Characterization of Bone Explants by Magnetic Resonance Microscopy

I. E. Chesnick[1], C. B. Fowler[1], F. A. Avallone[2], and K. Potter[1]
[1]Department of Biophysics, Armed Forces Institute of Pathology Annex, Rockville, MD, United States, [2]Department of Genitourinary Pathology, Armed Forces Institute of Pathology, Washington, DC, United States

Introduction: There are numerous examples in the literature of the application of MRI to the evaluation of engineered bone constructs in vitro [1-3]. Importantly, the behaviour of water proton MRI parameters with the accumulation of collagen with mineralized deposits is well understood [4]. The current challenge is to characterize bone constructs post-implantation. MRI studies of ectopic bone formation clearly demonstrate the utility of the MRI technique for monitoring the bone formation process in vivo [5]. However, the detection of ectopic bone formation is not as challenging as orthotopic bone formation, which is a clinically relevant end-point. In an attempt to evaluate bone formation in vivo, we present our MRI finding for cell-seeded scaffolds implanted onto the chorioallantoic membrane (CAM) of a chick embryo. The CAM model is an excellent in vivo model system to study the vascularization of tissue engineered constructs because of the accessibility of the vascular network and its lack of immuno-competence permits the use of xenogenous implants [6].

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 [7]. First-passage osteoblasts were resuspended in tissue culture medium and 2.5 x 10^5 cells were seeded onto OPLA® cylinders (BD Biosciences). After one week of growth, scaffolds were reseeded with an additional 2.5 x 10^5 osteoblasts and allowed to incubate for 24 hours. Cell-seeded (SIM) and control (CIM) scaffolds were implanted onto the CAM of day 6 chick embryos. At the same time, cell-seeded (SIV) and control (CIV) scaffolds were mineralized in vitro by the addition of 1% β-glycerophosphate to the culture medium. SIM and CIM scaffolds were explanted after 7 days and submitted to MRM with SIV and CIV scaffolds maintained in vitro. MR 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 8 μm. Serial cryosections were subsequently treated with the following: fluorescently labeled alendronate (FL-ALN)–Alexa Fluor® 350 for mineral [8], anti-αvβ3(Chemicon)-Alexa Fluor® 555 expressed by angiogenic vessels, anti-collagen I (DSHB)-Alexa Fluor® 555, phalloidin-Alexa Fluor® 488 (Invitrogen) for actin, and DAPI (Invitrogen) for nuclei.

Results and Discussion: Cell-seeded scaffolds maintained in vitro have limited tissue growth throughout the scaffold due to a lack of nutrient transport to the center of the scaffold in static culture. In contrast, when scaffolds are implanted onto a CAM, blood vessels invade the scaffold and provide nutrients and oxygen for the proliferation of the osteoblasts and the recapitulation of bone throughout the scaffold [9]. High-resolution T2 images of SIM (A), CIM (C), and SIV (E) scaffolds are shown in Figure 1. The reduction in water proton T2 values with a concomitant increase in MTR values (data not shown) compared to CIV scaffolds were observed for all scaffolds. This observation is consistent with the formation of bone-like tissue within the scaffold. However, sections stained with FL-ALN revealed mineral deposits in SIM (B) and SIV (D) scaffolds but not in CIM (E) or CIV (data no shown) scaffolds. To explain the dark annulus on the CIM T2 map (E), scaffolds were stained with anti-αvβ3-Alexa Fluor® 555. Both the SIM (B) and CIM (F) scaffolds were positive for new blood vessels while the SIV scaffold (D) did not retain any red stain. This result confirms the presence of angiogenic vessels for the SIM and CIM scaffolds and the presence of these vessels can confound MRI findings of mineral deposition. Therefore, on-going studies in our laboratory aimed at evaluating bone formation in tissue engineered scaffolds post-implantation will use targeted contrast agents for studying mineral deposition and blood vessel infiltration.

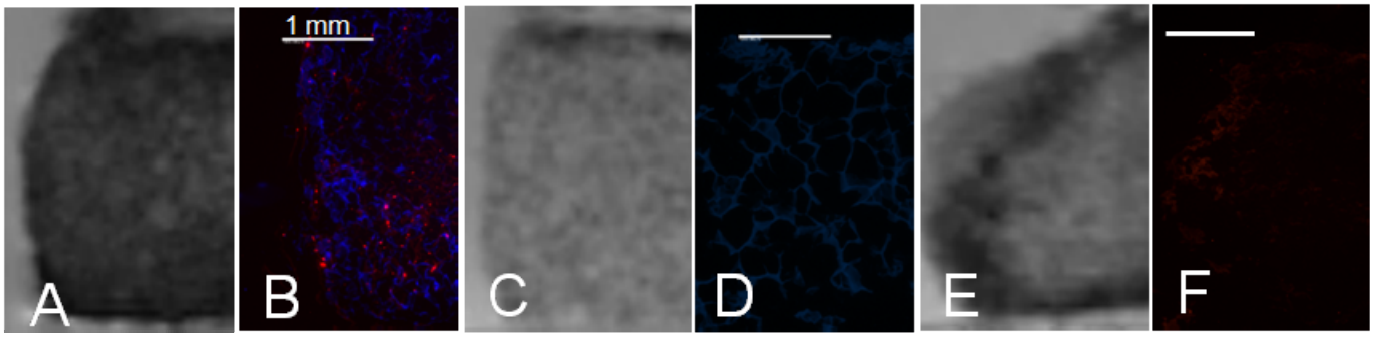

Figure 1. Representative T2 maps and composite fluorescence images of (A, B) SIM, (C, D) SIV, and (E, F) CIM scaffolds, respectively. Contiguous sections stained with anti-αvβ3 Alexa Fluor® 555 (red) for angiogenic vessels and FL-ALN (blue) for mineral deposits were used to generate composite fluorescence images.

Acknowledgements: This work was supported in part by NIH grants AR51446 (KP).

References: **1.** Chesnick, I.E., et al., Bone, 2007. **40**(4): p. 904-12. **2.** Peptan, I.A., et al., Tissue Eng, 2006. **12**(4): p. 843-51. **3.** Washburn, N.R., et al., J Biomed Mater Res, 2004. **67A**: p. 738-747. **4.** Chesnick, I.E., et al., Biophys J, 2008. **95**(4): p. 2017-26. **5.** Hartman, E.H., et al., Tissue Eng, 2002. **8**(6): p. 1029-1036. **6.** Borges, J., et al., Tissue Eng, 2003. **9**(3): p. 441-50. **7.** Gerstenfeld, L.C., et al., Dev Biol, 1987. **122**(1): p. 49-60. **8.** Thompson, K., et al., Mol Pharmacol, 2006. **69**(5): p. 1624-32. **9.** Mancini, L., et al., Ann N Y Acad Sci, 2007. **1116**: p. 306-15.