Online Monitoring of Tissue-Engineered Cartilage Development in a Dynamic Compression Bioreactor

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Statement of Purpose*: Bioreactors with online, nondestructive measurement capabilities offer great advantages to the production of engineered tissue. We have developed a dynamic compression bioreactor instrumented with five load cells, an ultrasonic transducer and a video microscope for assessing development and mechanical properties of tissue-engineered constructs. The objective of this research was to evaluate mechanical stimulation of chondrocyte-laden poly(ethylene glycol) dimethacrylate (PEGDM) hydrogels which are cultured for one week in the bioreactor.

Methods: The bioreactor consisted of five sample wells to hold 3 mL of media and a 125 mm^3 construct. Five 9.8 N load cells with surgical steel platens were attached to a 22 N actuator. A 30 MHz ultrasonic transducer was situated under the well rotation stage. The bioreactor was placed in an incubator at 37 ˚C and 5 % CO_2 during mechanical stimulation and measurement and was placed in a test stand for ultrasound analysis for less than 10 min before being returned to the incubator. Bovine chondrocytes were isolated and encapsulated in PEGDM at a density of 5×10^7 cells/mL. Constructs were subjected to a ramp (10 % strain, 0.5 mm/min), 1 h of sinusoidal compression (1 Hz, 10 % strain), and 11 h with no compression. This regime was repeated twice per day for seven days. Load and displacement data were collected during the ramp and compression stages. Gels were nondestructively evaluated with ultrasound before and after the seven-day compression regime. Glycosaminoglycan (GAG) and collagen content were biochemically measured and stained on days 1 and 7.

Results: Chondrocyte/hydrogel constructs were subjected to displacement-controlled intermittent cyclic load for seven days, resulting in maximum loads of approximately 0.022 kg (Fig. 1a).

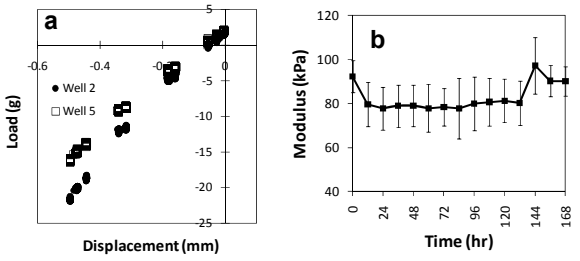

Figure 1. (a) Real-time measurements of load vs. displacement for two representative wells and (b) construct strength versus time. Points represent the mean modulus of n=5 samples ± standard error.

Compressive modulus was calculated from data collected during the ramp stage. Modulus did not significantly change over the course of seven days in the bioreactor (Fig. 1b). Histological staining demonstrated an increase in pericellular GAG deposition between days 1 and 7 (Fig. 2). However, there was no visible difference between controls and bioreactor constructs at day 7.

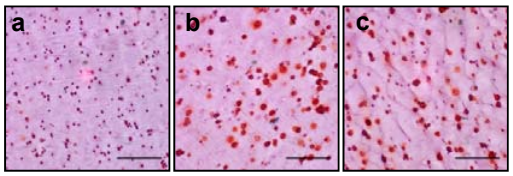

Figure 2. Histological sections stained with Safranin O, (a) day 1 control, (b) day 7 control, and (c) day 7 bioreactor. Scale bars are 100 μm.

GAG content was quantified with dimethylmethylene blue, indicating a significant increase in GAG content after seven days, independent of compression. Collagen was not detected with staining or the hydroxyproline assay (data not shown).

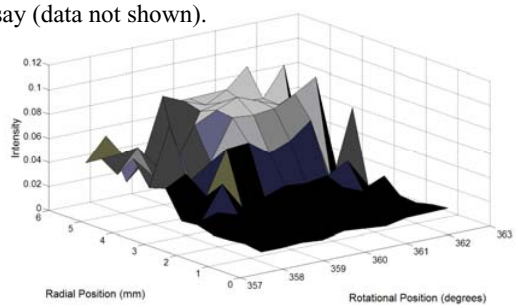

Figure 3. Ultrasonic intensity map of a chondrocyte-hydrogel construct on day 7.

Bioreactor samples were evaluated by ultrasound on days 1 and 7 (Fig. 3). The ultrasonic intensity through the construct was normalized by the ultrasonic intensity through media only to calculate the attenuation coefficient. There was a slight decrease in attenuation coefficient between day 1 (1.100 ± 0.320) and day 7 (0.934 ± 0.258), likely due to the increase in GAG. However, the difference was not significant.

Conclusions: This study successfully demonstrates the capability of the bioreactor for mechanical stimulation, real-time strength measurements, and ultrasonic evaluation. Increases in pericellular GAG were seen after seven days. However, no significant differences were measured in modulus, collagen content, or ultrasonic attenuation coefficient during this period. Such a short-term study with nondegrading hydrogels was not expected to produce large amounts of extracellular matrix. We expect the bioreactor to be particularly powerful when combined with degradable hydrogels in long-term studies. Continuing work focuses on the use of the video microscope for construct strain validation and live imaging of ECM development.

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