

Magnetic Resonance Molecular Imaging of Bone Resorbing Osteoclasts

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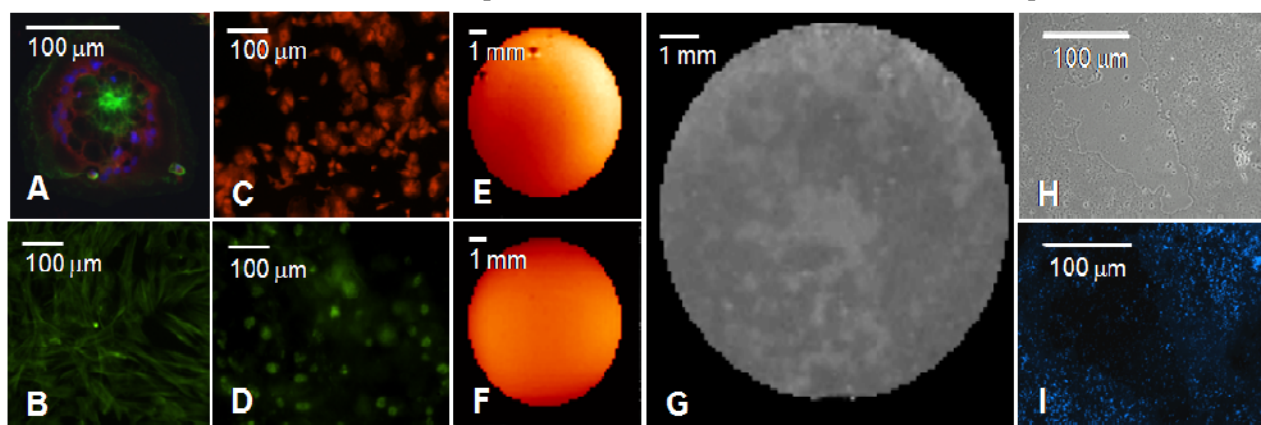
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Introduction: Magnetic resonance molecular imaging has been widely adopted for studies of tumor biomarkers and tumor angiogenesis because protein expression levels are significantly higher compared to healthy tissue [1]. In this work, we demonstrate the ability of a targeted Gd-liposomes to detect relatively low numbers of active osteoclasts in the presence of a mixed population of cells. Additionally, we present a novel bisphosphonate contrast agent for imaging mineral pits generated by bone-resorbing osteoclasts. The ability to detect osteoclasts on the surface of bone will allow for the development of more sensitive screening tools for monitoring changes in bone metabolism, specifically bone resorption, in response to therapeutic interventions [2].

Materials and Methods: Osteoclast cells were prepared from bone marrow extracted from embryonic chick femurs [3]. Cells were cultured for 24 hours and trypsin-sensitive cells were transferred onto OsteologicTM discs (BD Biosciences, Chicago, IL) and maintained in culture for at least 2 to 4 weeks with 10nM vitamin D₃ (Calcitriol, Axxora, San Diego, CA) supplementation every 2 days. After fixation with paraformaldehyde, the samples were incubated with biotinylated anti- $\alpha_v\beta_3$ (Chemicon), washed with PBS, blocked with 1% BSA, and then incubated with Neutravidin (Pierce, Rockford, IL). After washing twice with PBS and unlabeled, blocking liposomes, the discs were incubated with biotinylated Gd-liposomes or AlexaFluor[®]488. Fluorescence images of rhodamine-containing Gd-liposomes or AlexaFluor[®]488 were used to confirm the successful labeling of the osteoclasts with the target antibody. Next, samples were subjected to high-resolution MR imaging in our 9.4T system equipped with a micro-imaging gradient set and a custom built triple-tuned (¹H, ³¹P, and ¹⁹F) probe (Bruker Biospin) for imaging thin tissue sections. High-resolution T1-weighted (T1w) images were acquired with a nominal in-plane resolution of 100 μ m. After MRM examination, cells were removed and the mineral disc was treated with admixture of two contrast agents that use a bisphosphonate to deliver GdDOTA (Gd-ALN) or AlexFluor[®]350 (FL-ALN) to the residual mineral islands.

Results and Discussion: A representative fluorescence image of a mature chick osteoclast is shown in Fig 1A. This large multinuclear cell has a classic actin ring (green) around its sealing zone where it actively resorbs mineral and it expresses $\alpha_v\beta_3$ (red) throughout its cytoplasm [4]. Accordingly, in a mixed population of cells labeled with phalloidin-AlexaFluor[®]488 (Fig 1B), anti- $\alpha_v\beta_3$ -Rd-Gd-liposomes (Fig 1C) and anti- $\alpha_v\beta_3$ -AlexaFluor[®]488 (Fig 1D) uniquely label differentiated osteoclasts. Similar wells treated with Gd-liposomes produced significant signal enhancement on T1w images (Fig 1E) compared to control wells treated with anti- $\alpha_v\beta_3$ -AlexaFluor[®]488 (Fig. 1F). To study the resorbing activity of the osteoclasts, the mineral disc was treated with both Gd-ALN and FL-ALN after the removal of cells. A T1 map of the disc is shown in Fig 1G. High T1s (bright) occur in the pits and low T1s (dark) occur in those locations not subjected to mineral resorption by active osteoclasts. On the bright field image (Fig 1H) the approximate location of mineral pits occurs in those locations that appear black on the FL-ALN image (Fig. 1I) of the same disc. In conclusion, targeted contrast agents for osteoclasts cells and for mineral can provide valuable information about bone resorption in vivo.

Figure 1. A fluorescence image of a mature osteoclast (A) and a mixed population of actin-labeled cells (B) derived from bone marrow. Fluorescence images of identical wells stained with anti- $\alpha_v\beta_3$ labeled with (C) Rd-Gd-liposomes (red) and (D)



AlexaFluor488 (green) and their corresponding T1w images (E) and (F), respectively. T1 map (G) of a mineral disc stained with both Gd-ALN and FL-ALN and corresponding bright field (H) and fluorescent (I) images of the mineral pits.

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