Monitoring Adipogenesis in Tissue-Engineered Fat *in vitro* Using Microscopic Magnetic Resonance Elastography

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INTRODUCTION

Facial injury due to trauma or cancer resection surgery often results in permanent disfigurement. Soft tissue engineering has the potential to generate and repair the key tissue components needed for reconstructive surgery. For example, human mesenchymal stem cells (*h*MSCs) encapsulated in a biocompatible three-dimensional hydrogel matrix were capable of generating adipose tissue with predefined shape and dimensions *in vivo* (1). As tissue engineering advances, there is a critical need for real-time monitoring of the engineered tissues *in vivo*, including engineered adipose tissue for reconstructive surgery applications. Histological techniques such as Oil red O staining are capable of detecting adipogenesis but necessitate tissue destruction, thus not appropriate for *in vivo* and *in situ* monitoring. Current conventional MR techniques are not able to differentiate adipogenic development due to the small changes in tissue relaxivity and diffusivity occurring during tissue developments, thus a new contrast mechanism is needed (2). Microscopic magnetic resonance elastography (μMRE) is a new high resolution phase contrast based MR imaging technique for visualizing low sonic frequency shear waves (typically less than 1 kHz) in soft materials and biological tissues (3). μMRE extends currently available MR-elastography techniques to the microscopic domain by utilizing stronger magnetic field and gradients systems. Magnetic resonance elastography (MRE) is a phase contrast based MR imaging technique for observing acoustic strain waves propagating in soft materials (4). Mechanical shear waves, typically with amplitudes of less than 100 μm and frequencies of 100-500 Hz, are induced using either a piezoelectric or a voice coil oscillator directly coupled to the acoustic propagation path of the region of interest. In this preliminary study, we monitored different stages of adipogenesis in tissue engineered constructs from human mesenchymal stem cells using microscopic magnetic resonance elastography. Measurement of the mechanical properties of different growth stages of tissue engineered fat is performed by visualizing the temporal shear waves using μMRE with a microscopic spatial resolution. μMRE was able to provide a contrast mechanism large enough to differentiate different growth stages.

MATERIAL AND METHOD

Human bone marrow derived mesenchymal stem cells (*h*MSCs) were isolated from commercially available marrow samples (AllCells, Inc.). The *h*MSCs were passaged three times using basal culture media (89% DMEM, 10 Fetal Bovine Serum, 1% Penicillin/Streptomycin). To induce adipogenic differentiation of *h*MSCs, the cultures were expanded to 100% confluence and supplemented with 0.5 μM Dexamethasone, 0.5 μM Isobutylmethylxanathine (IBMX), and 50 μM Indomethacin every other day for 7 days. The *h*MSCs were trypsinized, counted, and encapsulated in 0.5 % w/v agarose at a seeding density of 3 Million cells/ml. Stem cell-agarose constructs with a volume of 100 μl were fabricated using cylindrical molds (4mm height×6mm diameter). The stem cell-agarose gels solidified within 20 minutes at room temperature. Samples were placed in Pyrex tubes and surrounded on all sides by 0.5% w/v agarose which served as the acellular control for all adipogenic constructs. Tissue engineered adipogenic constructs were scanned at 7, 14, and 21 days following *in vitro* cell culture.

Experiments were conducted using a standard 11.74 T (500 MHz for protons) vertical bore magnet (Oxford Instruments, Oxford, UK) using a Bruker DRX 500 MHz Avance spectrometer (Bruker Instruments, Billerica, MA). The shear waves were generated using a piezoelectric transducer (Piezo system, MA) and amplifier. The shear wave signal frequency was fixed to 550 Hz. Acoustic signals were applied with synchrony with the NMR spin echo based phase contrast pulse sequence. RF saddle coil with a diameter of 10 mm was used to acquire phase difference maps. The field–of–view ranged from 10 to 14 mm. The μMRE system including the pulse sequence is shown in Fig.1.

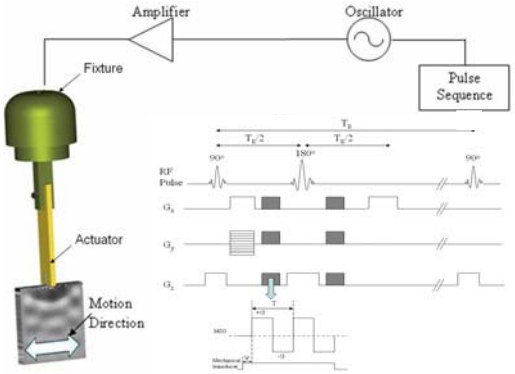


Fig. 1 A schematic diagram for the μMRE system. The mechanical piezoelectric actuator is coupled to the sample via a thin needle. The modified spin echo based phase contrast pulse sequence controls a gated square wave oscillator tuned to the actuator frequency which is synchronized with the toggled bipolar gradient waveforms. Bipolar pulses can be added to the read, slice select, or phase encoding gradient.

RESULTS

Fig. 2 shows a typical 3-dimensional shear wave image through a uniform agar gel phantom displaying uniform shear wave propagation. Fig. 3 shows the shear wave propagating through three growth stages of adipogenic differentiation and their corresponding vertical line profile passing through the tissue and the agar gel phantom. The shear wavelength changes when passing from the adipogenic tissue to the agar phantom. The wavelength in the agar phantom remained consistent during the entire study. Fig. 4 shows the changes in the mechanical properties during the three growth stages presented. The shear stiffness changed from 5.8 kPa at week 1 to 1.44 kPa at week 3.

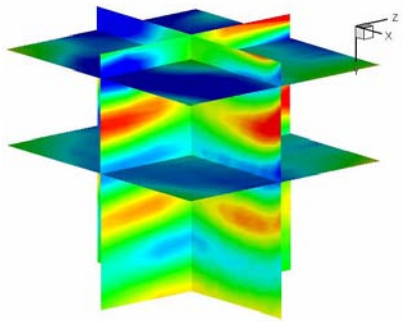
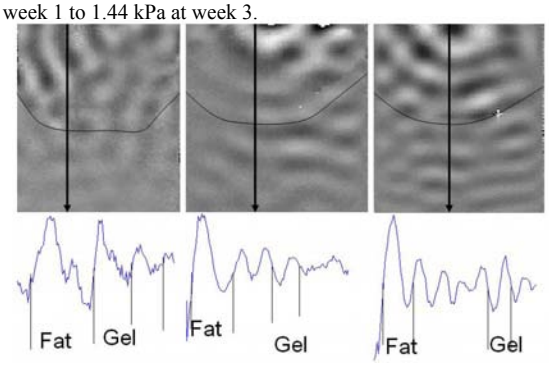


Fig. 2 Typical 3D shear wave propagating through a homogenous agar gel phantom (0.25 % w). In plane resolution = 109 μm x 109 μm, slice thickness = 0.5 mm



(a) (b) (c)

Fig. 3 Shear wave images and their corresponding vertical line profiles of adipogenic constructs at three growth stages of (a)1, (b) 2, and (c) 3 weeks, respectively. In plane resolution = 109 μm x 109 μm, slice thickness = 0.5 mm,,NEX = 1

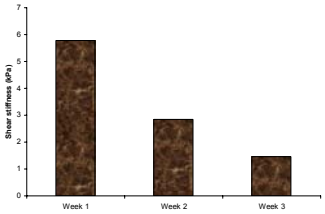


Fig. 4 Shear stiffness as a function of growth stage. The shear stiffness (μ) was calculated from the shear wavelength. $\mu = \rho c^2$, where c is the shear speed and it is dependent on the wavelength and the excitation frequency ($c = f\lambda$.).

CONCLUSIONS AND FUTURE WORK

μMRE was able to provide contrast between different stages of adipogenic development in tissue engineered constructs. μMRE distinguished stem-cell-derived adipose tissue from the surrounding scaffold by a factor of four reductions in tissue stiffness between growth stage at week 1 and week 3. The decrease in shear stiffness suggests that the engineered adipogenic tissue becomes 'softer' as adipogenic matrix production from the *h*MSC derived adipogenic cells gradually increases. Implanted adipogenic tissues from stem cells *in vivo* animal models are being tested to monitor their *in vivo*, *in situ* and real-time applications in reconstructive surgery.

REFERENCES: [1] Alhadlaq et al., Tissue Eng. 2005;11:556-566.. [2] Xu, University of Illinois at Chicago PhD dissertation 2005, Ch 4; 92-96. [3] Othman et al., MRM 2005;54:605–615 [4] Muthupillai et al., Science 1995;269:1854-1857. **ACKNOWLEDGMENTS:** The authors thank Dr. Kleps of the Research Resource Center for providing his expertise in NMR, NIH, Grant # EB004885-01.