Magnetic Resonance Microscopy of a Novel Mineralizing System

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Introduction: The limited supply of autograft material for the repair of skeletal defects has inspired a number of strategies for the production of replacement bone tissue. In the majority of cases, bone cells are required to attach and grow on a three-dimensional scaffold (1). While two-dimensional culture techniques can be applied to three-dimensional scaffolds, confluent cultures cannot be maintained for long, owing to insufficient nutrient diffusion within the scaffolds. To overcome this problem scaffolds were implanted onto the allantochorial membrane of chick embryos (2). The development of the bone-like tissue within explanted scaffolds was confirmed by Magnetic Resonance Microscopy (MRM) and verified using fluorescence microscopy.

Materials and Methods: Primary osteoblasts were obtained from the third population of cells released by serial collagenase digestion of the calvarial bones of 16-day old chick embryos (3). First-passage osteoblasts were resuspended in tissue culture medium and 1 x 10^5 cells were seeded onto OPLA® cylinders (BD Biosciences, Bedford, MA). Mineralization was initiated after one week by the addition of 1% β-glycerophosphate to the culture medium. After 3 weeks in culture, scaffolds were implanted onto the allantochorial membrane of day 6 chick embryos. Scaffolds were explanted after 7 days and submitted to MRM together with cell-seeded scaffolds maintained in culture during the implantation period. MRM images, with a nominal in-plane resolution of 70 μm, were acquired at 37ºC on a Bruker DMX spectrometer operating at 9.4T. The following parameters were measured: the water proton transverse (T2) relaxation time, the water proton density (PD), and the magnetization transfer ratio (MTR). After MRM examination, scaffolds were embedded in OCT and cryosectioned at 30 μm. Cryosections were subsequently treated with the following fluorescent dyes: DAPI for nuclei; calcein for mineral; and Alexa Fluor® 555 labeled antibodies against chick Type I collagen.

Results and Discussion: Long term cultures (8 to 10 weeks) of cell-seeded polymer scaffolds yield limited bone growth on the scaffold exterior and very little bone growth in the interior. To generate bone suitable for autograft material 3D OPLA® scaffolds were implanted onto the allantochorial membrane of chick embryos where blood vessels can invade the scaffold material and provide nutrients and oxygen for the proliferation of the osteoblasts and the recapitulation of bone within the scaffold. The infiltration of blood vessels into one such scaffold can be observed in the photograph shown in Figure 1A. High-resolution T2 and MTR images of an implanted scaffold (top) and a non-implanted scaffold (bottom) are shown in Figures 1B and 1C, respectively. The reduction in water proton T2 values and the increase in water proton MTR values for the implanted scaffold compared to the non-implanted scaffold were consistent with the formation of a bone-like tissue within the polymer scaffold. This result was confirmed by fluorescence microscopy. A representative fluorescence image is shown in Figure 1D, in which the cells appear blue, the mineral deposits are green, and the collagen matrix is red. Relatively thick sections were cut so as to keep the scaffold intact. To better understand the dynamics of the bone formation process, MRM will be used to monitor cell-seeded scaffolds throughout the implantation period *in ovo*.

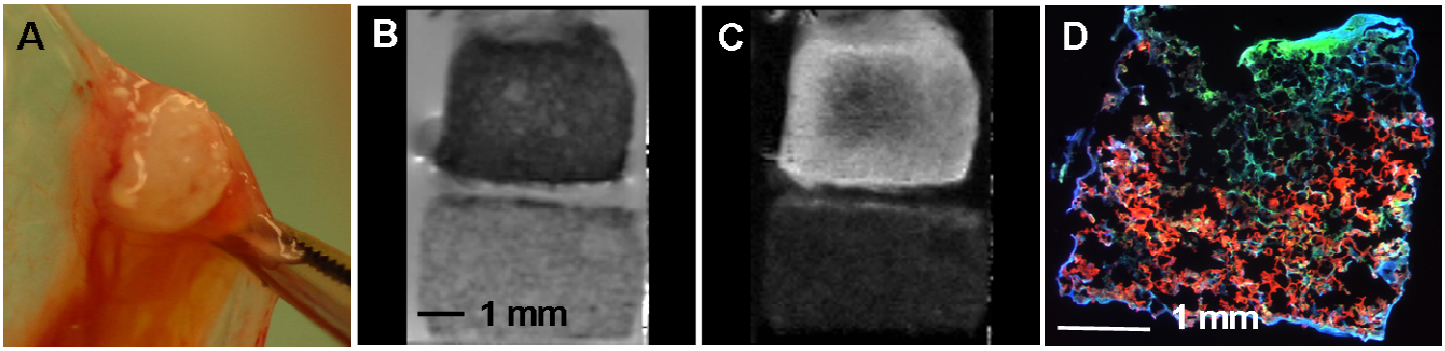

Figure 1. (A) Photograph of cell-seeded scaffold on the allantochorial membrane of a chick embryo, seven days post implantation. Representative T2 (B) and MTR (C) maps of an implanted scaffold (top) with a non-implanted scaffold (bottom). Fluorescence image (D) of a section from an implanted scaffold in which the cells appear blue, mineral deposits are green, and the collagen matrix is red.

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References: 1. Crane GM, et al. Nat Med 1995; **1:**1322-1324. 2. Mancini L, et al. Ann N Y Acad Sci. 2007 (in press). 3. Gerstenfeld LC, et al. Dev Biol 1987; **122:**49-60.