Evaluation of Bioreactor-Cultivated Bone by Magnetic Resonance Microscopy

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Introduction: To study the impact of various biochemical and biomechanical factors on the bone formation process, we have developed a three-dimensional mineralizing culture system using a hollow fiber bioreactor (HFBR) system. The HFBR system was inoculated with primary osteoblasts and the tissue formed by the cells was interrogated spatially and temporally by proton MRM. Unlike conventional techniques such as histomorphometry, X-ray diffraction, and scanning and transmission electron microscopy, MRM can be performed without destructive preparative techniques. Therefore, spatial maps of MRM properties of the intact tissue may be used to detect unique changes in collagen and mineral content with high specificity, which in turn can be used to monitor the mineralization process indirectly. This non-invasive methodology has been adopted to examine bone formation in various tissue-engineering applications (1-3).

Experimental: Primary osteoblasts were obtained from the third population of cells released by serial collagenase digestion of the calvarial bones from normal 16-day old chick embryos (4). HFBRs were seeded with 10 million cells and cultured for up to 9 weeks. Mineralization was initiated after one week by the addition of 1% β-glycerophosphate to the culture medium. High-resolution MRM images of the HFBR cross-section, approximately 12 mm from the outflow end, were acquired with a nominal in-plane resolution of 78 µm on a Bruker DMX spectrometer at 9.4T at 37ºC. The following measurements were performed: water proton transverse (T2) relaxation time, water proton density (PD), and the magnetization transfer ratio (MTR) at weekly intervals. To assess temporal changes in tissue MRM parameters, we used a region-growing segmentation tool to establish Region 1, the tissue around fibers 1, 2 and 3, and Region 2, the tissue around fibers 4, 5, 6 and 7 (**Figure 1A**). At the end of the growth period, reactors were submitted for FTIR microspectroscopy to confirm the presence of bone, and mineral deposits were subjected to selected area electron diffraction (SAED) and electron probe microanalysis (EPMA).

Results and Discussion: FTIR microspectroscopy confirmed the presence of a heterogeneous bone-like tissue in the HFBR system and SAED and EPMA verified the deposition of poorly crystalline apatite. The temporal evolution of the tissue formed within the HFBR system was monitored by MRM and mineralized tissue, visible on PD maps, increased with time in culture. Mineralized zones within a HFBR, 9 weeks post-inocuation, are indicated with red arrows on the representative PD map shown in **Figure 1A**. Typically, the tissue around fibers 1, 2, and 3 (Region 1) was more hydrated (**Figure 1B**) with higher T2 values (data not shown) and lower MTR values (**Figure 1C**) compared to the tissue around fibers 4, 5, 6, and 7 (Region 2). Between weeks 4 and 6, the hydration state for both tissue zones steadily decreased with a concomitant increase in T2 values. This result is likely attributable to a loss of proteoglycans during early mineral formation. Collagen dehydration caused by mineral formation might explain why spatially resolved MTR values peak at week 6. By week 7, the hydration state of both tissue zones increased, resulting in higher T2 and lower MTR values. This result was attributed to the formation of additional osteoid. Finally, spatially resolved parameters measured at weeks 8 and 9 were consistent with mineralization of the newly formed osteoid. In conclusion, the spatial mapping of tissue parameters by non-invasive MRM supports the role of MRM in monitoring the bone formation process in this model mineralizing system.

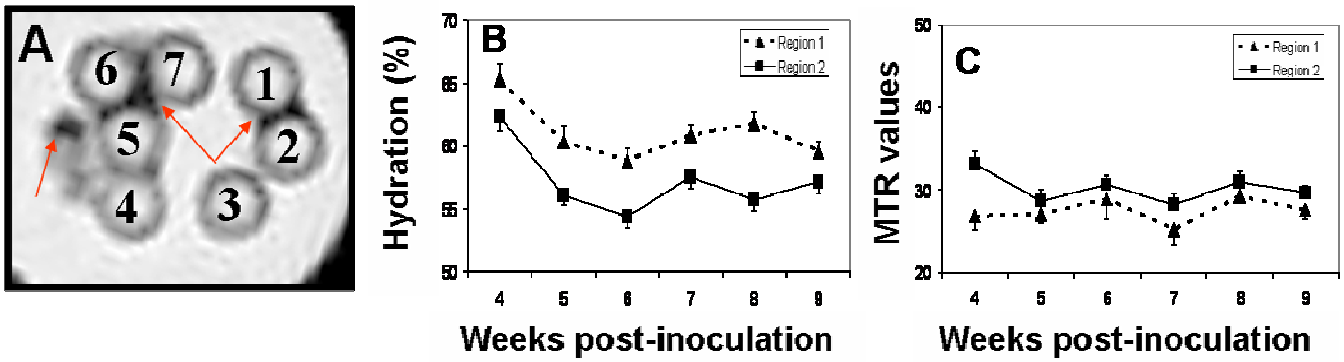

Figure 1. Proton density map of a single bioreactor, acquired at 9 (A) weeks post-inoculation, with a nominal in-plane resolution of 78 µm. Dark regions with red arrows correspond to heavily mineralized tissue. Spatially averaged tissue hydration (B) and MTR (C) values for tissue around fibers 1, 2, and 3 (Region 1, triangles connected with a dashed line) and for tissue around fibers 4, 5, 6, and 7 (Region 2, squares connected with a solid line) measured at weekly intervals.

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