A Focused Ultrasound Technique for Modulating Local Tissue Properties for Articular Cartilage Tissue Engineering

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INTRODUCTION: In cartilage tissue engineering, successful integration (i.e., 'meshing') with the bone poses a problem. Mature cartilage is anchored to and separated from underlying vascularized bone by a mineralized subchondral plate that provides an impermeable interface. This impermeable interface promotes elevated interstitial fluid pressurization needed for load-bearing and lubrication mechanisms in articular cartilage [1-4]. The challenge of creating such an interface in engineered tissue constructs may be addressed through the use of Harmonic Motion Imaging (HMI) for Focused Ultrasound (HMIFU), a new method of focused ultrasound that allows for simultaneous tissue monitoring and controlled protein denaturation [5]. Current applications for ultrasound include clinical modalities for imaging and for ablation of uterine fibroids (FDA approved), and as a method for material testing (e.g., elastography). This study investigates the feasibility of utilizing this technique for sealing the cartilage-bone interfaces by demonstrating the technique's ability to affect tissue changes in a spatially-controlled (x,y,z) fashion in gel phantoms (Study A), tracking the mechanical properties of cartilage in real time through the denaturation process (Study B), and by demonstrating the technology's localized thermal effects on cell viability within the engineered cartilage (Study C).

METHODS: Study A: Acrylamide-albumin phantoms were fabricated and subjected to HMIFU. The lesions were generated inside the phantom by moving the HMI transducer in a 2D raster-scan fashion, Figure 1, using 450 mV per point, 42 W power and an excitation period of 2 seconds/point, 50 cycles for a 10x10 mm^2 lesion (1 mm spacing). The peak-positive pressure was approximately equal to 4 MPa during heating.
Study B: Explants were taken from bovine cartilage. These explants were monitored using a 7.5-MHz single-element, pulse-echo transducer (Panametrics, Waltham, MA, USA) before, during, and following controlled protein denaturation at 35 W with a 4.5-MHz HIFU transducer (Imasonics, Besancon, France). The explants were placed on a silicon rubber/absorber (McMaster-Carr, Dayton, New Jersey, USA) and immersed in phosphate-buffered saline solution (PBS). Explants were also thermally affected in a raster fashion.
Study C: Juvenile bovine chondrocytes were cast in an agarose (Type VII, Sigma-Aldrich, St. Louis, MO, USA) slab of 2.38 mm thickness (30 x 10^6 cells/mL). The slab was placed on a bed of silicon rubber/absorber over polyurethane foam (McMaster-Carr, Dayton, New Jersey, USA) within a 100 mm culture dish. PBS was added on top of the slab and the dish was covered with cling wrap. The slab was subjected to denaturation at 33 W for 5 seconds using the HIFU transducer focused at the bottom surface (Figure 1). Heated and non-heated regions were excised using a scalpel. Cell viability was compared between heated and non-heated regions using live/dead staining two days after ultrasound. Images of the slab's surfaces were obtained using a tile-scan acquisition method on a Leica TCS SP5 multiphoton confocal microscope.

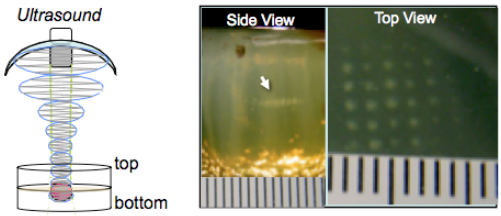

Figure 1. Schematic of HMIFU application. Gel phantom showing rasterized pattern of denaturation (Study A). (scale: 1 mm)

RESULTS: Study A: Denatured regions became opaque as the albumin became thermally cross-linked. Figure 1 demonstrates the xyz control of the heating application. Study B: HMIFU protein denaturation of the cartilage explant was monitored before, during, and following heating; it displayed softening following denaturation, indicated by an increase in HMI displacement (Figure 2). Rasterized thermal denaturation of the explanted cartilage yielded a color change in the pattern of the raster.

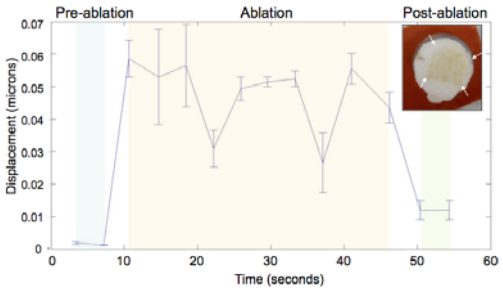

Figure 2. HMIFU of articular cartilage showing monitoring of the cartilage properties at all stages of treatment (denaturation corresponding to HMI displacement increase); Inset: Same cartilage tissue with denatured region (square region with discoloration).

Study C: Live/dead staining of the non-heated cell-seeded agarose construct (control) showed a homogenous distribution of nearly all live cells on both the top and bottom surfaces (Figure 3, left). Constructs subjected to HMIFU showed a viable top surface similar to control, but a bottom surface with a dark area (Figure 3, right *) surrounded by a homogenous distribution of mostly live cells. The dark region contained dead cells towards the edges, but no living cells.

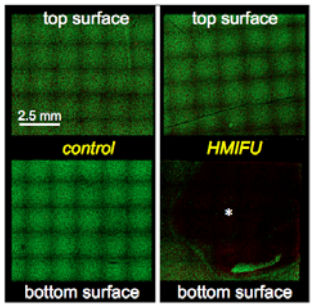

Figure 3. Live/dead images of control (left) and thermally affected constructs (right). The checkered pattern is due to the tiling of multiple images.

DISCUSSION: In this study, we performed several studies aimed at determining the feasibility of using HMIFU, a new focused ultrasound technique, as a methodology for affecting local tissue property changes in a targeted fashion. Our preliminary studies demonstrate the ability to induce thermal changes in tissues and alter their properties in a spatially-controlled fashion with real-time monitoring (Study A, B), accompanied by corresponding cell death to heated regions only (Study C). Ultrasound technologies provide a significant advantage over alternative (optical) laser-based approaches in general because they are not restricted by tissue opacity (permitting focusing of several centimeters in depth), Figures 1,3. The underlying rationale for our approach, using HMIFU, is to apply controlled thermal changes to tissue regions. Temperatures of ≥50˚C result in melting/denaturation of the agarose scaffold, the cartilage collagen, and the proteoglycan matrix [6-9]. Such thermally induced tissue changes are the principle behind the clinical use of Radio-Frequency (RF) energy probes as a minimally invasive technique for debriding fibrillated articular cartilage surfaces and annealing the edges of lesions, potentially improving joint biomechanics and providing tissue stability [10]. Future studies will entail development of HMIFU as a non-invasive method of generating and monitoring the sealing of the cartilage-bone interface, with the spatial flexibility to follow the contours of anatomic surfaces and temporal flexibility to be applied at any point in the culture duration (and level of tissue formation).

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