Magnetic Resonance Microscopy of Collagen Mineralization

I. E. Chesnick[1], J. T. Mason[1], and K. Potter[1]

[1]Magnetic Resonance Microscopy Facility, Dept. of Biophysics, Armed Forces Institute of Pathology Annex, Rockville, MD, United States

Introduction: It is widely accepted that collagen-containing tissues give rise to a significant magnetization transfer (MT) effect (1,2). In our experience, the MT effect of collagen is enhanced in mineralizing tissues, such as the avian growth plate (3), calcifying cartilage (4), osteoblast-seeded polymer scaffolds (5), and tissue-engineered phalanx model (6). We hypothesize that as mineralization progresses the MT effect is enhanced due to the shrinkage of the collagen lateral packing arrangement caused by the loss of water from the collagen fibrils and the formation of mineral deposits. To test our hypothesis, we subjected a model mineralizing system to MRM. In this model system, amorphous calcium carbonate, produced by exposing a $CaCl_2$ solution to the decomposition products of ammonium carbonate, is stabilized by poly-acrylic acid. This polymer-stabilized mineral precursor flows into the gaps and groves of collagen fibrils and, upon solidification, forms plate-like crystals between the collagen fibrils (7).

Experimental: A Cellagen™ sponge (ICN Biomedicals, Inc., Aurora, OH), cut into 2 x 10 mm rectangular strips, was rehydrated in 12 mM $CaCl_2$. At the start of the mineralizing process strips were placed in petri dishes with 12 mM $CaCl_2$ and 0.33 mg/ml polyacrylic acid and the dishes were transferred to a dessicator where they were exposed to the decomposition products of ammonium carbonate. Every three days the calcifying solution was changed and additional strips were added to the chamber. This process was repeated until 24 days had passed. At the end of the mineralization process, collagen strips were imaged between two glass slides with spacers to prevent them for being crushed. 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. The following MRM parameters were measured for collagen sponges at each time point: the water proton density (PD), the water proton transverse (T2) relaxation time, and the magnetization transfer ratio (MTR). The final mineral content of the collagen strips was measured using an Alizarin red-based calcium assay (8) and the state of mineral deposits was assessed by X-ray diffraction.

Results and Discussion: A representative proton density image of collagen strips exposed to mineralizing conditions for 0, 3, 6, 13, 15, and 18 days, moving from the bottom to the top of the stack, is shown in **Figure 1A**. We also present MRM graphs of the hydration state (**Figure 1B**), T2 (**Figure 1C**), and MTR (**Figure 1D**) values of the collagen sponges from all time points up to day 24. Based on X-ray diffraction data (not shown) and MRM-derived parameter data (shown below), we found that the mineralizing process can be sub-divided into three phases. During the initial phase, between days 0 and 3, both the hydration state ($p = 0.007$) and the T2 values ($p = 0.02$) were markedly reduced compared to those of control sponges due to the replacement of fibrillar water with amorphorous calcium carbonate. X-ray diffraction data confirmed the earliest appearance of small mineral deposits. In the second phase, between 6 and 13 days, calcium carbonate crystallites grew between the collagen fibrils resulting in a gradual reduction in T2, but little change in sponge hydration. X-ray diffraction data confirmed the increase in the crystallinity of the calcite phase as well as the unchanged hydration state of collagen. We speculate that MTR values increase from 0.26 ± 0.02 for controls strips to a maximum value of 0.33 ± 0.04 at day 15 because mineral accretion reduces the mobility of the collagen fibrils. This result was consistent with the increased MTR values observed for cartilage specimens fixed with formalin (9). In the third and final phase of the mineralizing process, after day 15, mineral crystals proceed to encapsulate the collagen fibrils, which explains the overall reduction in the absolute intensity of the collagen diffraction pattern and the reduction in MTR values after day 15. In conclusion, this work supports the use of MTR values as a surrogate measure of collagen mineralization, which in turn can be used to gain further insights into the maturation state of mineralizing tissues.

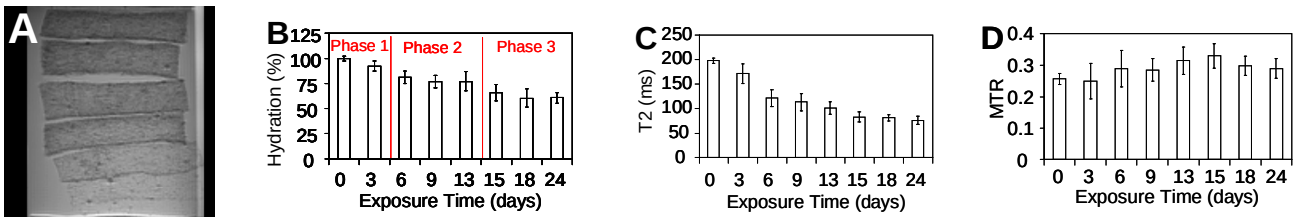

Figure 1. Proton density map (A), acquired at 37°C with a nominal in-plane resolution of 109 µm, for collagen strips exposed to mineralizing conditions for 0, 3, 6, 9, 13, 15, and 18 days (from bottom to top). Graphs of the hydration (B), T2 (C), and MTR (D) values of collagen sponges exposed to mineralizing conditions for different lengths of time.

Acknowledgements: The authors thank Matt Olszta and Dr. Laurie Gower (University of Florida) for their advice on running these experiments.

References: 1. Eng J, et al. Magn Reson Med (1991) **17:**304-314. 2. Ceckler TL, et al. J Magn Reson (1992) **98:**637-645. 3. Potter K, et al. J Bone Miner Res (2001) **16:**1092-1100. 4. Potter K, et al. J Bone Miner Res (2002) **17**(4)**:**652-660. 5. Washburn NR, et al. J Biomed Mater Res (2004) **67A:**738-747. 6. Potter K, et al. Bone (2006) **38:**350-358. 7. Olszta MJ, et al. Connect Tissue Res (2003) **44:**326-34. 8. Gregory CA, et al. Anal Biochem (2004) **329:**77-84. 9. Fishbein KW, et al. Magn Reson Med (2007) **57:**1000-11.