Imparting Physical Load to Synthetic Extracellular Matrices Using Ultrasound

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Statement of Purpose: Considerable work has been done evaluating the efficacy of low intensity pulsed ultrasound (LIPUS) as a treatment for bone repair. Clinical data has demonstrated a 38% reduction in healing time for fresh fractures, and as high as 85% success in healing non-union defects [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. Studies performed by the PI in which LIPUS administration resulted in active β-catenin upregulation in MC3T3 cells [2], a phenomenon that had previously been linked to the administration of a physical force to cells [3], suggest that LIPUS may be imparting a physical load on objects within its sound field. The goal of this study was to evaluate the physical effects of LIPUS in an aqueous environment, and to examine whether LIPUS may be used as a means to apply physical load to particles estimating cellular bodies, embedded in a synthetic extracellular matrix (ECM).

Methods: *LIPUS Force and Heating Measurement:* Ultrasonic radiation force was produced using a 1.2 MHz unfocused immersion transducer with a 1" nominal element size (Olympus NDT, Inc. Waltham MA) while submerged in distilled, deionized water (DIH_2O). An ultrasound signal was generated with a carrier frequency of 1.0 MHz and amplitude that ranged from 20mV–300mV, which was pulsed at 1kHz. Radiation force produced by LIPUS was measured using a mass balance (Mettler, Inc. Toledo OH) modified for use as a radiation force balance. Temperature change of 3ml phosphate buffered saline in a 6-well culture dish was assessed using a thermocouple (Oakton, Inc.). *Particle Displacement Evaluation:* A commercially available peptide hydrogel (BD PuraMatrix) was used as a synthetic ECM and was reconstituted according to manufacturer's instructions. Briefly, the hydrogel stock solution (1% w/v) was sonicated for 30 minutes and pipetted into a culture well plate. The solution was then diluted into three separate concentrations (1%, 0.5%, 0.25%), pipetted onto a glass cover slip, mixed with salt solution and allowed to gel for 30 minutes at room temperature. The salt solution was replenished twice over 60 minutes. To visualize the movement of the matrix under LIPUS stimulation, 10 μl of a fluorescent 1-μm diameter polystyrene bead suspension (Fisher Scientific, Inc.) was pipetted into each well prior to gelation. Hydrogels were exposed to ultrasound with a carrier frequency of 1.2MHz and an amplitude of 500mV, pulsed at 1kHz. Duty cycle of the pulse ranged from 20%-100%. Imaging of beads within the ECM 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.).

Statistical Evaluation: One way ANOVA ($p<0.05$) was used to determine statistical differences in measured load. Confidence interval was used to characterize particle displacement within hydrogels.

Results: Increasing both amplitude of LIPUS and exposure time resulted in an increase in DIH_2O temperature, but only in the most extreme case did this increase exceed 1ºC, the limit of acceptable temperature increase by the FDA. Load was observed to increase as high as 40 mg with a continuous waveform, but was found to be approximately 5-10mg at intensities commonly used for fracture repair. These measured forces translated into the physical movement of encapsulated beads within the hydrogel, with displacements approaching 4μm (figure 2) as duty cycle was increased. Velocity of particles moving within the hydrogel increased as hydrogel viscosity decreased (data not shown).

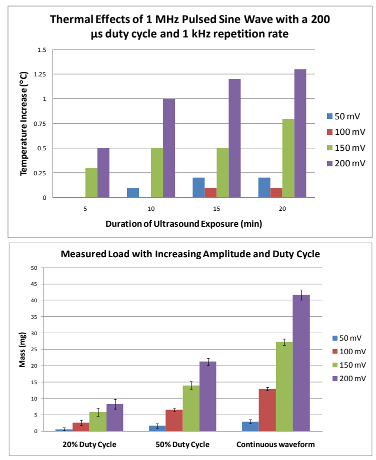
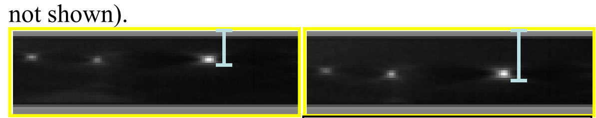


Figure 1. Signal amplitude and resulting temperature increase over time (top). Changes less than 1ºC were noted for all but the highest amplitude signal. Measured load in-creased with in-creasing duty cycle and amplitude of ultrasound signal (bottom). All mass changes were statis-tically significant except 50mV vs 100 mV for the 20% duty cycle.



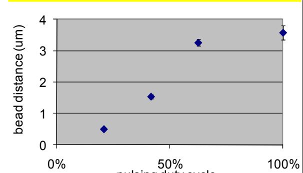


Figure 2. Z-plane of individual particles within the hydrogel prior to ultrasound (top left) and after a continuous duty cycle (top right). Particle distance was positively correlated with duty cycle (bottom left).

Conclusions: These studies illustrate how LIPUS can be used to apply physical forces to scaffolds designed to mimic the natural ECM with no thermal damage. Control over magnitude of applied force and resulting particle displacement through controlling the nature of the waveform have been demonstrated.

References:[1] Khan Y. JBJS 2008;90;138-144. [2] Khan Y. 2010 Orthop Res Society annual meeting abstract [3] Robinson JA. J Biol Chem. 2006;281;31720-28