Spatial Mapping of Collagen Deposition in Bone Cultures by Magnetic Resonance and FTIR Micro-Imaging

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Introduction: Collagen is the predominant macromolecular constituent in cartilaginous tissues that gives rise to a significant magnetization transfer (MT) effect (*1*,*2*). Using this parameter, calibration curves have been derived that allow for the mapping of the collagen content of articular cartilage (*3*) and of engineered cartilage (*4*). However, there are few studies in the literature that attribute the measured MT effect of bone to its collagen content. In this work, the amount of collagen deposited by osteoblasts during the mineralization process will be assessed with quantitative magnetic resonance microscopy (MRM)-derived magnetization transfer ratio (MTR) maps and validated against collagen maps derived by FTIR reflectance-transmission microspectroscopy (FTIR-RTM) (*5*).

Experimental: To develop a suitable MRM-FTIR calibration curve, osteoblasts were grown directly on poly (*D*,*L*-lactide-co-glycolide) (PLGA) films (*6*), and the PLGA films were subjected to both MRM and FTIR-RTM mapping. MRM images were acquired on a Bruker DMX spectrometer coupled to a wide-bore magnet operating at 9.4T. MTR maps were calculated using the equation: [1 – *Mso*/*Mo*], where *Mso*/*Mo* gives the ratio of image intensities acquired with (*Mso*) and without (*Mo*) the application of a 5-s, 12-μT saturation pulse, 6000 Hz off-resonance. After MRM imaging, the PLGA films were placed on reflective glass slides and subjected to FTIR-RTM mapping using a Nicolet Magna-IR 550 FTIR spectrophotometer interfaced with a Nic Plan microscope. The microscope is equipped with a video camera, a liquid nitrogen cooled-mercury cadmium telluride detector, a computer-controlled mapping translation stage, and Atlus software. Using pure component spectra for collagen and PLGA, a multivariate approach was used to extract maps for the spatial distribution of collagen and PLGA from the FTIR-RTM data. The resulting collagen calibration curve was used to establish the amount of collagen deposited by calvarial osteoblasts in a three-dimensional mineralizing system based on a hollow fiber bioreactor (HFBR).

Results and Discussion: A representative FTIR-RTM image of a PLGA film is shown in **Figure 1A**. Using pure component spectra for collagen and PLGA and a multivariate approach, maps for the spatial distribution of collagen and PLGA were extracted from the FTIR dataset. The FTIR-derived collagen map in **Figure 1B** was comparable to the MTR map in **Figure 1C**, which was acquired from the same film. By plotting the MTR values for different regions on the PLGA film against the FTIR-derived collagen content of each region, we were able to generate an MTR-FTIR calibration curve (**Figure 1D)**. HFBR mineral deposits, which typically formed at the surface and between fibers, had low T2 and high MTR values. FTIR-RTM maps of tissue sections taken from the same approximate location as that of the MRM slice data confirmed the presence of high levels of collagen in zones containing bone-like mineral. In conclusion, spatial maps of collagen content derived from MTR maps were consistent with the FTIR-derived collagen maps.

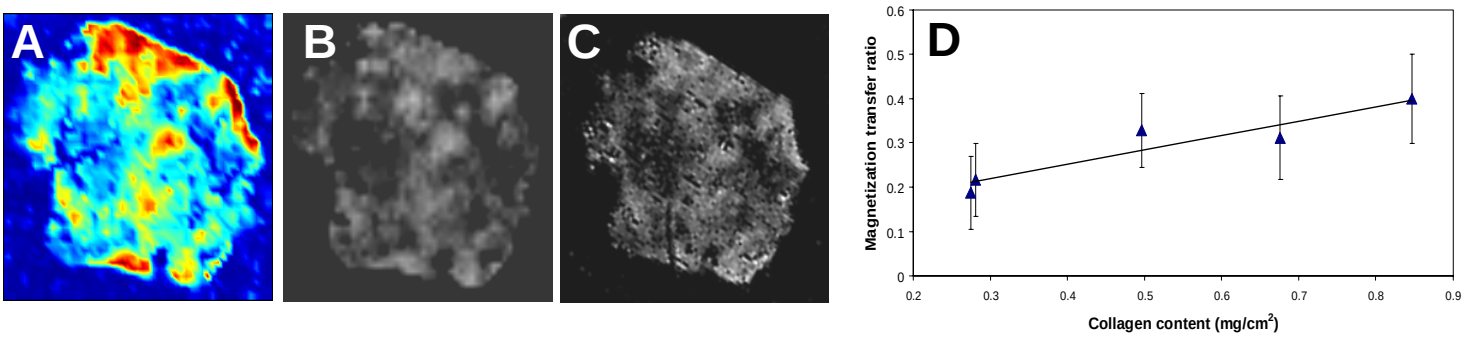

Figure 1. (**A**) Amide I plane extracted from the FTIR hyperspectral dataset (low -> high values = blue -> red), (**B**) FTIR-derived collagen map, and (**C**) MTR map of a PLGA film 4.5 weeks post-inoculation. Five regions of interest were selected from the images in **B** and **C**, and their mean values were used to generate the MTR-FTIR calibration curve shown in (**D**).

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