Effects of Focused Ultrasound on Cell Viability in Its Application to Articular Cartilage Engineering

A. B. Nover[1], G. D. O'Connell[1], G. A. Ateshian[1], E. G. Lima[2], E. E. Konofagou[1], and C. T. Hung[1]
[1]Columbia University, New York, NY, [2]The Cooper Union, New York, NY

Introduction: A primary contribution to the load-support mechanism of articular cartilage is the pressurization of its interstitial fluid. This pressurization is critically dependent on the integrity of the subchondral plate, the virtually impermeable bony interface between cartilage and underlying trabecular bone [1-4]. Cartilage tissue engineering strategies should include methods to seal this interface for the purpose of restoring normal interstitial fluid pressurization. Focused ultrasound (FUS), may offer the ability to thermally affect changes in local tissue properties on demand, aimed at sealing the interface, during in vitro growth,. of Harmonic Motion Imaging for Focused Ultrasound (HMIFU) allows for exquisite spatiotemporal control of thermal changes and real-time monitoring of tissue mechanical properties properties [5]. Its ability to penetrate several centimeters in depth gives FUS an advantage over many current methods of affecting cartilage thermally, such as radio-frequency probes and lasers. Some collateral loss in cell viability is anticipated due to thermal effects. Our previous study has reported that this cell death is localized to the targeted ultrasound area immediately after application [6]. The current study further examines the effect of HMIFU on long-term cell viability, as a step toward optimizing this methodology for sealing the osteochondral interface.

Methods and Materials: Juvenile bovine chondrocytes were cast in a 2% w/v agarose (Type VII) of 2.3 mm thickness (30 x 10^6 cells/mL). Albumin from egg white was also incorporated, as it is commonly used in FUS phantoms for visualization; once heated, albumin changes color from clear to opaque white. The slab was placed on a silicon rubber/absorber over polyurethane foam within a 100 mm culture dish and immersed in phosphate buffered saline. The slab was subjected to focused ultrasound using a 4.5-MHz FUS transducer at ~7.8 W power and 400 mV peak-to-peak, with an excitation time of 3 seconds per point. The transducer was aimed toward the bottom of the slab, and multiple points were heated across the slab, leaving space in between points. The slab was punched using a Ø4 mm biopsy punch in thermally treated and untreated (control) areas; focused ultrasound lesions were kept within each cylindrical construct. Over six weeks, constructs were fed with chondrogenic media. Parallel samples from each group were cut in half and stained for viability with Live/Dead kit (Invitrogen). Top and bottom surfaces and the cross section were imaged with confocal microscopy.

Results and Discussion: On the day the ultrasound was applied, the thermally affected constructs showed a region of cell death (red; Figure 1, *left*), corresponding to the white regions of heated albumin. This cell death was localized at the bottom of the constructs with the above region remaining viable (green). The control constructs appeared viable throughout. Over the six week period, the region of cell death remained localized, (Figure 1, *right*), with some cell repopulation observed, an effect which is supported by other studies [7].

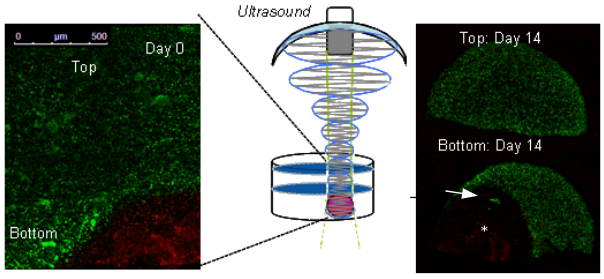

Figure 1. (*left*) Cross-section (axial) of a constructs stained for viability on day 0, immediately following the ultrasound treatment. Red (asterisk) indicates cell death corresponding to the targeted region. (*right*) Cell viability of the construct's top and bottom surfaces on day 14 (lateral plane). Arrow indicates possible cell repopulation.

Conclusions: The on demand spatial control of FUS has been demonstrated, along with cell death at the target site of ultrasound heating, which remains localized without spreading during culture. This observation supports the feasibility of using FUS to modulate tissue properties, suggesting that cell viability effects can be mitigated through optimization of ultrasound parameters and lesion spacing while modulating tissue properties. Studies underway investigate FUS's effects on mechanical, biochemical, and transport properties of tissues.

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