Ultrasound As a Physical Force for Enhanced Scaffold-Based Bone Repair

J. Veronick[1], Y. Kutes[2], B. D. Huey[2], Y. Khan[1]
[1]Raymond and Beverly Sackler Center for Biomedical, Biological, Physical and Engineering Sciences, University of Connecticut Health Center, Farmington, CT, [2]University of Connecticut, Institute of Materials Science

Statement of Purpose: Fracture non-unions occur in as many as 10% of simple bone fractures [1]. Low intensity pulsed ultrasound (LIPUS) has shown efficacy in both reducing fracture healing times by 38% and in healing non-union defects (with an 85% success rate) [1]. Given the correlation between LIPUS and bone repair considerable work has been done to examine how osteoblasts respond to LIPUS, and considerable progress has been made. What is less clear is the mechanism behind the healing effects of LIPUS, and how LIPUS may be used in conjunction with scaffold-based tissue engineering. We hypothesize that LIPUS applies a physical force to the fracture site and thus contributes to healing through mechanical loading. Here we demonstrate the effect of this physical force on cells encapsulated in a tissue-engineering collagen hydrogel, both through visualizing LIPUS-induced hydrogel deformation and through measuring osteoblast gene expression. Fluorescent beacons were observed to deflect within the hydrogel after LIPUS exposure, while osteoblasts encapsulated within the hydrogel were noted to upregulate both alkaline phosphatase and osteocalcin expression, both well-established osteoblast phenotype markers.

Methods: Collagen scaffolds (0.1%, 0.2%, 0.3%) were made using rat tail Collagen Type I (BD Biosciences) as directed by protocol. All reagents were cooled and mixed on ice, including α-MEM media (Invitrogen) and Fluoro-Max beads (Thermo Scientific), adding the collagen concentrate last. Imaging of collagen scaffolds with the encapsulated fluorescent beads under LIPUS was performed using a Nikon optical microscope equipped with epifluorescence, and a water-cooled digital camera (Hammamatsu, Inc.). Images were collected and evaluated using Volocity Software, (Improvision, Inc.). Gels were subjected to LIPUS (1MHz carrier frequency, 1 kHz pulse-rate frequency) with 50% and 100% duty cycles for 60 seconds: 0-20sec (off); 20-40sec (on); 40-60sec (off). For cell studies, collagen scaffolds were made as indicated above; however, in place of fluorescent beads, MC3T3 cells suspended in α-MEM media supplemented with 10% FBS and 1% penicillin/streptomycin were mixed on ice. After thorough mixing, 3mL of the collagen solution (containing 100,000 cells) were injected per well into 6-well plates (BD Biosciences). Cells were treated with LIPUS (20% duty cycle, 30mW/cm^2 intensity) for 20 minutes per day (experimental group) and no LIPUS (control group) for 1, 3, and 7 days. At each time point, cells were washed with PBS (Invitrogen) and RNA was extracted with the RNeasy Kit (Qiagen) and samples stored at -20°C. Genes of interest (Alkaline Phosphatase and Osteocalcin) and house-keeping genes (GAPDH, Actb, 18s) TaqMan probes (Applied Biosystems) were used. Gene expression analysis was performed using iCyclerIQ PCR plates on the MyIQ2 Real-Time PCR Detection System (BioRad).

Results: Imaging of hydrogels before, during, and after LIPUS reveals the displacement of fluorescent beacons within the hydrogel, indicating that the applied force from LIPUS results in hydrogel deformation. Figure 1 demonstrates this optically (top) and statistically (lower left) for several hundred beads exposed to progressively stronger duty cycles, quantitatively correlating deformation and LIPUS. Gene expression analysis of

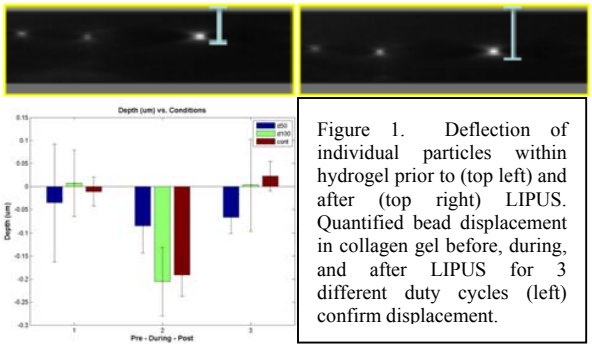

Figure 1. Deflection of individual particles within hydrogel prior to (top left) and after (top right) LIPUS. Quantified bead displacement in collagen gel before, during, and after LIPUS for 3 different duty cycles (left) confirm displacement.

MC3T3 cells encapsulated in 0.1% collagen scaffolds treated with LIPUS showed an upregulation of alkaline phosphatase and osteocalcin at day 7 in comparison to the untreated group (control) (Figure 2).

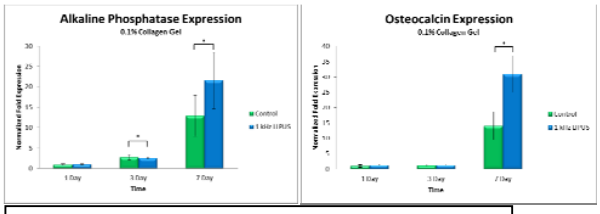

Figure 2. MC3T3 gene expression over 1, 3, and 7 days from cells in 0.1% collagen scaffolds is enhanced with LIPUS exposure over cells not exposed to LIPUS.

Conclusions: Results show varied hydrogel deformation depending on the LIPUS duty cycle applied, suggesting that a controllable physical force can be applied to the hydrogel using LIPUS. Upregulation of alkaline phosphatase and osteocalcin gene expression of MC3T3 cells in 0.1% collagen scaffolds as a result of LIPUS-based mechanical loading does indeed affect the behavior of bone forming cells, perhaps toward accelerated healing. Further investigation of gene expression and extended time points will provide a better understanding of osteoblast response to LIPUS and how this may correlate to enhanced fracture healing.

References: [1] Khan Y. JBJS 2008;90;138-144.