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Effect of Competitive Surface Functionalization on Dual-Modality Fluorescence and Magnetic Resonance Imaging of Single-Walled Carbon Nanotubes

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Abstract

It is well-known that ionic surfactant coated single-walled carbon nanotubes (SWNTs) possess higher near-infrared fluorescence (NIRF) quantum yield than nonionic polymer functionalized SWNTs. However, the influence of surface functionalization on the magnetic properties of SWNTs for T_2 -weighted magnetic resonance imaging (MRI) has not been reported. Here, we demonstrate that SWNTs functionalized by nonionic polymers display superior T_2 relaxivity for MRI as compared to those coated by ionic surfactants. This difference may indicate that micelle structures formed by ionic surfactants are sufficiently tight to partially exclude water protons from the iron catalysts attached to the ends of SWNTs. On the basis of the different effects of the two types of suspension agents on NIRF and MRI of functionalized SWNTs, we further explore the

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Notes

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competitive surface functionalization between ionic surfactants and nonionic polymers by stepwise replacing ionic surfactant molecules in a nanotube suspension with nonionic polymers. The superior NIRF of ionic surfactant coated SWNTs gradually quenches whereas no improvement on T_2 relaxivity is observed during this replacement process. This result may indicate that nonionic polymers wrap around the outside of micelle structures to form small nanotube bundles rather than replacing ionic surfactants in the micelle structures to directly interact with the SWNT surface. Finally, we demonstrate the feasibility of dual-modality NIRF and MRI of nonionic polymer functionalized SWNTs in brain cells.

Single-walled carbon nanotubes (SWNTs) have shown promising potential in biomedical applications due to their small size and unique physical properties. They have been used to deliver various drugs, ligands, and other molecules both in vitro and in vivo.^{1–6} SWNTs have also been utilized as optical imaging agents due to their intrinsic nonbleaching near-infrared fluorescence (NIRF)^{7–12} and as magnetic resonance imaging (MRI) contrast agents (CAs) owing to magnetic metal catalysts attached to the ends of SWNTs.^{13–18} All of these unique properties make SWNTs ideal candidates for drug delivery as well as simultaneous labeling and tracking via a variety of imaging methods.

Various suspension agents such as surfactants,¹⁹ polymers,¹ DNA oligonucleotides,²⁰ and proteins²¹ have been used to functionalize hydrophobic as-grown SWNTs in aqueous media for better imaging contrast and higher biocompatibility. It is well-known that such surface functionalization greatly influences the NIRF quantum yield of those SWNTs.^{22–26} Micelle-encased nanotubes exhibit sharp peaks with high intensity as small ionic molecules completely cover the nanotube side-walls.²⁷ Polymer-functionalized SWNTs present broadened peaks as polymers are partially wrapped around the nanotubes by noncovalent stacking interactions and leave some areas uncovered.^{11,28} However, the influence of surface functionalization on the T_2 -weighted MRI of SWNTs has not been reported. SWNTs have been used as T_2 -shortening MRI agents because they reduce the T_2 of protons nearby, where T_2 is the spin–spin relaxation time. Previous studies have demonstrated that iron catalysts attached to the ends of SWNTs may be the main reason for their T_2 -shortening property.^{16,17} Because MRI measurements of SWNTs are influenced by the protons in their vicinity, surface functionalization of SWNTs may also change their MRI efficiency. Conducting dual-modality imaging on SWNTs requires a balanced performance of both NIRF and MRI. It is therefore critical to understand how different types of functionalization agents compete with each other in nanotube coating, how these interactions affect dual-modality imaging, and how to achieve the best surface functionalization of SWNTs for both NIRF and MRI detection.

In this study, we suspended SWNTs with various ionic surfactants and nonionic polymers and investigated their effects on nanotube T_2 -relaxation efficiency for MRI. Nonionic polymer-functionalized SWNTs have shown superior T_2 shortening ability as compared to ionic surfactant-functionalized SWNTs. This difference is likely due to different surface functionalization mechanisms as nonionic polymers wrap around the nanotube surface via noncovalent stacking forces, whereas ionic surfactants form micelle structures that thoroughly mask its surface. In the latter case, water protons have limited access to the iron

catalysts attached to the micelle-encased nanotubes and thus the T_2 shortening ability of the iron catalysts is reduced. Utilizing differences in surface functionalization, we also investigated the competitive surface interaction of functionalization agents with the nanotubes. In SWNT functionalization, we started with ionic sodium cholate, which provides high NIRF quantum yield and gradually replaced it with a nonionic phospholipid-polyethylene glycol (PL-PEG) polymer using dialysis. The brightness of NIRF reduced over time during this process, but the nanotube T_2 relaxivity remained essentially the same. This observation indicates that the surfactant-formed micelle structure still tightly covers the nanotube while nonionic polymers wrap around the outside of the micelle structures by noncovalent stacking. As such, the small nanotube bundles that form during the replacement process may be the primary reason for fluorescence quenching. On the basis of these mechanistic studies, we conclude that nonionic polymer functionalized SWNTs are potentially a better choice for dual-modality biomedical imaging. Finally, we demonstrate the feasibility of in vitro dual-modality imaging of SWNTs in live brain cells.

RESULTS AND DISCUSSION

SWNTs as T_2 -Weighted CAs for MRI

Various ionic surfactants and nonionic polymers were used to suspend as-grown HiPco SWNTs via a method adapted from a previous report.¹⁹ The various surfactants and polymers used for functionalization can be sorted into two categories. The first is ionic small molecules, such as sodium cholate, sodium dodecylbenzenesulfonate (SDBS), and sodium dodecyl sulfate (SDS), which cover SWNTs with a micelle structure that prevents nanotube aggregation by charge repulsion (Figure 1a top).²⁷ The second is nonionic polymers, including PL-PEG, PEG-PPG-PEG Pluronic F-108 (PF108), and PEG-PPG-PEG Pluronic F-68 (PF68), which suspend nanotubes by stacking and wrapping around SWNT sidewalls (Figure 1a bottom).^{11,27}

For T_2 -weighted MRI, each SWNT sample was prepared with series dilution and imaged in NMR tubes (Figure 1b). T_2 relaxivity is related to the spin–spin relaxation efficiency of an MRI CA. Image contrast increases with increasing T_2 relaxivity due to better interactions between CAs and nearby water protons. T_2 relaxivity, and the slopes of their linear fitting versus SWNT concentration, or r_2 , were also calculated (Figure 1c and Table 1). The r_2 for micelle-encased SWNTs was around $0.05\text{--}0.06\text{ s}^{-1}\text{ (mg/L)}^{-1}$; polymer-suspended nanotubes exhibited r_2 higher than $0.10\text{ s}^{-1}\text{ (mg/L)}^{-1}$, with PF68 yielding the highest r_2 at $0.16\text{ s}^{-1}\text{ (mg/L)}^{-1}$. As reported in previous literature,^{13,16–18} T_2 -shortening properties of SWNTs are mainly attributed to iron catalysts attached to the ends of nanotubes: small-diameter iron nanoparticles (a superparamagnetic material) contribute to proton spin dephasing by atomic nuclei energy exchange. Therefore, differences in r_2 may result from different iron concentrations in these SWNT suspensions. However, all nanotube suspensions were prepared from the same batch of HiPco SWNTs. Iron nanoparticles in as-grown SWNTs account for about 25 wt % (Figure 2a), but only those iron nanoparticles (1–2 nm in diameter) attached to the ends of nanotubes are left in SWNT suspensions after centrifugation (Figure 2b). Assuming each SWNT is 1 nm in diameter and 100 nm in length, and assuming iron nanoparticles with an average 1.5 nm diameter are only present at the two

ends of SWNTs, we calculated the iron weight percentage to be 10.43%. This estimation was further confirmed by inductively coupled plasma optical emission spectrometry (ICP-OES) measurements, where the iron concentrations in different SWNT suspensions were not significantly different (12.5 ± 1.5 wt %). It is clear that the distinct r_2 of nanotubes functionalized by the two types of suspension agents are not caused by different iron concentrations. The r_2 difference is likely due to varying amounts of water protons with access to the iron nanoparticles attached to the nanotubes; this would thus impact proton spin dephasing. For micelle-encased SWNTs, small ionic surfactants thoroughly cover the nanotubes, including the two ends where iron catalysts are attached; therefore, water molecules have limited access to the iron catalysts.¹⁹ In contrast, polymers suspend SWNTs by helical wrapping via π - π stacking and hydrophobic forces,²⁹ allowing more space for water to directly approach the nanotube and hence shorten the spin dephasing time. In addition, we measured the T_2 relaxivity of purified SWNTs with an iron concentration less than 2 wt % (Figure 2c,d).³⁰ Sodium cholate functionalized purified SWNTs displayed an extremely low r_2 of $0.008 \text{ s}^{-1} (\text{mg/L})^{-1}$, about 7 times lower than as-grown nanotubes functionalized by the same surfactant. The r_2 of SWNTs with similar functionalization is proportional to their iron concentration. It is thus clear that r_2 of the SWNT suspension is influenced by not only the amount of iron catalysts present but also the interactions between the iron catalysts and the water environment, which varies for different surface functionalization.

Competitive Surface Functionalization between Surfactants and Polymers

Ionic surfactants and nonionic polymers display very distinct effects on the optical and magnetic properties of SWNTs in aqueous media. Ionic surfactants suspended SWNTs have bright NIRF, whereas nonionic polymer functionalized SWNTs exhibit higher r_2 for MRI. It is therefore critical to address how functional groups competitively interact with SWNTs and affect their performance in dual-modality imaging. Here, SWNTs were initially functionalized by ionic surfactants to achieve high NIRF quantum yield and nonionic polymers were gradually introduced to enhance their T_2 relaxation efficiency. This process was performed by adding 1 mg/mL PL-PEG into a sodium cholate-functionalized SWNT suspension, which was then dialyzed to remove sodium cholate in the suspension. In our experiment, the SWNT suspension was removed after 8 h, 1 day, 2 days, and 4 days of dialysis and immediately centrifuged to remove extra bundles that formed during dialysis. Absorption spectra of the samples were obtained and investigated. Two of the sharp E_{11} absorption peaks at a wavelength of about 1000 nm were measured and plotted in Figure 3a. Remarkable differences were observed among different samples. The original sharp absorption peaks seen in the cholate suspended SWNTs became broadened and red-shifted toward the peak of PL-PEG functionalized SWNTs (PL-PEG-SWNTs). The broadened and red-shifted peaks indicate that PL-PEG polymer molecules gradually wrapped around the SWNTs while small bundles were forming.

NIRF of SWNTs after dialysis was measured in the 1150–1700 nm range on a liquid-nitrogen cooled InGaAs camera. A step-by-step change in emission was clearly observed during the dialysis process (Figure 3b), the emission became weaker with increased dialysis time, corresponding to a NIRF quantum yield drop for the nanotubes. Nevertheless, the

SWNT suspensions still had a reasonable fluorescence quantum yield. After the 4 day dialysis, the NIRF signal of two-step functionalized SWNTs became close to that of PL-PEG-SWNTs. Despite the gradual change in NIRF during dialysis, the r_2 of each sample unexpectedly showed almost identical values (Figure 3c). Linear fitting revealed that after dialysis, the r_2 of SWNTs was almost identical to that of micelle-encased nanotubes and was distinct from that of PL-PEG-SWNTs. This may indicate that nanotube surfaces were still thoroughly covered by micelle structures of ionic molecules, shielding the iron catalysts from water protons. The binding force between ionic surfactants and nanotubes is stronger than that between nonionic polymers and nanotubes. Therefore, PL-PEGs may only wrap around the sodium cholate coating rather than replacing it. The reduction of NIRF quantum yield of two-step functionalized SWNTs may largely result from the formation of nanotube bundles, which is typical for PL-PEG-SWNTs.

Dual-Modality NIRF and MRI of SWNTs in Brain Cells

The studies in previous sections indicate that nonionic polymer functionalized SWNTs are potentially a better choice for dual-modality imaging due to their high T_2 relaxivity for MRI and reasonable NIRF brightness. Here, PL-PEG-SWNTs were selected to demonstrate their dual-modality in NIRF and MRI in primary cultures from rodent brains. Mammalian brain cells, mainly neurons and glial cells, were prepared as described in previous literature.³¹ For MRI, PL-PEG-SWNTs were added into the culture medium at a concentration of 3.56 mg/L and incubated for 24 h. We did not observe any morphology change in the cells treated with PL-PEG-SWNTs. Equivalent amounts of solvent without SWNTs were used as a sham control. The cells treated with or without the SWNTs were then washed with phosphate buffered saline and mixed with agarose gel to form a semisolid cell solution. The cell-containing solution was transferred to a 96-well plate and imaged using a 4.7 T MRI scanner as described before. The final T_2 map is shown in Figure 4a, and a region of interest was manually drawn to calculate the relaxivity of each sample. The T_2 relaxivity of cells treated with SWNTs was 5.06 s^{-1} , higher than that of the sham control (4.76 s^{-1}).

For NIRF imaging, cells were incubated with the PL-PEG-SWNTs added culture medium for 72 h and fixed by 4% paraformaldehyde. The SWNT fluorescence was measured on the InGaAs camera setup. Several images along the z -axis were overlapped to decrease noise levels and to reveal a more comprehensive SWNT uptake profile (Figure 4b). Many fluorescent puncta residing in the brain cells can be observed. The sizes of those puncta are limited by the resolution of the detector ($1 \mu\text{m}$, much bigger than SWNTs). Moreover, SWNT fluorescence ranging from 1150 to 1700 nm is not absorbed by biomolecules and is easily distinguished from the autofluorescence of biological samples ($\sim 500 \text{ nm}$), making it ideal for deep tissue or even whole animal imaging.^{7,12,32} The NIRF of SWNTs may therefore be employed to investigate SWNT uptake or labeling in intact brain.

CONCLUSION

In this work, a series of ionic surfactants and nonionic polymers were used to functionalize HiPco SWNTs, and to study their different effects on nanotube MRI T_2 relaxation efficiency. Polymer-functionalized SWNTs show higher T_2 relaxivity than ionic surfactant

coated SWNTs. This is different from their NIRF quantum yields, where ionic surfactant coated SWNTs are known to yield higher quantum efficiency in the near-infrared region than polymer-functionalized SWNTs. This difference may be attributed to different mechanisms of surface functionalization. Ionic surfactants can thoroughly and tightly coat nanotubes via micelle structures, protecting the fluorescence of SWNTs from environmental factors. On the other hand, water protons have limited access to the metal catalysts attached to the micelle-encased nanotube ends and thus reduce MRI T_2 relaxation efficiency. The competitive surface functionalization mechanisms between ionic surfactants and nonionic polymers were also investigated on the basis of the different effects of ionic surfactants and nonionic polymers on NIRF and MRI of SWNTs. To functionalize nanotubes, we started with ionic sodium cholate, which provides high NIRF quantum yield, and gradually replaced it with nonionic PL-PEG polymer via dialysis. The brightness of NIRF was gradually reduced over time by this process, but the nanotube T_2 relaxivity remained the same. This observation indicates that the surfactant-formed micelle structure still tightly covers the iron catalysts and nanotube surface while nonionic polymers wrap around the outside of the micelle structures by noncovalent stacking. Thus, the small nanotube bundles that formed during the replacement process may be the primary reason for fluorescence quenching. Together, our data indicate that SWNTs suspended in nonionic polymer solutions are potentially a better choice for dual-modality imaging. Finally, we demonstrate the feasibility of dual-modality detection of PL-PEG-SWNTs in live brain cells by NIRF and T_2 -weighted MRI, a novel technique for in vivo brain imaging.

MATERIALS AND METHODS

Functionalization of SWNTs

Five milliliters of surfactant or polymer solution was added to 1 mg of as-grown SWNTs and bath sonicated for 6 h, followed by ultracentrifugation (Beckman L8-70 M ultracentrifuge with SW55Ti swing bucket rotor) at 133000g for 4 h. Surfactant or polymer solution concentration varied from 0.1 to 2 wt % to obtain SWNT suspensions with a typical mass concentration of 10–20 mg/L. The surfactants and polymers used are sodium cholate (Fluka), sodium dodecylbenzenesulfonate (SDBS, Aldrich), sodium dodecyl sulfate (SDS, Aldrich), PL-PEG (Avanti Polar Lipids, Inc.), PEG-PPG-PEG Pluronic F-108 (PF108, Aldrich), and PEG-PPG-PEG Pluronic F-68 (PF68, Aldrich).

T_2 -Weighted MRI of SWNTs

The efficiency of SWNTs as T_2 -weighted MRI CAs is measured using a Carr–Purcell–Meiboom–Gill (CPMG) spin–echo pulse sequence in a 4.7 T MRI scanner (Varian Inc.). The pulse repetition time (T_R) is 3 s with an echo spacing of 8 ms. The signal from each voxel was fit to a monoexponential signal decay model to determine T_2 for each voxel. A region of interest (ROI) was manually drawn using Matlab (MathWorks, Inc.) for the first imaging time point and translated to the images from later echoes. The mean T_2 and standard deviation was then calculated from all voxels within this ROI. A 250 μ L nanotube sample was placed in NMR tubes and imaged in a 32 $\times$ 32 mm field of view and at 1.5 mm slice thickness.

Surfactant/Polymer Dialysis

PL-PEG (1 mg/mL) was added into a sodium cholate-functionalized SWNT suspension, which was then dialyzed against a 3500 MWCO membrane to remove sodium cholate in the suspension. In our experiment, the SWNT suspension was removed after 8 h, 1 day, 2 days, and 4 days of dialysis and immediately centrifuged at 24000g for 6 h to remove extra bundles that formed during dialysis. Absorption spectra of the samples were obtained by a Cary 5000 spectrophotometer (Agilent Technologies) in a 1.5 mL cuvette.

NIRF Imaging of SWNTs

SWNT suspensions were thoroughly mixed with 2 wt % agarose gel (Sigma) and sandwiched between a coverslip and a glass slide. The samples were then left at 4 °C for 2 h to allow the agarose gel to solidify fully. NIRF microscopy was performed on an Olympus IX-71 inverted microscope coupled with a liquid-nitrogen cooled OMA-V:2D NIR InGaAs camera (Princeton Instrument). A 785 nm laser beam (CrystalLaser) was expanded and focused on the sample (approximately 20 mW output power). An 1150 nm long pass filter (Thorlabs) was placed before the camera to filter out excitation light.

Cell Preparation and Imaging

Mammalian brain cells, mainly neurons and glial cells, were prepared from postnatal day 0–2 pups and maintained at 37 °C, 5% CO₂ and 100% humidity as previously described.³¹ For MRI, PL-PEG-SWNTs were added into the culture medium at a concentration of 3.56 mg/L and incubated for 24 h. We did not observe any morphology change in the cells treated with PL-PEG-SWNTs. Equivalent amounts of solvent without SWNTs were used as a sham control. The cells treated with or without the SWNTs were then washed with phosphate buffered saline (in mM: NaCl 137, KCl 2.7, Na₂HPO₄ 4.3, and KH₂PO₄ 1.47) and mixed with equal volumes of 0.8% agarose gel (Sigma) to form a semisolid cell solution. The cell-containing solution was transferred to a 96-well plate and imaged using a 4.7 T MRI scanner as described before. For NIRF imaging, cells were incubated with the PL-PEG-SWNTs added culture medium for 72 h and fixed by 4% paraformaldehyde. The SWNT fluorescence was measured with 10 s exposure time on the InGaAs camera setup.

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Figure 1.

(a) Schematic diagram of SWNTs functionalized by ionic surfactants (top), which form a micelle structure, and by nonionic polymers (bottom), which wrap around nanotubes to form a suspension. (b) MRI T_2 map and (c) T_2 relaxivity fitting for SWNTs functionalized by different surfactants or polymers with various SWNT concentrations.

Figure 2. Transmission electron microscopy (TEM) images showing catalyst residues on as-grown SWNTs (a) before and (b) after separation and centrifugation. Nanotube bundles and large metal impurities were removed, and catalyst impurities were mostly at the two ends of each nanotube. For purified SWNTs (c) before and (d) after separation and centrifugation, few catalysts can be observed.

Figure 3.

(a) Absorption spectra of selected peaks showing intensity and position change during surfactant/polymer dialysis. (b) NIRF intensity change in thin films. After 4 days of dialysis, the fluorescence intensity is close to PL-PEG-SWNTs. The scale bar is 15 μm . (c) T_2 relaxivity (R_2) of SWNT samples. The red line and blue line are the linear fitting of sodium cholate and PL-PEG-SWNTs, respectively.

Figure 4.

(a) MRI T_2 map of brain cells with or without the PL-PEG-SWNT treatment. (b) NIRF image and (c) optical image of brain cells incubated with a PL-PEG-SWNT added culture medium for 72 h. The NIRF image of cells was coded with false color and the scale bar is 30 μm .

Table 1

T_2 relaxivities of SWNTs functionalized with different suspension agents for MRI

suspension agents	molecular weight	type	$r_2, s^{-1} (mg/L)^{-1}$
PF108	14600	nonionic polymer	0.104
PF68	8400	nonionic polymer	0.159
PL-PEG	5000	nonionic polymer	0.106
sodium cholate	430.6	ionic surfactant	0.057
SDBS	348.5	ionic surfactant	0.051
SDS	288.4	ionic surfactant	0.057