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Technical note

High intensity focused ultrasound as a tool for tissue engineering: Application to cartilage

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ABSTRACT

This article promotes the use of High Intensity Focused Ultrasound (HIFU) as a tool for affecting the local properties of tissue engineered constructs *in vitro*. HIFU is a low cost, non-invasive technique used for eliciting focal thermal elevations at variable depths within tissues. HIFU can be used to denature proteins within constructs, leading to decreased permeability and potentially increased local stiffness. Adverse cell viability effects remain restricted to the affected area. The methods described in this article are explored through the scope of articular cartilage tissue engineering and the fabrication of osteochondral constructs, but may be applied to the engineering of a variety of different tissues.

1. Introduction

In the field of tissue engineering there exists a need to generate tissues with specific local tissue properties so to match native tissue architecture and therefore gain true functionality. For example, engineered osteochondral grafts [1,2] are being investigated as an alternative to osteochondral allografts, which are of scarce supply [3,4]. Native tissue contains a mineralized subchondral plate, which anchors mature cartilage, separating it from the underlying vascularized bone, providing a virtually impermeable interface. This subchondral interface promotes the elevated interstitial fluid pressurization needed for load-bearing and lubrication mechanisms in articular cartilage [5–9]. However, engineered osteochondral tissues, consisting of a chondrocyte-seeded hydrogel atop a nonliving porous, bone-like substrate, lack any sort of subchondral plate *a priori*. Incorporation of such an impermeable layer from the start, such as via a denser porosity region in the bony substrate localized to the gel-substrate overlap region [10], would block nutrient transport across the layer during tissue culture. Since nutrient transport is critical for the development of engineered tissue [11,12], a technology is required that can stiffen or seal the bone-cartilage interface at an optimal time during *in vitro* growth.

High intensity focused ultrasound (HIFU) may offer such an ability. Like other forms of ultrasound, HIFU is a minimally or non-invasive, non-contact, non-radiation, low-cost technology. It is under investigation for its use in soft-tissue cancer therapy [13] and is currently FDA approved for uterine fibroid [14,15,46,47]. HIFU can penetrate depths of at least 10 cm and reach temperatures of at least 80 °C [16,17]. Studies have shown that HIFU's thermal and mechanical effects can lead to a loss of *in vitro* viability [18], yet the thermal effects of HIFU remain the primary mechanism of damage [16] and repair tissues enter the periphery of the treated region after two weeks [17].

In conjunction with heat, an additional substance may be necessary for inducing a change in tissue properties. Heat-induced protein denaturation has been demonstrated in a number of tissues and protein-incorporated gel phantoms, often leading to color change, coagulation, and, after certain temperatures, irreversible stiffening [19–27]. Albumin is commonly used in gel phantoms for tissue

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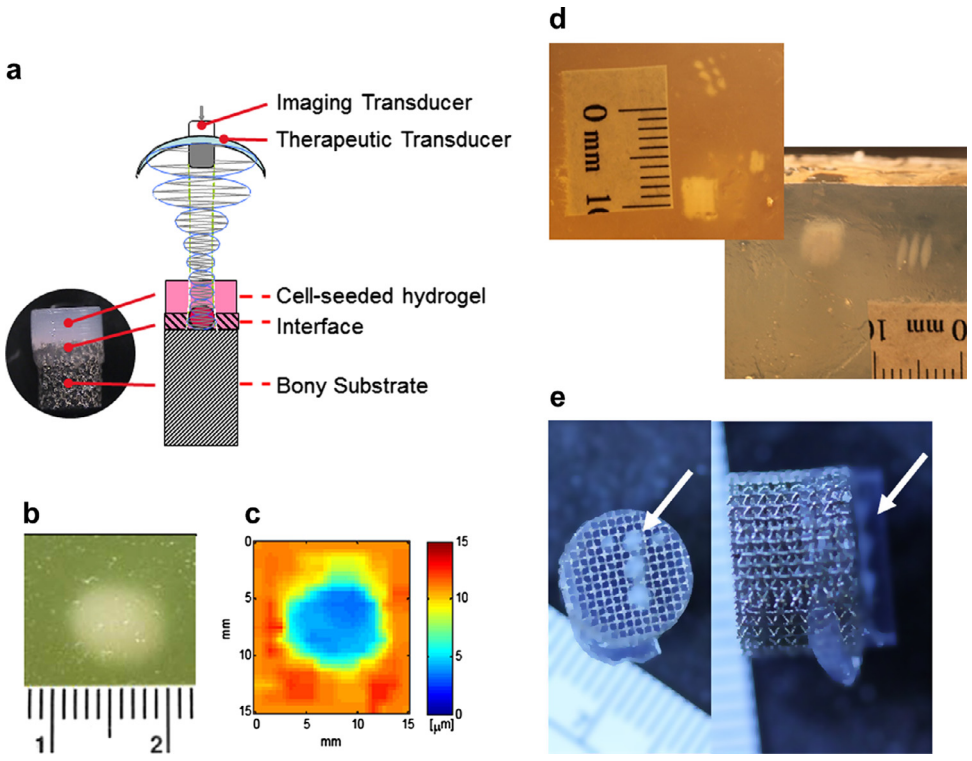

Fig. 1. (A) Schematic of HIFU treatment of osteochondral construct. The two-transducer system is attached to a computer-controlled positioner. (B–C) Circular thermally affected region induced in an egg white gel by HIFU with a 25 s duration in the center and 10 s duration in the surrounding ring (~6 MPa peak *in situ* positive pressure), which was then mapped using HMI using a rastered step size of 1 mm across a 15 × 15 mm^2 region (peak-positive pressure of 1.98 MPa, 500 ms/point duration). The circular egg-white pattern indicated that the thermally affected region was three times stiffer than the surrounding gel. (B) Gross Image and (C) HMI map of HIFU-induced thermally affected region. Color bar shows oscillatory displacement in μm (lower displacements correspond to stiffer regions). (D) Thermally affected region patterns beneath the surface of agarose-egg white gel (Top and Side views). (E) Osteochondral constructs (Top and Side views) displaying thermally affected regions created at different powers. Arrows indicate 11.6 W total acoustic power for 20 s. Scale bars indicate millimeters.

ablation studies via thermal treatment modalities, such as HIFU [23–26,28]. Albumin from bovine serum (BSA) or egg white, a more economical option, coagulates at 67 ± 1 °C via intermolecular β-sheet formation, changing from a clear color to an opaque white, providing easy visualization of the heated region [20,24]. Outside of phantoms, albumin has been utilized as a biological solder [29]. With respect to articular cartilage, albumin is relevant; it is the chief protein component of synovial fluid [30]. For these reasons, the addition of albumin may aid the alteration of internal properties of tissue engineered constructs, especially prior to cellular protein elaboration.

While most research utilizing HIFU technology aims to induce pressures and/or thermal elevation over large (cm scale) areas of dense tissue *in vivo*, such as in full tumor ablation, here HIFU is investigated as a potential tool for precisely modulating the spatial properties of small (mm scale) tissue engineered constructs during *in vitro* growth, which increase in density during culture (agarose gel scaffold is 98% w/v water on initial seeding day). Here, the aim of this study is to test the feasibility of HIFU as a method of altering local tissue properties as a tool for tissue engineering, namely forming a biomimetic subchondral plate in engineered osteochondral constructs by thermally affecting the interface region.

2. Methods

2.1. HIFU setup

HIFU treatment was conducted according to Maleke and Konofagou [27], utilizing a setup conducive to later incorporation of Harmonic Motion Imaging (HMI), an ultrasound-based method of monitoring elastographic changes in tissues [27,31–33]. Briefly, this setup consists of an imaging transducer centrally positioned inside a confocal therapeutic transducer (Fig. 1A) and attached to a computer-controlled positioner (Velmex Inc. Bloomfield, NY, USA). The two transducers were self-contained by attaching to a coupling cone containing degassed, distilled water and sealed with an acoustically transparent latex membrane [34,35], which allows the membrane to be coupled to the sample through degassed culture media, phosphate buffered saline (PBS), water, or ultrasound gel. For imaging, a 7.5 MHz single-element pulse-echo transducer (Panametrics, Waltham, MA, USA) connected to a pulser/receiver (Panametrics 5051PR or Olympus 5072PR, Waltham, MA, USA) was used to target and monitor treatment produced by the therapeutic transducer. For thermal treatment, either a 4.755 MHz (Riverside Research Institute, New York, NY, USA) or a 4.5 MHz (Imasonics, Besancon, France) therapeutic transducer was used. Heating parameters vary; these parameters represent an aspect of this treatment which will require further optimization. Two function generators (Agilent (HP) 33120 A and 33220A, Palo Alto, CA, USA) were used to drive the therapeutic transducer with an amplitude-modulated waveform, combining sine waves: one of the therapeutic transducer's excitation frequency at a varied amplitude with a 25 Hz wave of amplitude 5.086 $V_{peak\text{-}to\text{-}peak}$. The signal was then amplified by 50 dB using a power amplifier (Model 3000L, ENI®, NY, USA). For HIFU treatment, constructs or cell-seeded slabs were submerged in degassed PBS or culture media.

2.2. Gel construct fabrication and media formulation

As in our previous cartilage tissue engineering studies, gels were cast with final concentrations of 2% w/v agarose (Type VII or IX-A, Sigma-Aldrich, St. Louis, MO, USA) and 30 million cells/mL as gel-alone or osteochondral constructs according to Nover et al. [1]. In studies where albumin was incorporated, equal volumes of egg