 1000x), **B)** HA detected only in Phase B (EDAX line scan spectra (Ca – green, P – red)*

Table 1*: *: $p<0.05$

Table 1 Scaffold Properties	*Phase A*	*Phase B*	*Biphasic*
Tensile Modulus (MPa)	341±31*	166.4±17	324.4±24
Yield Strength (MPa)	9.8±1.1*	3.1±0.2	7.7±0.4
Ultimate Strength (MPa)	12.0±1.5*	4.1±0.5	9.2±0.3
Compressive Modulus (MPa)	0.21±0.02	0.76±0.2*	-

Fig. 2*:Histological analysis of biphasic scaffolds following 3 weeks of implantation. L-R) Picrosirius red, Alcian blue, von Kossa (32x, bar=20 μm).*

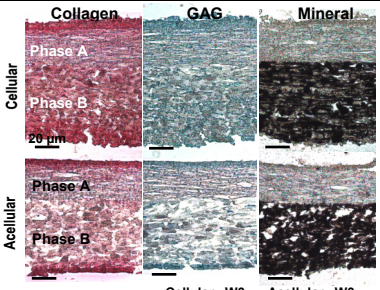

Fig. 3*:Scaffold Osteointegration. Scaffold-bone core construct **(A)** stereo-micrograph, **(C)** MicroCT scan (bar=0.25 cm); **B)** Push-out strength (*:$p<0.05$ between groups, ^:$p<0.05$ over time); D) Normalized mineral density at scaffold-bone core interface as a function of time ^:$p<0.05$ over time).*