## Development of a High-Throughput Ultrasound Technique for the Analysis of Tissue Engineering Constructs

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**Statement of Purpose:** Ultrasound is an attractive technique for quantitative characterization of tissue engineering constructs because it is nondestructive, highly sensitive, and allows for simultaneous imaging of multiple samples [1]. These attributes are ideal for rapid screening of multiple factors influencing cell maintenance within 3D constructs. In addition, if properly validated, ultrasound can eventually enable continuous in situ monitoring of cell integration within a scaffold to ensure tissue repair and regeneration. In this study, we developed a quantitative technique using clinical B-mode ultrasound to differentiate fibroblast morphology and cell density and collagen content within poly(ethylene glycol) (PEG) hydrogels and validated it in an in vitro phantom environment.

Methods: Collagen (0-2.5 mg/mL) was mixed into 5 and 10% (w/v) PEG-diacrylate (3300 MW) solutions prior to crosslinking. Fibroblasts were directly seeded in 10% (w/v) PEG solutions with 0.1 mg/mL collagen at  $0-1x10^6$ cells/mL. One set was fixed immediately resulting in round cells while the other was cultured for 24 hours before fixation, resulting in stretched cell morphology. To characterize echogenicity of the constructs, 4 or 5 samples were placed in a water-filled acrylamide mold. The gels were imaged using a clinical ultrasound system (Toshiba Aplio500) with a linear array transducer centered at 12 MHz. Cell morphology was characterized via fluorescent microscopy on a Zeiss inverted microscope. The cells were permeabilized and nuclei-labeled with Hoechst 33342 and actin-labeled with Alexa Fluor 488 Phalloidin. Statistical significance was determined using a 2-way ANOVA and Tukey post hoc test, with p<0.05 considered significant.

**Results:** Greyscale image analysis (Figure 1) revealed that round cells showed a maximum signal increase of  $199\pm160\%$  at  $0.5\times10^6$  cells/mL compared to the no cell control, while stretched cells (Figure 2) showed an increase of  $320\pm163\%$  at the same cell concentration and a maximum increase of  $459\pm160\%$  at  $1\times10^6$  cells/mL (Figure 3). Both groups of cells showed an increase in signal intensity as cell density increased. No significant differences were observed in ultrasound intensity between gels with varying collagen concentrations (Figure 4).



Figure 1. A) Representative ultrasound images of stretched and B) round cells in PEG gels, and collagen in C) 5% and D) 10% PEG gels.



**Figure 2.** Cell morphology. Cells were fixed immediately (A) or cultured for 24 hours (B, C) prior to fixation. Scale bar =  $20 \ \mu m$ . Cells cultured for 24 hours had more extensions than the cells fixed immediately after seeding, similar to literature of fibroblast morphology in PEG based scaffolds [2].



**Figure 3.** Ultrasound intensity of round (A) and stretched (B) cells. ANOVAs were run in SAS 9.4 on all data, including outliers. Bars with different letters are significantly different at p<0.05.



Figure 4. Ultrasound intensity of varying collagen concentration in 5% (A) and 10% (B) PEG gels. No significant differences were noted.

**Conclusions:** The increased intensity measured in the stretched cell samples is likely due to both increased cell number and area. No significant differences were observed in ultrasound intensity between gels with varying collagen concentrations. While previous studies have investigated high frequency ultrasound for use with hydrogels [3], these results suggest that clinical ultrasound can distinguish various cell densities and morphologies in PEG hydrogels. Additional studies are required to conclusively identify if differences exist with protein concentration. This technique can eventually be used to create a predictive model and have applications imaging implants in vivo for the characterization of tissue engineering scaffolds for regenerative medicine.

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**References:** [1] Solorio, L, Drug Deliv Transl Res. 2012; 2(1): 45-55; [2] Rizzi SC, Biomaterials. 2010; 31: 8454-8464; [3] Anseth KS, Acta Biomater. 2009; 5(1): 152-161