The Novel Use of Acoustic Radiation Force to Mechanically Load Hydrogel-Encapsulated Osteoblasts for Bone Repair

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Statement of Purpose: In the field of orthopedics, low intensity pulsed ultrasound (LIPUS) has shown clinical efficacy in both reducing fracture healing times by 38% and in healing non-union defects (with an 85% success rate) [1]. Despite the positive correlation between LIPUS and bone repair, the mechanism behind the healing effects of LIPUS therapy still remains unclear. While LIPUS for bone repair has been studied extensively in 2-dimensions, we have developed collagen hydrogel tissue mimetics with encapsulated cells to evaluate cellular responses to acoustic forces in a three-dimensions. Acoustic radiation force was applied using a custom-made ultrasound system with precise control over ultrasound intensity. Collagen hydrogels of varying viscosities were loaded and visualized in deformation through fluorescent beads that were tracked in real time. Here we demonstrate the precise control over loading and resulting hydrogel deformation, and how this deformation influences cellular response, suggesting the ability to control cell response based on acoustic radiation force and hydrogel synthesis.

Methods: Acoustic radiation force was applied using a 1.2 MHz unfocused immersion transducer and calibrated with a mass balance/cone setup and a needle hydrophone. A 1 MHz carrier frequency pulsed at 1 kHz with a 20% duty cycle were used to supply 30mW/cm^2 spatial intensity to cells. MC3T3 osteoblast-like cells were encapsulated in Type I Collagen hydrogels of 1, 2, and 3 mg/mL concentration and exposed to acoustic force for 1, 3, and 7 days at 20 minutes per day (experimental group) and compared to no LIPUS treatment (control group). qRT-PCR was performed to quantify the gene expression of osteogenic markers alkaline phosphatase, osteocalcin, type I collagen, RUNX2, and osteopontin. Additionally, deformation of collagen hydrogels (0.5, 0.75, 1, and 2 mg/mL) was imaged with encapsulated 2 µm fluorescent beads over a 90-second time interval at 20% (30mW/cm^2), 50% (72mW/cm^2) and 100% duty cycle (140mW/cm^2). Image acquisition and analysis was performed with the Volocity software (PerkinElmer).

Results: Collagen hydrogels showed clear evidence of scaffold deformation when exposed to ultrasound treatment (figure 1). The lowest viscosity hydrogel tested (0.5 mg/mL collagen) experienced the highest amount of deformation as indicated by mean bead displacement in the X, Y, and Z-direction. Within the 0.5 mg/ml hydrogel displacement was dependent on duty cycle, with 100%, 50%, and 20% all showing deformation in decreasing quantities (see figure 1) respectively. Displacement decreased for a given intensity as collagen concentration, and subsequently storage modulus (data not shown), increased to the point where 2 mg/ml collagen hydrogel showed very little deformation (figure 1 bottom). However, increasing ultrasound intensity did prove that deformation was possible for the highest concentration hydrogel tested (not shown). MC3T3 cells experienced a

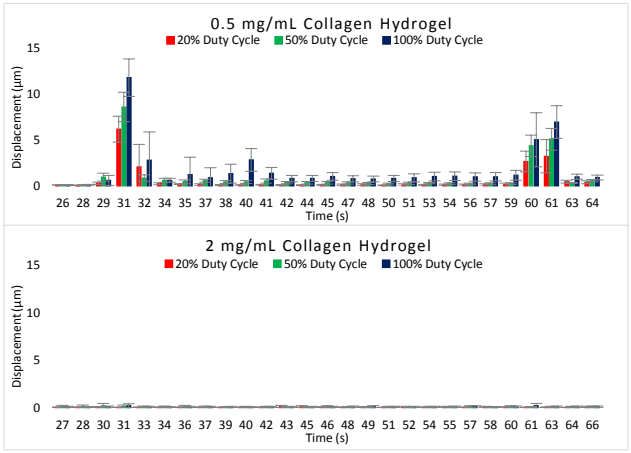

Figure 1. Collagen hydrogel (0.5 mg/mL – top; 2 mg/mL – bottom) mean bead displacement before (0-30s), during (30-60s), and after (60-90s) 20% duty cycle (red), 50% duty cycle (green), and 100% duty cycle (blue) ultrasound treatment.

varied genetic response when treated with LIPUS in collagen hydrogels of increasing mechanical viscosities (figure 2). Alkaline phosphatase was upregulated in the LIPUS group at 7 days compared to the control group, but as collagen concentration increased and deformation of the gel decreased the measured AP response also

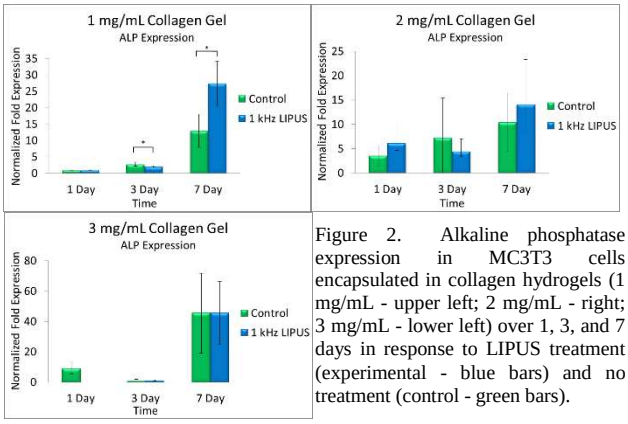

Figure 2. Alkaline phosphatase expression in MC3T3 cells encapsulated in collagen hydrogels (1 mg/mL - upper left; 2 mg/mL - right; 3 mg/mL - lower left) over 1, 3, and 7 days in response to LIPUS treatment (experimental - blue bars) and no treatment (control - green bars).

decreased, suggesting that the hydrogel, as it increased in modulus, began to shield the osteoblasts from the applied force and as such from the effects of the mechanical loading. Other markers demonstrated a similar response, but less of a dependence on hydrogel concentration.

Conclusions: We have developed a process for applying acoustic radiation force to encapsulated cells, and for measuring its effects on both the hydrogels and the cells within them. Certain markers of bone formation may be more or less susceptible to the physical forces resulting from low intensity pulsed ultrasound. Understanding the relationship between externally applied physical forces at the cellular level and the immediate physical surroundings may prove critical in developing novel transdermal bone repair treatment systems.

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