# Manganese-Enhanced Magnetic Resonance Microscopy of Mineralization Rates

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**Introduction:** Skeletogenesis in vertebrates is characterized by the precise spatial and temporal arrangement of mesemchymal cells and the expression of specific extracellular matrix genes, which can either promote, regulate, or inhibit mineral deposition. Our current knowledge of progressive events is based on highly informative, though static, studies of specially prepared sections of mineralizing tissues or from whole embryo staining [1]. There are very few non-invasive, non-ionizing techniques that are capable of spatially mapping the mineralizing activity of viable specimens. To address this need, we present the application of Mn-enhanced MRM to (i) measure the level of mineralizing activity in organ-cultured calvariae and (ii) map the spatial variation in mineralizing activity over the surface of embryonic skull bones. This study is an extension of our earlier work in which we established that organ-cultured calvariae can process the manganese present in the culture medium to mineralized deposits at high doses of manganese (1mM) and with chronic exposure [2]. In this work, organ-cultured calvariae will be treated with a low dose of manganese (100 µM) for just 24 hours to reduce the toxic effects of manganese. Even at this exposure level, organ-cultured calvariae sequester sufficient manganese to alter water proton relaxation times compared to untreated specimens [3].

**Experimental:** Calvariae from 16-day chick embryos were treated with 100 µM $MnCl_2$, 3 mM $CaCl_2$, 50 µg/ml ascorbic acid, and 1% β-glycerophosphate for 24 hours after 1, 8, 15, and 22 days in culture. MRM images were acquired 48 hours after manganese treatment on culture days 4, 11, 18, and 25. Mn-treated and control calvariae were imaged between two glass plates positioned in a tube filled with phosphate buffered saline. High-resolution MRM images, with a nominal in-plane resolution of 109 µm, were acquired at 37ºC on a Bruker DMX spectrometer operating at 9.4T. Water proton longitudinal (T1) relaxation times were measured with a saturation recovery sequence. After MRM, organ-cultured calvariae were analyzed by laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) [4]. Manganese uptake studies were performed on parallel organ-culture experiments and those specimens were analyzed by inductively coupled plasma optical emission spectrometry (ICP-OES).

**Results and Discussion:** ICP-OES studies confirmed the overall increase in mineral content for both Mn-treated and control specimens with time in culture. More importantly, the Mn content was significantly higher in specimens treated on day 1 (965 ± 56 ppm) compared to those treated on day 22 (688 ± 38 ppm). Representative T1 maps of specimens treated on days 1 and 22 are shown in Figures 1A and 1B, respectively. These images confirm that more Mn is sequestered on day 1 compared to day 22. Additionally, a detailed analysis of the regional changes in T1 values, with respect to age-matched controls, confirm that specimens treated on day 1 sequester Mn on all surfaces while specimens from the later time points sequester Mn on the more posterior regions of the superficial (S) surface. These results were validated against Mn elemental maps acquired using LA-ICP-MS. The Mn maps of specimens treated on days 1 and 22 are shown in Figures 1C and 1D, respectively. These maps confirm the reduced levels of Mn and the preferential uptake of Mn by the posterior regions of the superficial surface for the later time point. In areas with T1 values higher than the surrounding bone (arrows), the Mn content was highest on the LA-ICP-MS map. On close inspection, it was found that the T1 values for those areas were markedly reduced compared to similar regions in untreated samples, which is consistent with the LA-ICP-MS findings. This work supports the application of Mn-enhanced MRM to the study of different treatment paradigms on mineralization rates.

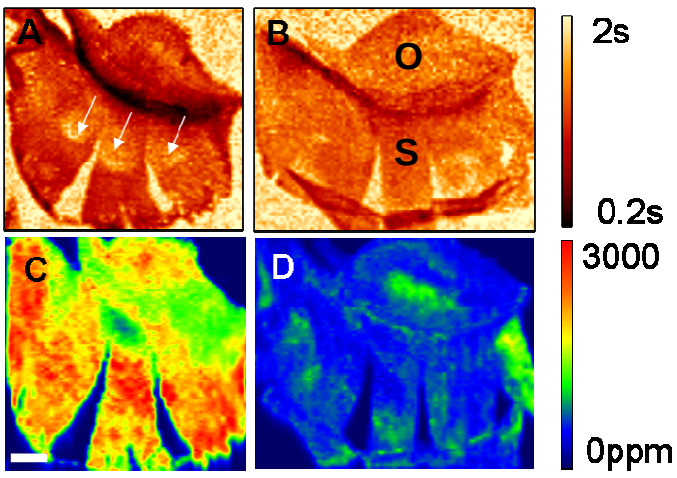


**Figure 1.** Representative T1 maps of organ-cultured calvariae treated with 100µM Mn on day 1 (A) and day 22 (B). Corresponding LA-ICP-MS maps of the Mn content of the mineral deposited on the extracranical surface of calvariae treated on day 1 (C) and day 22 (D). Scale bar represents 1mm. The superficial (S) and orbital (O) regions of the calvariae are indicated

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**References:** 1. Tuan RS, et al. Dev Biol 1983; 100: 374-386. 2. Chesnick IE, et al. Magn Reson Imaging 2007; 25: 1025-1104. 3. Chesnick IE, et al. Proc Intl Soc Mag Reson Med 2007; 15: 2672. 4. Günther D, et al. Trends Analyt Chem 2005; 24: 255-265.