Relaxometry of Bacterially Derived Organelles: A Novel Class of MRI Contrast Agent for Cell Labeling and Tracking

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Purpose: Superparamagnetic iron oxide (SPIO) contrast agents have been used for a number of years to label cells for tracking by MRI[1-3]. However, these particles are significantly disadvantaged in longitudinal imaging because, as cells divide, the amount of iron per cell decreases and eventually the labeled cells can no longer be detected. MR reporter genes exist that allow cells to produce iron particles, but these have met with limited success[4,5]. These reporters also require modification to the host cell genome, making them less translatable.

Recently, Bell Biosystems Inc. (Palo Alto, CA) has developed a bacterial-derived magnetic pseudo-organelle known as the "Magnelle®," from magnetotactic bacteria, building on previous work that explored bacteria as a potential MR contrast agent[6]. Since magnetotactic bacteria naturally generate magnetosomes (chains of lipid bilayer enclosed compartments containing magnetite crystals) through the coordination of over 100 genes, they may have certain advantages as MR contrast agents. Some MR reporter gene candidates are based on magnetotactic bacterial genes such as magA and mms6[4,5]. Since Magnelles were derived from bacteria, they have the ability to self-replicate, making them interesting candidates for labeling and longitudinal evaluation of cells. This is crucial for many emerging applications, particularly evaluation of stem cell and other cell-based therapies. This work presents initial characterization of the MRI relaxivity properties (both r1 and r2) of Magnelles, their cell loading, as well as *ex vivo* imaging characteristics using a model breast cancer cell line.

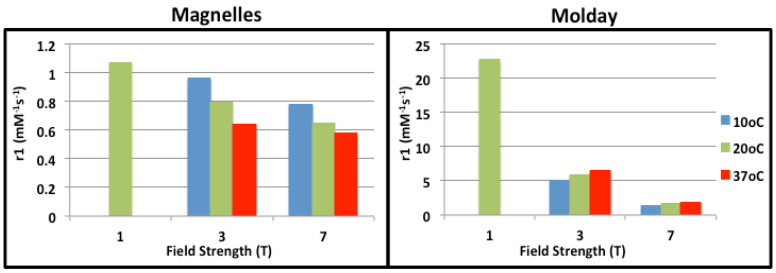

Figure 1 – Longitudinal relaxivity (r1) characteristics for both Magnelles and Molday at different field strengths and temperatures. Error bars are the std dev of the r1 calculation.

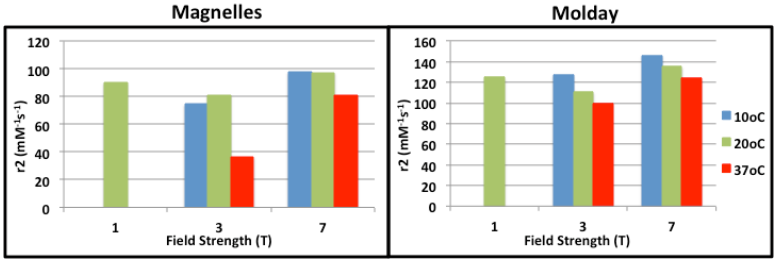

Figure 2 – Transverse relaxivity (r2) characteristics for both Magnelles and Molday at different field strengths and temperatures. Error bars are the std dev of the r2 calculation.

Methods: Magnelles were obtained from Bell Biosystems, Inc. MR relaxation properties of Magnelles were measured and compared to Molday ION Rhodamine B SPIO particles (BioPAL, Worcester, MA). All samples were prepared by suspending Magnelles or Molday particles in 200ul of 4% gelatin in mini-PCR tubes. For *ex vivo* imaging, MDA-MB-231 breast cancer cells were labeled with Magnelles overnight using manufacturer instructions, then suspended in PBS. A Balb/c nude mouse with two shoulder-mounted A549 human lung carcinoma subcutaneous tumors was humanely sacrificed. One million magnelle labeled cells were injected intratumorally into the right tumor and 1 million unlabeled cells into the opposite tumor.

MR relaxivity characterization was done at 1T, 3T and 7T (using Bruker ICON, GE MR750 and MR950 imagers, respectively). At 7T and 3T, a single channel receive-only surface coil was used, and a single channel T/R volume coil at 1T. T_1 mapping was done using an IR-FSE sequence with ETL=8, TE/TR= min/6000ms. Eight TIs were used: 4000, 2500, 1500, 800, 400, 200, 100 & 50ms. T_2 mapping was done using a 16-echo spin-echo sequence with TR=1600ms, echo spacing= 6.6ms (7T), 7.7ms (3T), or 8ms (1T). *Ex vivo* mouse MR images were done at 1T with a T_1-weighted FLASH, FOV=3cm, 128x128, 1mm slice thickness, TR/TE=174/6ms, NEX=4, FA=70°. Iron measurements were done using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES).

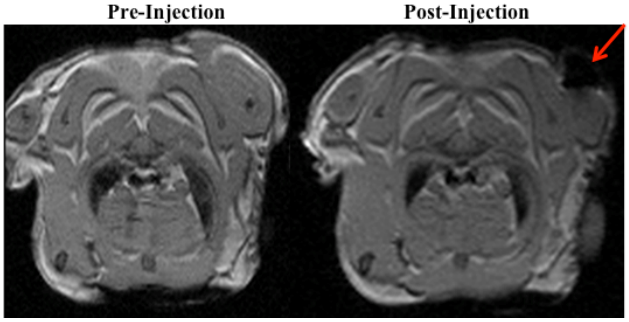

Figure 3 – *Ex vivo* images of Magnelle labeled MDA-MB-231 breast cancer cells injected into the right subcutaneous tumor (unlabeled cells injected into tumor in opposite shoulder exhibit no observable signal).

Results & Discussion: Longitudinal relaxivity (r1) values found in Fig. 1 show that, although Magnelles exhibit typical decreasing r1 with increasing field strength, the absolute r1 values and their relative field-dependent change are far smaller than for conventional iron oxide nanoparticle agents such as Molday. As well, the temperature dependence for Magnelles shows the opposite trend to Molday, i.e. r1 decreases with temperature. The decreased r1 for Magnelles compared to Molday at all field strengths is potentially due to the encapsulation of the magnetite particles, either inside of the magnetosome, or inside the Magnelle itself. Sample transverse relaxivities (r2) at 7T and room temperature were ~100$mM^{-1}s^{-1}$ for Magnelles and ~135$mM^{-1}s^{-1}$ for Molday. Magnelles exhibited field and temperature dependence comparable to Molday (Fig 2). Cellular uptake for MDA cells was approximately 0.7pg iron/cell for Magnelles and 2-3pg iron/cell for Molday. Magnelle-labeled breast cancer cells (MDA-MB-231) exhibited very strong MR contrast *ex vivo* as shown in Fig. 3.

Conclusions: These bacterial-derived pseudo-organelles, "Magnelles", have potential for use as novel self-replicating magnetite-based MR contrast agents. They have r2 relaxivity values comparable to traditional iron-oxide nanoparticle contrast agents, and demonstrate strong MR contrast when loaded into cells and implanted in tissue. Further exploration and characterization of the relaxivity properties of Magnelles (and their active magnetosome components) could help direct the optimization and application of this novel class of MRI cell-labeling probe.

References: [1] Long & Bulte. *Expert Opin Biol Ther* (2009) **9**:293. [2] Dekaban et al. *J Immunother* (2009) **32**:240. [3] Heyn et al. *MRM* (2006) **55**:23. [4] Goldhawk et al. *WIREs Nanomed Nanobiotech* (2012) **4**:378. [5] Robeledo et al, *Proc. ISMRM* (2013) 0681. [6] Benoit et al. *Clin Cancer Res* (2009) **15**:5170.

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