The magnetic, relaxometric, and optical properties of gadolinium-catalyzed single walled carbon nanotubes

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(Received 6 September 2012; accepted 8 March 2013; published online 4 April 2013)

We report the magnetic behavior, relaxometry, phantom magnetic resonance imaging (MRI), and near-infrared (NIR) photoluminescence spectroscopy of gadolinium (Gd) catalyzed single-walled carbon nanotubes (Gd-SWCNTs). Gd-SWCNTs are paramagnetic with an effective magnetic moment of $7.29 \mu_B$. Gd-SWCNT solutions show high r_1 and r_2 relaxivities at very low (0.01 MHz) to clinically relevant (61 MHz) magnetic fields ($r_1 \geq 130 \text{ mM}^{-1} \text{ s}^{-1}$, $r_2 \geq 160 \text{ mM}^{-1} \text{ s}^{-1}$). Analysis of nuclear magnetic resonance dispersion profiles using Solomon, Bloembergen, and Morgan equations suggests that multiple structural and dynamic parameters such as rotational correlation time τ_R , rate of water exchange τ_M , and the number of fast-exchanging water molecules within the inner sphere q may be responsible for the increase in r_1 and r_2 relaxivity. The T_1 weighted MRI signal intensity (gradient echo sequence; repetition time (TR) = 66 ms, echo time (TE) = 3 ms, flip angle = 108°) of Gd-SWCNT phantom solution is 14 times greater than the Gd-based clinical MRI contrast agent Magnevist. Additionally, these nanotubes exhibit near infrared fluorescence with distinct E_{11} transitions of several semiconducting SWCNTs. Taken together, these results demonstrate that Gd-SWCNTs have potential as a novel, highly efficacious, multimodal MRI-NIR optical imaging contrast agent. © 2013 American Institute of Physics. [<http://dx.doi.org/10.1063/1.4796183>]

I. INTRODUCTION

The development of contrast-enhancement products, called contrast agents (CAs), for imaging modalities such as confocal microscopy, magnetic resonance imaging (MRI), X-ray computed tomography (CT), and positron emission tomography (PET) has led to rapid advances in our understanding of human and animal anatomy and physiology.¹ Especially with MRI, improved disease detection via increased sensitivity and diagnostic confidence has resulted in the use of CAs in one of every three clinical MRI procedures.² CAs used in clinical MRI procedures typically alter the T_1 or T_2 relaxation times of water protons. An important parameter that quantifies their efficacy is relaxivity ($r_{1,2}$), defined as the change in relaxation rate of water per unit concentration of CA, and reported as units of $\text{mM}^{-1} \text{ s}^{-1}$.^{3,4} The lanthanoid element gadolinium (Gd), as well as the transition elements iron (Fe) and manganese (Mn) are the most widely used elements in MRI CAs,^{5–7} and gadolinium-chelate-based T_1 MRI CAs are used in the majority of clinical MRI procedures involving CAs.⁷ Theoretical studies predict the possible development of novel MRI CAs with 50–100 times greater relaxivity than current clinical gadolinium-chelate

complexes.^{4,8} Thus, a major focus of MRI CA research has been to utilize relaxometry and other characterization tools to further understand the molecular, structural, dynamic, and magnetic properties of MRI CAs that affect water proton relaxation times.⁸ This improved understanding facilitates the development of rational synthetic design strategies to improve the relaxivity of next-generation MRI CAs.

The significant limitations of current T_1 MRI CAs require the development of new high sensitivity MRI CAs, and/or co-validation of *in vivo* MRI experiments using multimodal imaging.⁹ Currently, optical fluorescence imaging has better sensitivity than T_1 MRI CAs, at extremely low detectable concentrations (e.g., optical fluorescence CAs can be detected at picomolar (pM) concentrations,¹⁰ while T_1 MRI CAs are only detectable at micromolar (μM) concentrations¹¹). Additionally, MRI and optical imaging are complementary techniques.⁹ MRI is capable of deep tissue penetration and millimeter (mm) resolution. Optical imaging is capable of submicron resolutions, but is limited by optical aberration and light scattering to the outer 100–300 μm of a specimen. Unfortunately, concomitant use of different optical imaging and MRI CAs is not workable unless, both CAs have comparable pharmacokinetic properties. Therefore, development of a novel multifunctional chemical agent that shows high efficacy as an integrated MRI-optical CA is highly desirable. To date, multimodal MRI-optical imaging CAs have been developed either by covalent or non-covalent functionalization of a T_1 MRI CA (typically Gd-chelate small

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molecule complexes) and fluorescent reporter (organic fluorescent molecules, quantum dots, and lanthanoid chelates) to macromolecules such as polymers and proteins.⁹

Gd³⁺ ion carbon nanotube (CNT) complexes show promise as high-performance T_1 MRI CAs.^{12–16} Wilson and colleagues developed a synthesis method, wherein pristine commercial single-walled carbon nanotubes (SWCNTs, length $>1\ \mu\text{m}$) are chemically cut into ultra-short (US) SWCNTs (US-tubes; length, 20–80 nm); Gd³⁺ ions are then loaded into these US-tubes through defects along the side-walls.² Richard and coworkers coordinated Gd³⁺ ions to multiple ligands, which were non-covalently adsorbed onto multi-walled carbon nanotubes (MWCNTs).¹⁶ Both of these methods are “top down” synthesis strategies involving the addition of Gd³⁺ ions to bulk commercial CNTs, and result in 3–90 times (depending on magnetic field and Gd³⁺ concentration) increase in r_1 relaxivity compared to the clinical MRI CA Magnevist (Gd-DTPA). To date, these MRI CAs have not been demonstrated to be concomitantly suitable as optical CAs.

Recently, we have developed a novel “bottom up” chemical strategy for synthesis of SWCNT Gd³⁺ complexes (hereafter called Gd-SWCNTs).¹⁷ In general, the synthesis protocol of Gd-SWCNTs first involves deposition of Gd₂O₃ nanoparticles catalysts (average size 1.8 nm). Next, during chemical vapor deposition of a carbon feedstock gas (ethylene), the curvature of the lanthanoid nanoparticle serves as a template around which SWCNT caps of five- and six-member rings are formed, which in turn acts as a nucleus to assemble carbon into the cap edge atoms to form a graphite sheet cylinder—a nanotube.¹⁸ The lanthanoid nanoparticles are embedded into, and located at one end of the SWCNTs (SWCNT average diameter 2.05 nm, length 500 nm–1.5 μm).¹⁹ Thus, the synthetic strategy in the development of Gd-SWCNTs is distinctly different compared to previous approaches to develop SWCNT Gd³⁺ complexes as MRI CAs,^{12–16} or multimodal MRI–optical imaging CAs. Here, we report the magnetic behavior, T_1 and T_2 nuclear magnetic resonance dispersion (NMRD) profiles, MRI phantom imaging, and near-infrared (NIR) photoluminescence spectroscopy of water solubilized Gd-SWCNTs toward development as novel multimodal MRI–optical CAs.

II. EXPERIMENTAL METHODS

A. Preparation of Gd-SWCNT dispersions

Previous methods involved Gd-SWCNT dispersion in 1% Pluronic F127 (1% Pluronic = 1 g of Pluronic in 100 g of water) solutions.^{19,20} Pluronic F127 has been employed to water solubilize the hydrophobic Gd-SWCNTs and to prevent their aggregation. The Si wafer (square-shape, size = 1 cm²) on which Gd-SWCNTs were synthesized was placed in a 50 ml centrifuge tube and filled with 15 ml of Pluronic F-127 and then sonicated in a CV33 Ultrasonic Processor (Cole Palmer, Vernon Hills, Illinois) at 300 W for 10 min. The shear force of sonication facilitated release of Gd-SWCNTs along with any free nanoparticle catalysts present on the Si wafer into the solution. The Si wafer was then removed, and vials were centrifuged at 15 000 rcf for 1 h. As a result of centrifugation, solubilized Gd-SWCNT aggregates and dense

Gd₂O₃ nanoparticles settled to the bottom. Centrifugation was repeated to enable proper separation of solubilized Gd-SWCNTs in the supernatant from the non-dispersed carbon nanomaterials that remained in the pellet.

Relaxivity and phantom MRI measurements were obtained for solubilized Gd-SWCNTs at a concentration of 0.1 mg/ml. Inductively coupled plasma optical emission spectroscopy (ICP-OES) analysis was performed on the Gd-SWCNT sample to determine Gd³⁺ ion content. Gd³⁺ ion concentration of Gd-SWCNT solution was determined to be 34 mg/l or ppm (equivalent to 0.22 mM). Gd₂O₃ nanoparticles dispersed in 1% Pluronic F127 solution were used for comparison in NMRD profiles and relaxivity studies. Magnevist[®] was used for comparison in MR phantom imaging.

The samples for ICP-OES were prepared as follows. A 5 ml solution of 70% HNO₃ was added to 1 ml of Gd-SWCNTs. The acid digests the sample and oxidizes the carbonaceous materials into CO₂ and H₂O. The solution was heated to vaporize the excess water, and stirred until a solid residue was obtained; that residue was treated with 5 ml of 30% H₂O₂ to oxidize any trace carbonaceous materials into CO₂ and H₂O, heated and then stirred again until a solid residue was again obtained. The final solution was dissolved in 10 ml of 2% HNO₃. ICP-OES was performed at an independent research laboratory (Galbraith Laboratories, Knoxville, TN) on an Optima 5300 ICP Emission Spectrometer (Perkin Elmer, Waltham, MA). ICP analysis was performed on three samples for each experimental group.

B. Super conducting quantum interference device (SQUID)

A SQUID magnetometer with a sensitivity of $\sim 10^{-8}$ emu was used to study the magnetic states of Gd-SWCNT samples. Samples were weighed and loaded in gelatin capsules, and measured for changes in magnetization as a function of applied magnetic field. Applied magnetic fields varied from 5000 Oe to -5000 Oe at three temperatures (30 K, 150 K, and 300 K). In the field cooled (FC) and Zero Field cooled (ZFC) mode, the applied coercive field was 5000 Oe for the magnetization measurements as a function of temperature.

C. Proton relaxivity measurements

Single point relaxivity (r_1) was calculated during NMRD measurements. Relaxivity values (r_1), were calculated using the formula $r_i = (R_i - R_{io})/[Gd^{3+}]$; where R_i and R_{io} are the longitudinal or transverse relaxation rates of the samples and 1% Pluronic F127 surfactant solution ($i = 1$ for longitudinal relaxation, $i = 2$ for transverse relaxation), respectively, and $[Gd^{3+}]$ is the concentration of gadolinium in the volume of solution used for relaxation measurements. Relaxation rate ($1/T_1$) NMR dispersion (NMRD) profiles at magnetic fields corresponding to a proton Larmor frequency range from 0.01–20 MHz were obtained using a fast field cycling relaxometer (Spinmaster FFC2000, Stelar Inc, Pavia, Italy). A high field relaxometer (Stelar Inc, Pavia, Italy) consisting of a superconducting dipole electromagnet (HTS Inc., Wellington, New Zealand) was used to acquire relaxation data ranging

from 25 to 80 MHz of the proton Larmor frequency. Temperature was fixed at 25 °C, and controlled by a Stellar VTC-91 airflow heater, equipped with a copper-constantan thermocouple; temperature calibration in the probe head was ensured with a Delta OHM digital thermometer (absolute accuracy of 0.5 °C). $1/T_2$ NMRD profiles at magnetic fields corresponding to a proton Larmor frequency range from 1–60 MHz at 25 °C were obtained using a custom-designed variable-field relaxometer (Southwest Research Institute, San Antonio, TX) at the Laboratory of Diagnostic Radiology Research, National Institutes of Health, Bethesda, MD. This relaxometer consists of a nuclear magnetic resonance (NMR) console and custom-made permanent magnets corresponding to the various magnetic field strengths. A nonlinear least squares algorithm (FindFit in Mathematica[®]) was used to fit r_1 and r_2 NMRD data by applying the Solomon, Bloembergen, and Morgan (SBM) equations. A simultaneous curve fitting method was used (see supplementary information for details).

D. Magnetic resonance imaging

A 3T Philips MR imager was used for phantom imaging. Image acquisition was performed at room temperature (25 °C), and a quadrature head coil was used for signal reception. Imaging parameters of the gradient echo protocol were adapted from previous MRI studies with gadofullerenes,²¹ and were as follows: repetition time (TR)/echo time (TE)/flip angle = 12 ms/3 ms/108°; FOV and matrix sizes were chosen to yield an acquired voxel size of $2 \times 2 \times 6$ mm³. The inversion pulse was repeated every 3.6 s, and longitudinal recovery was sampled at 28 ms temporal resolution (128 time points).

E. Near infra-red fluorescence spectroscopy

The NIR fluorescence spectra of the Gd-SWCNT solution (0.1 mg/ml nanotube concentration) were acquired with a Horiba Jobin Yvon Nanolog spectrofluorometer with a built-in liquid-nitrogen-cooled Symphony InGaAs-1700 detector. The exposure time was 30 s. The excitation and emission slit widths was 10 nm. The data were analyzed with Nanosizer software.

III. RESULTS AND DISCUSSION

A. Magnetic characterization

Figure 1 shows the magnetization data for Gd-SWCNTs at 30 K, 150 K, and 300 K. The M vs. $\mu_0 H/T$ data were fit using the function $M = ngJ\mu_B B_J(x)$, where the Brillouin function $B_J(x) = (1 + 0.5/J) \coth(x + 0.5/J) - 0.5/J \coth(0.5x/J)$ and $x = gJ\mu_B H/k_B T$, n is the number of Gd³⁺ per kilogram of sample, g is the electron spin factor or the Lande factor, J is the total angular momentum, μ_B is the Bohr magneton, and k_B is the Boltzmann constant.²² The fitted data yielded $J = 3.18$. Thus, the effective magnetic moment μ_{eff} given by $g[J(J + 1)]^{1/2}\mu_B$ was calculated to be $7.29\mu_B$. The ground electronic state of Gd³⁺ is $^8S_{7/2}$ with theoretical $J = 7/2$ and the calculated effective magnetic moment is $\mu_{eff} = 7.94\mu_B$. This calculated μ_{eff} value is lower than the theoretical value. The difference between the experimental and theoretical J values could be due to transfer of electron spins onto the carbon nanotubes similar to a process

FIG. 1. Magnetization vs. $\mu_0 H/T$ plot fit to a Brillouin function of the Gd-SWCNTs.

postulated for gadofullerenes.²³ Further electron paramagnetic resonance (EPR) investigations are necessary to confirm this spin transfer, since trace organic impurities or carbon debris present in the sample could also be the cause of the difference in μ_{eff} .²²

Figure 2 shows the magnetization (ZFC + FC) vs. temperature plot at 5 T; measurements were obtained at temperatures ranging from 10–300 K. No evidence of ferromagnetic transitions was detected in this temperature range. Ferromagnetization of bulk gadolinium has been widely reported to occur below its Curie temperature T_c (292.5 K). For gadolinium nanoparticles at a size of 10 nm, T_c has been shown to shift to a lower value of 283 K, while particles with grain sizes of less than 2 nm have been theoretically predicted and experimentally shown not to have temperature-dependent spin orientation. In the case of Gd-SWCNTs, gadolinium nanoparticles have an average size of 1.8 nm, possibly explaining the absence of ferromagnetic transitions.

Reciprocal susceptibility between 10 K and 300 K, linearly fit using the Curie–Weiss equation $\chi = C/(T - \theta_p)$ resulted in a Curie constant $C = 476$ Am² K/kg T, and paramagnetic Curie temperature of $\theta_p = 18$ K. Using the theoretical effective magnetic moment per Gd³⁺ ion, $\mu_{eff} = 7.94\mu_B$, the Curie constant of Gd³⁺ ions is 501 Am² K/kg T.²² Thus, comparison of the two values of the Curie constant suggests that at least 95% weight of the gadolinium in Gd-SWCNT are Gd³⁺ ions.

FIG. 2. Magnetization vs. temperature plot for Gd-SWCNTs at applied field of 5 T (blue and red squares represent FC and ZFC data).

B. Relaxivity, nuclear magnetic dispersion profiles

NMRD measurements provide valuable insights and information about the dynamic, and structural parameters of MRI CAs that influence relaxivity.^{3–5,8} The r_1 NMRD profile (proton Larmor frequency = 1.15 to 61.73 MHz) for proton relaxivity at 25 °C for individually dispersed Gd-SWCNTs in 1% Pluronic F127 is presented in Figure 3(a). Gd-SWCNTs have distinctly different r_1 NMRD profiles than Gd₂O₃ nanoparticles dispersed in 1% Pluronic F127 solution (Figure 3(b)), and Gd³⁺-based small molecular and macromolecular complexes.^{4,5,24} The NMRD profile of Gd₂O₃ nanoparticles shows a bell-shaped relaxivity distribution at proton Larmor frequencies between 10 and 80 MHz with a peak value of $\sim 3 \text{ mM}^{-1} \text{ s}^{-1}$ at 40 MHz. At proton Larmor frequencies less than 10 MHz, relaxivity is constant at $\sim 2.4 \text{ mM}^{-1} \text{ s}^{-1}$. The shape of the profile is similar to previous reports.²⁵ Small molecule Gd³⁺ complexes such as Gd-DTPA (DTPA = diethylene triamine penta-acetic acid) show a constant value of $\sim 1.9 \text{ mM}^{-1} \text{ s}^{-1}$ at fields greater than 10 MHz, and marginal increases at fields less than 10 MHz.²⁴ Macromolecular complexes, Gd³⁺-DTPA-BSA (BSA = bovine serum albumin), show a bell-shaped relaxivity distribution at proton Larmor frequencies between 10 and 80 MHz with a peak value of $42 \text{ mM}^{-1} \text{ s}^{-1}$ at 20 MHz.²⁴ At proton Larmor frequencies less than 10 MHz, relaxivity is constant at $\sim 35 \text{ mM}^{-1} \text{ s}^{-1}$.²⁴ The Gd-SWCNT profiles are different than those of

FIG. 3. (a) r_1 NMRD profile of the Gd-SWCNTs. Circles represent experimental data and solid lines the best fit obtained using SBM theory. (b) r_1 NMRD profile of Gd₂O₃ nanoparticles dispersed in 1% Pluronic F127 solutions.

Gd³⁺ at C₆₀ (gadofullerenes), which are similar to those of Gd³⁺ macromolecular complexes.²³ At a clinically relevant 1.5 T magnetic field (proton Larmor frequency = 61.73 MHz), the r_1 value is $126 \text{ mM}^{-1} \text{ s}^{-1}$, which is an order of magnitude greater than any current T_1 Gd³⁺-based FDA approved MRI CA ($r_1 = 3.4\text{--}5.8 \text{ mM}^{-1} \text{ s}^{-1}$).²⁶ Further, r_1 relaxivities increase with decreases in proton Larmor frequency. These increases could be divided into two distinct linear regions: the region between 21.29 and 61.73 MHz, where the increase is moderate, and between 1.15 and 21.29 MHz, where the increase is steep. Qualitatively, the NMRD profiles above 1 MHz are similar to those reported by Wilson and co-workers.^{2,27} Below 1 MHz, the r_1 NMRD profile plateaus at a value of $\sim 310 \text{ mM}^{-1} \text{ s}^{-1}$ and does not increase further, while the NMRD profiles reported by Wilson and co-workers show further increased relaxivity with decreases in proton Larmor frequency. A possible reason for this discrepancy is their presence as individual nanotubes at this low Gd-SWCNT concentration ($0.1 \mu\text{g/ml}$ nanotube concentration). The NMRD profiles of the Gd-SWCNTs at a higher concentration (0.5 mg/ml nanotube concentration) were similar to those reported by Wilson and co-workers (see supplementary information Figure S1²⁸). At this concentration, individual nanotubes are in close proximity, get entangled and aggregate. This aggregation also leads to complete quenching of NIR fluorescence emission (discussed below, Figure 6).

Figure 4 shows a representative r_2 NMRD profile (proton Larmor frequency = 1.15 to 61.73 MHz) for proton relaxivity at 25 °C for individual Gd-SWCNTs dispersed in 1% Pluronic F127 solution. This is the first report of r_2 NMRD profiles for any Gd³⁺ carbon nanotube complex. These r_2 profiles follow a trend similar to r_1 profiles for all proton Larmor frequencies—a moderate increase in the region from 21.29–61.73 MHz, and a steep increase between 1.15 and 21.29 MHz. There are few published reports on the r_2 NMRD profiles of Gd³⁺-based small molecular or macromolecular complexes.^{29,30} Gd-SWCNTs have distinctly different r_2 NMRD profiles than Gd³⁺-based small molecular or macromolecular complexes. At clinically relevant 1.5 T magnetic field (proton Larmor frequency = 61.73 MHz), the r_2 value is $170 \text{ mM}^{-1} \text{ s}^{-1}$, which is an order of magnitude greater than any current Gd³⁺-based FDA-approved MRI

FIG. 4. r_2 NMRD profile of the Gd-SWCNTs. Circles represent experimental data and solid lines the best fit obtained using SBM theory.

CA ($r_2 = 3.2\text{--}4.2\text{ mM}^{-1}\text{ s}^{-1}$).²⁶ The r_2 value at this field is ~ 4 times greater than that of the FDA-approved iron oxide nanoparticle MRI CA, Ferridex.²⁶ However the r_2/r_1 ratio for Gd-SWCNTs is 1.34, which is within the range reported for Gd³⁺-chelate complexes (r_2/r_1 between 1 and 2),²⁶ and substantially lower than that of gadonanotubes ($r_2/r_1 \sim 4$),¹⁴ and iron-based CAs ($r_2/r_1 = 10$ or more).⁶ Thus, Gd-SWCNTs may be better suited as T_1 contrast agents. This assessment is further corroborated by MRI analysis of the Gd-SWCNT phantom solutions.

C. SBM equations

Overall relaxivity is influenced by both inner sphere and outer sphere relaxation mechanisms, and corresponding structural, magnetic, and molecular dynamic parameters.⁴ Equations based on the classical theory developed by SBM for MRI CAs were fit to the r_1 and r_2 NMRD data to obtain parameters governing these relaxation mechanisms.^{4,24} We restricted analysis to inner-sphere interactions for both r_1 and r_2 NMRD data, since reports² show that the contribution of the outer sphere is negligible compared to inner sphere contributions for Gd³⁺ ion SWCNT complexes. The r_1 and r_2 NMRD results are influenced by a number of common parameters. Thus, these results were subjected to a simultaneous least-squares fitting procedure. (A discussion of the fitting approach is presented in the supplementary information section (Ref. 28)). Simultaneous fitting procedures have previously been reported to improve the reliability of parameters governing relaxivity.^{31,32} Additionally, this provides a more rigorous test of the theory and its applicability to the materials under investigation. The best fits, shown as solid lines in Figures 3 and 4, necessarily involve shared parameters simultaneous fit. Table I lists the computed parameters, their definitions and values.

All computed parameters were within the range of values reported for other Gd³⁺-based complexes, except for the hydration number q . Additionally, the values of two parameters, namely the rotational correlation time, τ_R , and the water-residence lifetime, τ_M , were independently estimated (see supplementary information (Ref. 28)), and were in line with computed values. The parameter Δ^2 represents the zero-field splitting energy of the paramagnetic metal electrons, and the correlation time for this splitting is termed τ_V . These two parameters are important in determining the effectiveness of the paramagnetic center. The Δ^2 and τ_V values are within the generally accepted range of

$10^{18}\text{--}10^{20}\text{ s}^{-2}$, and 1–100 ps, respectively.²⁴ The rotational correlation time, τ_R , is within the microsecond range reported for other Gd³⁺ macromolecule complexes including gadonanotubes,² and was independently estimated using dynamic light scattering and theoretical rotational dynamic models developed for prolate ellipsoids (see supplementary information). For the parameter, τ_M , the water-residence lifetime is within the range of values (between tens to hundreds of ns) reported for Gd³⁺ macromolecules such as paramagnetic liposomes,³³ gadofullerenes,²³ and gadonanotubes.² Additionally, this value is close to the independently determined τ_M estimate by variable temperature ¹⁷O measurements (see supplementary information (Ref. 28)). The parameter q , with a value of 76, represents the number of fast-exchanging water molecules within the inner sphere. The q value falls outside the range (between 1 and 9) obtained for free Gd³⁺ ion solution, and various Gd³⁺ paramagnetic complexes. However, q values as high as 20 have been reported for gadofullerenes.²³

We estimated that gadolinium nanoparticles modeled as spheroids with an average radius of 0.9 nm would contain ~ 67 Gd atoms along the surface (number of Gd atoms = surface area of Gd nanoparticles/surface area of single Gd³⁺ ion; Gd³⁺ ionic radii of 0.11 nm). Results to date on lanthanoid-catalyzed SWCNTs show that the lanthanoid ions can interact with water, and thus observed relaxivity can be attributed to a strong inner-sphere relaxation mechanism.¹⁹ Assuming one binding site is available per Gd³⁺ ion, a gadolinium nanoparticle present in a single Gd-SWCNT should simultaneously interact with at least 67 water molecules, which is close to the value obtained from the model. While the SBM equations provided good fits for the NMRD profiles, and the values of two computed parameters have been independently estimated, additional temperature-dependent NMRD analysis needs to be performed, and the value of q and other parameters needs to be corroborated by other techniques⁴ before a firm conclusion can be drawn on the suitability of the SBM theory as a satisfactory model for evaluation of Gd-SWCNTs.

D. Phantom MRI

Figure 5 displays representative T_1 -weighted images of Gd-SWCNT (left image) solution dispersed in 4% gelatin (4 g of

TABLE I. Computed parameters representing best fit to SBM equations.

Parameters	Definition	Simultaneous fit parameters
Δ^2	ZFS	1.33×10^{19}
$\tau_{V(s)}$	Correlation time for splitting	2.78×10^{-11}
$\tau_{R(s)}$	Tumbling time of complex	7.26×10^{-6}
$\tau_{M(s)}$	Residence time of the inner sphere water molecules	5.75×10^{-8}
q	Hydration number	76

FIG. 5. Contrast-enhanced T_1 -weighted (gradient echo sequence; repetition time (TR) = 66 ms, echo time (TE) = 3 ms, flip angle = 108°) MRI phantom images of Gd-SWCNT (green circle), and Gd-DTPA (Magnevist, purple circle) solutions dispersed in 4% gelatin (4 g of gelatin in 100 g of distilled water). Both phantoms have equimolar concentrations of gadolinium ($[\text{Gd}^{3+}] = 0.22\text{ mM}$).

gelatin in 100 g of distilled water) and Gd-DTPA (Magnevist, right image) dispersed in 4% gelatin, respectively. Both phantoms have equimolar concentration of gadolinium ($[\text{Gd}^{3+}] = 0.22 \text{ mM}$). The Gd-SWCNT phantoms show substantially higher contrast enhancement compared to Gd-DTPA, which showed negligible enhancement. The T_1 -weighted MRI signal intensity (gradient echo sequence; repetition time (TR) = 66 ms, echo time (TE) = 3 ms, flip angle = 108°) of Gd-SWCNT was 14 times greater than Gd-DTPA. The T_1 relaxation time of Gd-SWCNT, and Magnevist solution were 35 ms and 1110 ms, respectively. Phantom MRI results indicate that Gd-SWCNTs could achieve *in vivo* contrast enhancement at substantially lower dosages compared to current clinical Gd^{3+} -based MRI CAs (at least an order of magnitude lower).

E. NIR fluorescence spectroscopy

A near infrared photoluminescence (PL) spectrum at wavelengths between 825 and 1375 nm of individual water-solubilized Gd-SWCNTs is shown in Figure 6. Gd_2O_3 nanoparticles dispersed in 1% Pluronic F127 solution (control) do not show any NIR fluorescence. The spectra show distinct E_{11} transitions of several semiconducting SWCNTs.³⁴ The bandgap between the first peaks above (lowest unoccupied van Hove singularity) and below (highest occupied van Hove singularity) the Fermi level represents the E_{11} transition. The distinct spectral features in the E_{11} region correspond to different types of semiconducting SWCNTs represented by the chiral vectors (n,m). As shown in Figure 6, seven distinct peaks were identified, and assigned to various semiconducting nanotube species. These peaks are red-shifted by 5–20 nm, compared to SWCNTs coated with ionic surfactants or DNA.^{34–36} Fluorescence intensity between 800 and 1100 nm is lower than the intensity beyond 1100 nm. Previous studies show that semiconducting SWCNTs at wavelengths above 1200 nm have higher diameters ($>1.4 \text{ nm}$).³⁶ The higher intensity peaks beyond 1100 nm suggest that the Gd-SWCNT solutions either contain a greater number of large-diameter SWCNTs ($>1.4 \text{ nm}$), or that more fluorescence quenching occurs in smaller diameter SWCNTs. In this study, a bath sonicator was used which does not cut the SWCNTs, or reduce their PL intensity.³⁷ Previous analysis of Gd-SWCNTs by atomic force microscopy and high resolution transmission electron microscopes showed that their average diameter is $\sim 1.9 \text{ nm}$.¹⁷ Taken together, that analysis and the PL results

reported here suggest that Gd-SWCNT solution contains a greater number of large-diameter SWCNTs.

IV. PERSPECTIVES AND CONCLUSIONS

The above results indicate that the Gd-SWCNTs show potential as highly sensitive multimodal MRI-optical probes. The relaxivity results indicate that the SWCNTs do not “passively” interact with the Gd^{3+} ion, but “actively” modulate the interaction of water molecules with Gd^{3+} ions, and thus, amplify the relaxivity (At 1.5 Tesla magnetic field, r_1 of Gd-SWCNTs = $126 \text{ mM}^{-1} \text{ s}^{-1}$ vs. Gd_2O_3 nanoparticles = $2.7 \text{ mM}^{-1} \text{ s}^{-1}$). Conversely, the multi-dentate ligands (chelates) used in Gd^{3+} -based small-chelate MRI CAs mainly sequester the toxicity of the naked metal ion. They play no or minor (“passive”) role in modulating the relaxation rate of the water protons (relaxivity r_1 at 1.5 Tesla magnetic field for Gd^{3+} -DTPA = $4.5 \text{ mM}^{-1} \text{ s}^{-1}$). The NMRD results for Gd-SWCNTs indicate that multiple structural and dynamic parameters such as rotational correlation time τ_R , rate of water exchange τ_M , and the number of fast-exchanging water molecules within the inner sphere q may be responsible for the increase in r_1 relaxivity. Gd^{3+} -macromolecular complexes (e.g., Gd^{3+} -DTPA-BSA) show increases in relaxivity mainly due to modulation of one parameter; a decrease in the rotational correlation time.⁹ Additionally, the NIR fluorescence of the Gd-SWCNTs is unaffected by photo-bleaching, tunable, and not significantly hindered by background auto-fluorescence.³⁶ Thus, the high relaxivities and NIR fluorescence, combined with the possibility that the exterior surface of the Gd-SWCNTs acts as a scaffold for attachment of groups to target biomacromolecules, cells or tissues make them excellent candidates for use in molecular imaging applications. Furthermore, the Gd-SWCNTs due to their large NIR absorption cross-sections can be employed for photothermal therapy.¹²

The use of Gd-SWCNTs as imaging agents will facilitate integration of the advantages of both MRI and optical imaging. This integration should allow the same biological specimen to be imaged at multiple scales, resolutions, and depths. Additionally, this multimodal CA could provide valuable insights during preclinical animal imaging with applications including co-localization of migrating stem cells, co-validation of targeting CA accumulation, enzyme activity detection, and visualization of tumors before and/or during treatment.⁹

In conclusion, Gd-SWCNTs are paramagnetic with an effective magnetic moment of $7.29 \mu_B$, and their NMRD profiles show high r_1 and r_2 relaxivities at very low (0.01 MHz) to clinically relevant (1.5 T) magnetic fields ($r_1 \geq 130 \text{ mM}^{-1} \text{ s}^{-1}$, $r_2 \geq 160 \text{ mM}^{-1} \text{ s}^{-1}$). Analysis of NMRD profiles using the SBM equations suggests that multiple structural and dynamic parameters such as rotational correlation time τ_R , rate of water exchange τ_M , and the number of fast-exchanging water molecules within the inner sphere q may be responsible for the increase in r_1 and r_2 relaxivity. Gd-SWCNTs exhibit NIR fluorescence, and their NIR spectra show distinct E_{11} transitions of several semiconducting SWCNTs. Taken together, our results indicate that Gd-SWCNTs show potential as novel,

FIG. 6. Near-infrared fluorescence emission spectra of the Gd-SWCNT solution excited at 662 nm.

highly efficacious, multimodal MRI-NIR optical imaging contrast agents.

ACKNOWLEDGMENTS

This research was supported by the Office of the Vice President of Research of Stony Brook University, Carol M. Baldwin Fund, National Institutes of Health (Grant No. 1DP2OD007394-01), Department of Defense Breast Cancer Research Program (W81XWH-10-1-0521). This work was also supported in part by the Clinical Center intramural research program at the National Institutes of Health.

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