Manganese-Enchanced Magnetic Resonance Microscopy of Mineralization

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Introduction: At present, detailed analyses of bone formation require specimens to be harvested at different time points and subjected to invasive techniques, such as histomorphometry, X-ray diffraction, and scanning and transmission electron microscopy. These techniques are of limited value because specimen preparation may disrupt labile mineral phases, or the detection of calcium may be limited to mineral deposits. Non-invasive X-ray imaging can be used to study the dynamics of bone development (*1*), but this modality does not have the required sensitivity to monitor very low levels of calcium inside cells or within extruded matrix vesicles. In this work, we propose that manganese can be processed like bone calcium and sensitize the MRM experiment to mineral formation at a mineralizing front.

Experimental: To verify that primary osteoblast cells take up manganese, cells were grown to confluence in T-75 flasks, treated with 1 mM $MnCl_2$ for 48 hours, and subsequently subjected to MRM and inductively coupled plasma mass spectrometry (ICP-MS). To test the hypothesis that manganese can substitute for calcium in a mineralizing tissue, a series of calvariae from 16-day chick embryos were cultured in medium supplemented with 1 mM $MnCl_2$, 3 mM $CaCl_2$, and 1% β-glycerophosphate for up to 3.5 weeks. The uptake and immobilization of manganese was monitored with MRM, electron probe microanalysis (EPMA), and ICP-MS.

Results and Discussion: For manganese-treated cells, the water proton longitudinal relaxation time (T_1 = 1.06 ± 0.15 s) and the magnetic transfer ratio (MTR = 0.19 ± 0.02) values were reduced compared to the untreated cells (T_1 = 2.67 ± 0.16 s, MTR = 0.39 ± 0.04). The observed reduction in T_1 was attributed to the presence of intracellular manganese. When subjected to ICP-MS, the manganese content of the manganese-treated cells was 7.8 ppm compared to 0.1 ppm in the control cell pellet. This result confirmed that osteoblasts take up manganese present in the culture medium. MRM images of organ-cultured calvariae revealed an overall increase in calvarial size and an increase in the amount of mineral deposited at longer culture times. In manganese-treated calvariae, a banding pattern of high and low transverse relaxation time (T_2) values was observed radiating out from the calvarial ridge (**Figures 1A-1C**). Such a pattern was not observed in control specimens (**Figure 1D**). Low-magnification EPMA of organ-cultured calvariae confirmed the deposition of manganese on the extra-cranial surface. ICP-MS data confirmed that untreated specimens did not contain detectable levels of manganese, and that manganese supplementation resulted in increasing levels of manganese (0.9 – 2.5%) with longer times in culture (1 – 3.5 weeks). In conclusion, these studies support the use of manganese-enhanced MRM for studying the process of bone formation in mineralizing cell and organ culture systems.

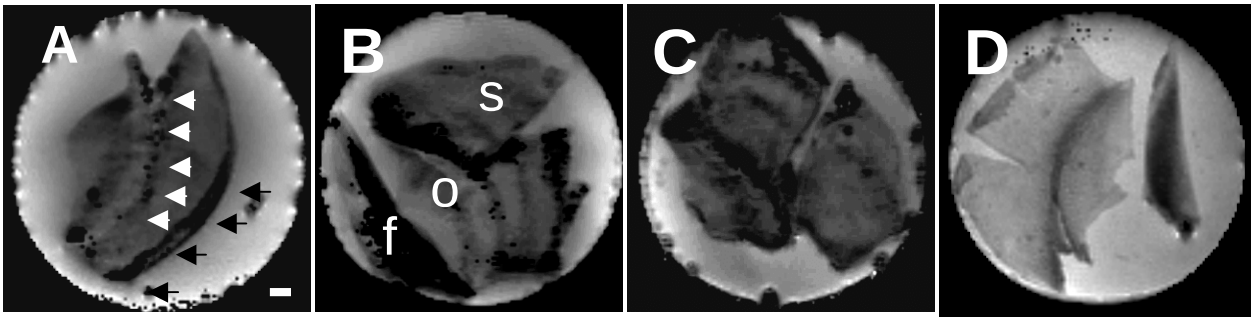

Figure 1. Quantitative T_2 maps acquired for mineralizing calvaria maintained in culture medium, supplemented with 1 mM $MnCl_2$, 3 mM $CaCl_2$, and 1% β-glycerophosphate for 1 (A), 2 (B), and 3.5 (C) weeks. MRM images were acquired at room temperature with a nominal in-plane resolution of 120 microns. The calvarial ridge (white arrows), mid-line suture (black arrows), and the superficial (s), orbital (o), and frontal (f) regions of the calvaria are indicated. The scale bar represents 1 mm. (D) T_2 map of control calvaria organ-cultured for 2 weeks without any manganese added to the culture medium.

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Reference: (*1*) Stock, S. R., et al., Dev Dyn (2003) **226**(2): 410-417.