Magnetic Resonance Properties of Gd-conjugated Microbubbles for use in MRI-guided Focused Ultrasound: Distinguishing Intact and Fragmented Microbubbles by Relaxivity

Michael A. Boss[1], Jameel A. Feshitan[2], and Mark A. Borden[2]

[1]National Instiute of Standards and Technology, Boulder, CO, United States, [2]Mechanical Engineering, University of Colorado, Boulder, Boulder, CO, United States

Purpose: Gas-filled microbubbles exhibit great potential for use in MRI-guided focused ultrasound (MRIgFUS) surgery [1]. In order to more easily track the microbubbles in vivo, it is useful to conjugate Gd-chelates to enhance the local relaxation rate of water protons in the vicinity of the microbubbles. This study was conducted to characterize the relaxation behavior of microbubbles when conjugated with a Gd-chelate, and whether cavitation state can be determined by changes in relaxivity.

Methods: 1-2 μm microbubbles were synthesized in aqueous solution from lipid precursors. They contained a gas core of perfluorobutane (PFB), and were coated in a brush layer of polyethylene glycol (PEG) to prevent agglomeration. DOTA complexes were conjugated either to the lipid surface of the microbubbles or to the end of the PEG chains; gadolinium was then introduced into the solution and sequestered by the DOTA (Fig. 1). These solutions were characterized via NMR relaxometry at 1.5 T using inversion recovery and CPMG to measure T_1 and T_2, respectively. The microbubbles were then subjected to ultrasound at 45 kHz to induce cavitation and fragmentation. Relaxometry was performed post-fragmentation. This process was repeated for dilutions of the stock microbubble solution at 25, 50 and 75% concentration. A sample with no Gd-conjugation was used as a control to isolate the contributions of the PFB gas.

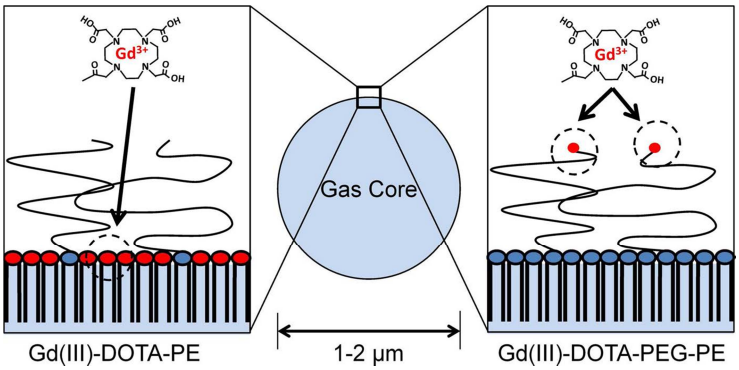

Figure 1: Conjugation of Gd-DOTA to microbubbles: conjugation to the lipid headgroups (left) and to the end of the PEG brush layer (right).

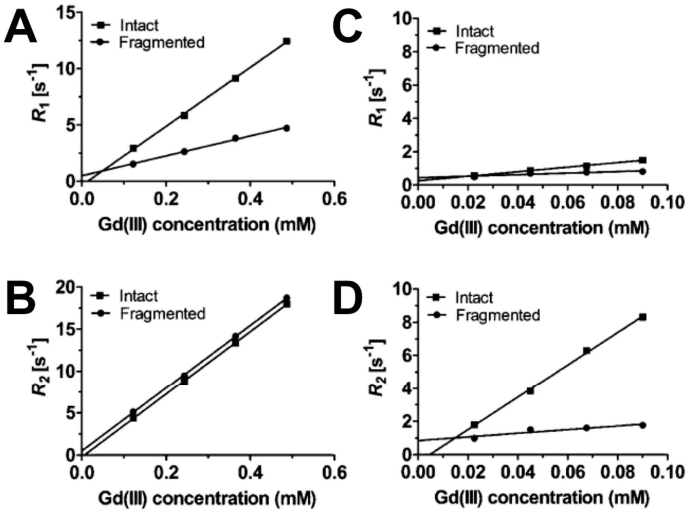

Figure 2: Microbubble solution relaxation rates. (A and B) R_1 and R_2, respectively, of lipid-bound Gd-DOTA microbubble dilutions. (C and D) R_1 and R_2, respectively, of PEG-bound Gd-DOTA microbubble dilutions.

Results: Typical relaxometry results from each of the two types of microbubbles are shown in Fig. 2. Lipid-bound Gd-DOTA microbubbles exhibited an increase in longitudinal relaxivity, r_1, by an average factor of 2.4; there was little change in their transverse relaxivity, r_2. In contrast, PEG-bound Gd-DOTA microbubbles exhibited a *decrease* in r_1 by an average factor of 2.1, as well as a decrease in r_2 by an average factor of 8.0. The control microbubbles exhibited virtually no enhancement of longitudinal relaxation, but a very high transverse relaxation. R_2 decreased by a factor of 36 after fragmentation.

Discussion: The two different types of microbubbles, lipid- vs. PEG-bound Gd-DOTA, exhibited different behaviors. In both cases, however, the microbubble state, intact or fragmented, was discernible using r_1. The control microbubbles had a large R_2 due to a susceptibility effect from the PFB gas, as evidenced in previous studies [2-4]. Lipid-bound Gd-DOTA microbubbles exhibited increased r_1 post-fragmentation: this is likely due to increased water access to the previously "buried" Gd. r_2 remained similar in these microbubbles due to competing effects: greater access of water protons to the Gd-DOTA enhanced transverse relaxation, while the loss of the PFB gas decreased it: the net effect was one of cancelation. PEG-bound Gd-DOTA microbubbles did not allow for enhanced access to the gadolinium: decreases in the rotational correlation time post-fragmentation likely served to reduce the longitudinal relaxation, while transverse relaxation decreased due to the loss of the PFB gas, similar to the control microbubbles. Finally, fragmented lipid-bound Gd-DOTA microbubbles show promise as a relaxation agent *per se*, with relaxivities exceeding those of clinically-used Gd-based contrast agents.

Conclusion: Intact and fragmented microbubbles can be distinguished by their relaxivities. This behavior will allow for better MRIgUS by allowing non-invasive detection of microbubble state in real-time.

References: [1] FA Jolesz, J. Mag. Res. Imag. **27**:391-399 (2008), [2] AL Alexander et al., Magn. Res. Med. **35**:801-806 (1996), [3] KK Wong et al., Magn. Res. Med. **52**:445-452 (2004), [4] JS Cheung et al., Neuroimage **46**:658-664 (2009)