Dendrimeric Paramagnetic Chelates for BIRDS

Yuegao Huang[1,2], Meser M Ali[3], Daniel Coman[1,2], Garry E Kiefer[4], and Fahmeed Hyder[1,5]
[1]*Diagnostic Radiology, Yale University, New Haven, CT, United States,* [2]*Magnetic Resonance Research Center, Yale University, New Haven, CT, United States,* [3]*Radiology, Henryford Health System, Detroit, MI, United States,* [4]*Macrocyclics, Dallas, TX, United States,* [5]*Biomedical Engineering, Yale University, New Haven, CT, United States*

INTRODUCTION Assessment of microenvironmental parameters, such as temperature and pH, is crucial. Current MRI detection scheme of temperature is based on the water proton signal **[1-2]**. However, it has relatively low temperature sensitivity. Recently, we have developed a novel method, called *B*iosensor *I*maging *R*edundant *D*eviation in *S*hifts (BIRDS) using low molecular weight paramagnetic chelates, e.g., $TmDOTP^{5-}$ and $TmDOTMA^-$. The BIRDS method utilizes signals from nonexchangeable proton resonances in the paramagnetic lanthanide chelates. Temperature and/or pH sensitivities of proton chemical shifts emanating from lanthanide chelates have been demonstrated **[3-5]**. Despite the superior sensitivities of these low molecular weight chelates, efforts have been sought to improve the biocompatibility, circulation time, and potentially sensitivities of the chelates. Dendrimers have been shown to be good nanocarriers for their attractive translational features **[6]**. For example, conjugation of gadolium-based contrast agents to the dendrimer surface improves the relaxivities of the agent **[7-8]**. Similarly, herein, we test the feasibility of BIRDS using dendrimeric paramagnetic chelates, i.e., conjugating paramagnetic chelates onto the surface of the polyamido amine (PAMAM) dendrimers.

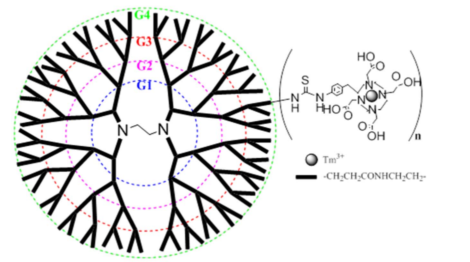

Figure 1. Structures of dendrimeric chelates.

MATERIALS AND METHODS Dendrimeric paramagnetic chelates were prepared by conjugating DOTA-Bn-NCS to the amine surface of the PAMAM dendrimers (1st-4th generation) followed by complexation of Tm^{3+} **[Fig. 1]**. The chelate–dendrimer conjugates were purified by repeated diafiltration using proper molecular weight cut-off filters until no low molecular weight materials could be detected by HPLC. The final dendritic conjugates were characterized by MALDI mass spectrometry and thulium ion contents were also determined by ICP-MS. NMR phantoms contained different concentrations of the dendrimeric chelates (i.e., 1.2 mM, 0.58 mM, 0.31 mM, 0.14 mM for G1-PAMAM-$(TmDOTA)_6$,G2-PAMAM-$(TmDOTA)_{11}$, G3-PAMAM-$(TmDOTA)_{26}$, G4-PAMAM-$(TmDOTA)_{45}$) at pH 7.4 were prepared. Temperature dependence of the resonances for the dendrimeric chelates was obtained for all the samples in the range of 25 °C to 40 °C in 11.7T Bruker vertical bore magnet. Longitudinal (T_1) and transverse (T_2) relaxation times were measured using inversion-recovery and spin echo methods with selective pulses at 35 °C, respectively. Intensities of the resonances for dendrimeric chelates were determined by peak integration methods. Amplification factors, as determined by peak intensities in NMR, were calculated relative to the H6 proton of $TmDOTA^-$ (2 mM).

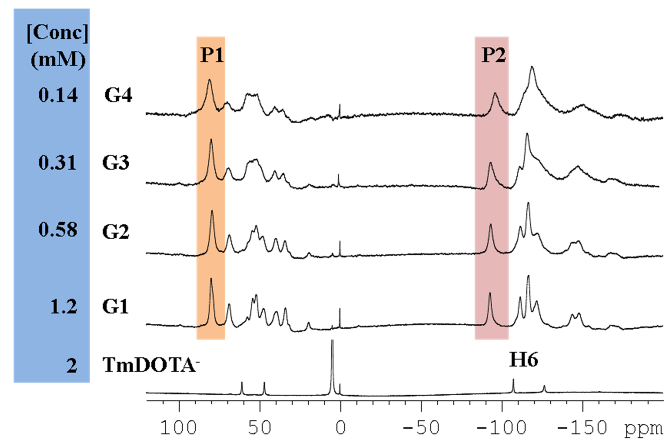

Figure 2. ^{1}H spectra of G1-4 chelates at 35 °C and pH 7.4.

RESULTS ^{1}H spectra of the dendrimeric chelates show similar pattern of resonances across all four generations of the chelates **[Fig. 2]**. Lower concentrations can be reached with higher generations of the dendrimeric chelates for similar signal intensities. Two distinguished resonances (P1 and P2) were chosen for BIRDS detection. These resonances are at least one order of magnitude more intense than the H6 signal of $TmDOTA^-$ indicating higher sensitivity is achieved. P1 and P2 have similar temperature sensitivities but opposite signs suggesting that combined sensitivities would be higher than that of H6 (0.4 ppm/°C) in $TmDOTA^-$ **[Table I]**. No significant difference in temperature sensitivity with increased generations of dendrimers. T_1 for both P1 and P2 are similar across G1-4 while T_2 decreased for higher generations of dendrimers.

Table I. BIRDS Properties of Resonances in Dendrimeric Chelates

Dendrimeric Chelates	P1			P2		
	C_T (ppm/°C)	T_1 (ms)	T_2 (ms)	C_T (ppm/°C)	T_1 (ms)	T_2 (ms)
G1-$(TmDOTA)_6$	-0.34	3.1	0.43	0.27	2.5	0.36
G2-$(TmDOTA)_{11}$	-0.37	2.7	0.34	0.26	2.1	0.31
G3-$(TmDOTA)_{26}$	-0.38	2.7	0.25	0.21	2.3	0.20
G4-$(TmDOTA)_{45}$	-0.31	3.0	0.12	0.17	1.9	0.12

DISCUSSION Our hypothesis was that by conjugating temperature sensitive lanthanide chelates to the amine surface of the PAMAM dendrimer will retain/improve BIRDS properties. For all dendrimeric chelates studied, they show certain symmetry such that well resolved resonances could be identified for BIRDS detection. Lower agent dosage can be achieved with higher generation dendrimeric chelate. The high temperature sensitivities for dendrimeric chelates hold their potential for in vivo studies. Because upon building larger dendritic chelates T_2 of the resonances are shortened, we need to consider ultra-short echo time CSI **[9]** for imaging the macromolecules. High molecular weight structures, such as our dendrimeric chelates, have been shown to have longer circulation time in the body that can be beneficial for in vivo imaging **[6]**.

REFERENCES

[1] Rieke V., et al., *J. Magn. Res.Imaging* **2008**, 27, 376-90.
[2] Senneville B.D., et al., *Int. J. Hyperthermia* **2005**, 21, 515-31.
[3] Coman D., et al., *NMR.Biomed.* **2009**, 22, 229-39.
[4] Coman D., et al., *NMR.Biomed.* **2010**, 23, 277-85.
[5] Coman D., et al., *NMR.Biomed.* **2011**, 24, 1216-25.
[6] Menjoge A.R., et al., *Drug Discov Today* **2010**, 15, 171-85.
[7] Ali M.M., et al., *Chem. Eur. J.* **2008**, 14, 7250-58.
[8] New K., et al., *Bioorg. Med. Chem.* **2010**, 18, 5925-31.
[9] Du J, et al., *Magn Reson Med*. **2007**, 58, 1001-1009.