Molecular Imaging Studies of a Robust Gd-Sucrose Scaffold Applied to MR-Colonography

Gary V. Martinez[1], Parastou Foroutan[2], Valerie E. Moberg[1], Suryakiran Navath[3], Roha Afzal[1], Robert J. Gillies[1], Eugene A. Mash[3], and David L. Morse[1]

[1]*Department of Cancer Imaging and Metabolism, H. Lee Moffitt Cancer Center & Research Institute, Tampa, Florida, United States,* [2]*Bruker Biospin, Billerica, Massachusetts, United States,* [3]*Department of Chemistry and Biochemistry, University of Arizona, Tucson, Arizona, United States*

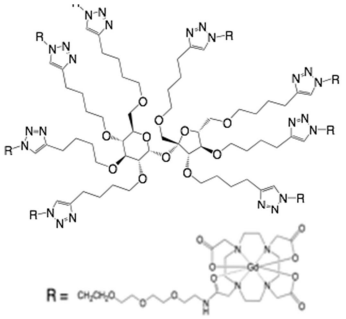

Figure 1. Molecular structure of the 8-Gd sucrose scaffold employed for these studies.

Introduction: Colorectal cancer (CRC) has a high incidence as it is the third most common cancer in both males and females, and the second leading cause of death from cancer in the United States (1). Despite these statistics, it is known that early detection can mitigate the risk and improve outcome dramatically, if detected early. CRC screening by colonoscopy is recommended for patients >50 years of age. However, compliance is < 20%, possibly because of the need for cathartic bowel preparation, conscious sedation, and the invasiveness of the procedure. Various screening methods for CRC, including fecal occult blood testing, flexible sigmoidoscopy and air-contrast barium enema examination have been available for decades, but, poor sensitivity and limited spatial coverage have often yielded false-positive results (1). Computed tomography colonography requires bowel cleansing and is as insensitive to small and flat polyps as standard colonoscopy (2), and radiation is a concern. Currently available MRI based Virtual Colonoscopy (VC) has not found wide acceptance, because of concerns with the inability to detect small lesions, insufflation, generation of false positives not detectable during follow-up colonoscopy, and the need for specialized training for the accurate interpretation of radiological results. In this work, we present an approach that may potentially mitigate all of the drawbacks of currently existing methods by using a Gd sucrose scaffold that is robust and resilient to the GI tract, has high spin-lattice relaxivity due to multiple Gd-DOTA groups, does not require bowel distension, and may be administered orally.

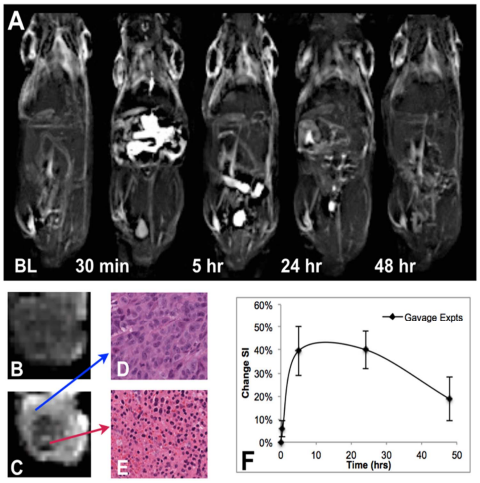

Figure 2. **(A)** Maximum Intensity Projections (MIP) generated from T_1-weighted Spin Echo sequences (3D) Zoomed in on the tumor solely, images of pre-contrast **(B)** and 5 hours post-gavage **(C)** H&E images of tumor rim (blue arrow) and dark core (red arrow). **(F)** Percent change in tumor signal intensity compared to baseline values before contrast.

Methods: Using an argon-degassed solution of azide octaalkyne in 9/1 THF/water, $CuSO_4$ and sodium ascorbate, a Gd-DOTA sucrose-derived agent was synthesized (Fig 1). A 7-T horizontal magnet (ASR 310, Agilent Technologies, Inc.) with 205/120/HDS gradients and 310 mm bore was used for MRI. Progressive saturation with 11 TR values exponentially spaced from 30 s to 60 ms was used to determine the spin lattice relaxation rate ($R_1 = 1/T_1$) in phantoms. Constants were obtained by nonlinear least squares regression using Matlab. Injections were done intra-rectally with $1x10^6$ HCT 116/luc cells (*i.e.* human colorectal cancer cells expressing luciferase) female SCID/beige mice (N = 6). Contrast agent was dissolved in 100 mM sodium phosphate buffer, pH 7.4, at a concentration of 2.5 mM for gavage administration (10⌈l/gram body weight). Mice were anesthetized with 2% isoflurane and restrained in a specific holder. Using a 35-mm Litzcage coil (Doty Scientific), coronal T_1-weighted gradient echo (GE3D) and T_2-weighted multislice spin-echo (SEMS) sequences were acquired with TE/TR=7.6/25 ms and 50/2750 ms, respectively. Slice thickness was 1 mm and spatial resolution kept at 350x350x100 μm^3 and acquired in 6 min. Scans were acquired at baseline and 0.5, 5, 24 and 48 hours following gavage. To evaluate potential intestinal absorption of the agent, the compound was dissolved in PBS (25 µM/kg body weight), and administered in 150 µL volume via a tail vein catheter during imaging, with acquisitions prior to, during and post injection. Tumor signal intensity was calculated using manually drawn regions of interest in VnmrJ.

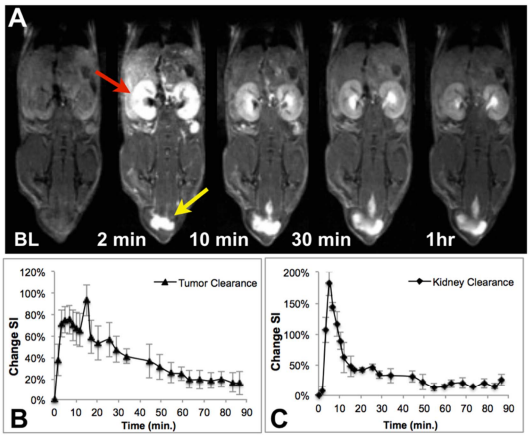

Figure 3. (A) T_1-weighted Spin Echo Multi Slice (SEMS) showing baseline (BL), 2 min, 10 min, 30 min, and 1 h post IV injection of contrast agent and corresponding quantitative data showing the signal intensity with time in the (B) tumor and (C) kidneys.

Results & Discussion: Relaxation studies on a phantom comprised of variable concentrations of Gd-DOTA-sucrose8 yielded a per Gd $r_1 = 26.6$ mM s^{-1} (3). In vivo gavage results are shown (Fig 2) where differences of pre-contrast and post-gavage indicate enhancement. H&E images demonstrated that area in the enhanced tumor rim (indicated by blue arrow in (C)) contains mainly viable cells (D) while the darker inner core (red arrow) is constituted by necrotic cells as shown in (E). Part (F) shows a time-series of enhancement resulting from gavage. Fig 3 shows that an i.v. injection rapidly extravasates and enhances the tumor, and is rapidly cleared via the kidney. These results indicate that this scaffold shows promise as an orally administered contrast agent for colorectal studies as it remains intact within the bowel and enhances the tumor. Further work will focus on adding a targeting moiety to the construct.

1. Walsh JM, Terdiman JP. Jama. 2003;289(10):1288-96. 2. Yee J. Radiographics. 2002;22(6):1525-31. 3. P. Foroutan, et al. Proc ISMRM. 2014.