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Non-invasive Evaluation of Engineered Cartilage Tissue Using Proton NMR Microscopy

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Introduction. The goal of this work was to determine whether biochemical changes in the extracellular matrix can be staged by NMR and whether NMR measurements can be correlated with biochemical parameters. For this purpose we have developed a NMR-compatible hollow fiber bioreactor (HFBR) for studying the NMR properties of three-dimensional cartilage tissue produced by isolated chick sternal chondrocytes.

Experimental. HFBRs were inoculated with 30 million chondrocytes isolated from distal sterna of day 16 chick embryos. After inoculation the bioreactors were perfused using a pin compression pump in a 5% CO_2 / 95% air incubator. This was noted as Day 0. The culture medium for all bioreactors was supplemented with 2% serum and 10 µg/mL ascorbic acid (AA) was added twice-weekly. The following experiments were performed: (i) control experiments, (ii) daily treatment with AA, (iii) treatment with 3 µg/mL trans-retinoic acid (RA) for 1 week at Day 21, and (iv) treatment with 10 ng/mL interleukin-1B for 1 week at Day 21.

All NMR experiments were conducted on a Bruker DMX spectrometer operating at 9.4 T (400.1 MHz for ^1H). HFBRs were maintained under incubator-like conditions while in the magnet. Each image had a nominal in-plane resolution of 60 microns and a slice thickness of 2 mm. The following quantitative NMR properties were measured for HFBR tissue: water proton T_1 and T_2 values, the magnetization transfer (MT) value, and the water diffusion coefficient (D). MT was calculated from the following equation:

$$MT = [1 - M_{SO}/M_O],$$

where M_{SO}/M_O gives the ratio of image intensities acquired with and without the application of a 5 s, 12 µT saturation pulse applied 6000 Hz off-resonance. Diffusion values are reported as percentages of the free water diffusion coefficient (D_W).

After NMR imaging the tissue was extracted from the HFBR, weighed, and submitted for biochemical analysis. Dried samples were digested with papain and the glycosaminoglycan (GAG) content was determined using the dimethylmethylene blue dye binding colorimetric assay (3). The collagen content of the tissue was derived from a colorimetric assay for hydroxyproline performed after hydrolysis of the papain digest with 6 N HCl at 110°C overnight (4).

Results and Discussion. Control bioreactors produced cartilage up to several millimeters thick around each fiber after two weeks of growth. Daily doses of AA, as opposed to treatment twice weekly, accelerated the formation of mature cartilage tissue. This matrix contained comparable levels of GAGs but significantly higher levels of collagen compared to controls. In contrast, treatment with RA resulted in a significant reduction in the GAG content of the tissue, but left the collagen content of the tissue unchanged. Treatment with IL-1B resulted in higher levels of GAGs compared to control bioreactors.

When the T_1 relaxation rate (R_1) was plotted against the GAG concentration of the matrix there was a good correlation between the two parameters ($R = 0.82$, $p < 0.001$). R_1 also correlated with the collagen concentration of the matrix ($R = 0.73$, $p < 0.001$), which was consistent with results for other collagen containing tissues (5). The T_2 relaxation rate (R_2) was found to correlate the GAG concentration of the tissue ($R = 0.81$, $p < 0.001$). This result was consistent with relaxation studies of polysaccharide gels (6). The correlation with tissue collagen concentration was weak. The correlation between MT and biochemically determined collagen content was fair ($R = 0.63$, $p = 0.003$). However, a similar relationship between MT and tissue GAG content was not observed. Finally, there was good correlation between the water diffusion coefficient in the tissue and its hydration level ($R = 0.64$, $p < 0.001$). This result was consistent with that obtained for explant cartilage (7).

Conclusions. We have developed correlations between NMR measurable parameters and tissue parameters for engineered cartilage tissue. This provides a method for monitoring tissue development non-invasively. These correlations may also be used to monitor the pathophysiology of cartilage degeneration as well as to the effects of various therapeutic interventions *in vivo*.

References.

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