Published in final edited form as:

Nanoscale. 2017 May 11; 9(18): 5948–5956. doi:10.1039/c6nr07706d.

Nano Emitters and Innate Immunity: The Role of Surfactants and Bio-Coronas in Myeloperoxidase-catalyzed Oxidation of Pristine Single-Walled Carbon Nanotubes

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Abstract

Single-walled carbon nanotubes (SWCNTs) are experimentally utilized in in-vivo imaging and photothermal cancer therapy for their unique physicochemical and electronic properties. For these applications, pristine carbon nanotubes are often modified by polymer surfactant coatings to improve their bio-compatibility, adding more complexity to their recognition and biodegradation by immuno-competent cells. Here, we investigate the oxidative degradation of SWCNTs catalyzed by neutrophil myeloperoxidase (MPO) using bandgap near-infrared (NIR) photoluminescence and Raman spectroscopy. Our results show diameter-dependence at the initial stages of the oxidative degradation of sodium cholate-, DNA-, and albumin-coated SWCNTs, but not phosphatidylserine-coated SWCNTs. Moreover, sodium deoxycholate- and phospholipid-polyethylene glycol coated SWCNTs were not oxidized under the same reaction conditions, indicating that surfactant can greatly impact the biodegradability of nanomaterial. Our data also revealed that possible binding between MPO and surfactant coated-SWCNTs was unfavorable, suggesting that oxidation is likely caused by hypochlorite generated through halogenation cycle of free MPO, and not MPO bound to SWCNTs surface. Identification of SWCNT diameters and coatings that retain NIR fluorescence during the interactions with the components of innate immune system is important for their applications in in-vivo imaging.

Graphical Abstract

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Electronic Supplementary Information (ESI) available:[details of any supplementary information available should be included here].

Introduction

Carbon nanotubes and graphene have unique electronic, optical and mechanical properties enabling many applications in materials and life sciences.^{1,2} Some of the promising medical applications are in the field of drug delivery and in-vivo imaging, where single-walled carbon nanotubes (SWCNTs) have their intrinsic bandgap photoluminescence (PL) in the near-infrared (NIR) region.³ The excitations and emissions of the SWCNTs often lay in the range of the “biological window”, 650 nm to 1350 nm, whereby tissue-light interaction is minimal.⁴ The NIR photoactivity of SWCNTs has minimal photobleaching,⁵ and avoids autofluorescence from biological molecules, which are typically excited by ultraviolet-visible light coinciding with small molecule dyes. The in-vivo imaging of tumors has been widely studied because of its medical values. The “search and destroy” approaches are of particular interest by coupling imaging with drug-release or thermal destruction to target cancer cells.^{6,7} The intensity of the emitted luminescence from the SWCNTs is critical for imaging, as well as for in-vivo chemical sensing.⁸

Carbon nanotubes utilized in optical imaging are pristine (not chemically modified or functionalized) as defect sites can quench their bandgap luminescence.⁹ However, previous studies have shown that even as-produced SWCNTs have structural growth defects and covalent derivatization of the CNT surface, which could quench the PL of SWCNTs from their maximal brightness.¹⁰ It has been determined that excitons in SWCNTs have a diffusional length of about 100 nm and are subjected to be attacked by reversible and irreversible reaction.¹¹ In order to preserve the luminescence properties, and to increase water solubility, pristine hydrophobic SWCNTs are often coated by surfactants,⁹ polymers,¹²⁻¹⁴ DNA,¹⁴⁻¹⁷ and/or proteins.¹⁸ These coatings had been shown to alter the cellular uptake and biodegradability of oxidized nanomaterials.^{13,18}

Enzymatic oxidative degradation of carbon nanomaterials has been demonstrated with different mammalian peroxidase systems such as neutrophil myeloperoxidase (MPO)¹⁸⁻²⁴ (Figure 1a), eosinophil peroxidase (EPO),²⁵ and lactoperoxidase (LPO)²⁰. This degradation can cause structural modification and destruction of carbon nanotubes.^{19,20} This enzymatic oxidation is irreversible as opposed to a reversible protonation at low pH.¹¹ MPO is an important enzyme in neutrophil antimicrobial responses and can undergo peroxidase and

halogenation cycles to oxidize a substrate (Figure 1b). For nanomaterials, it has been shown that hypochlorite (OCl^-) is the major oxidant and an important initiating factor in the MPO system.^{19,20} We have previously proposed that the OCl^- produced from MPO can oxidize the pristine SWCNT surface first, followed by MPO peroxidase cycle to further degrade the nanomaterials at defect sites.¹⁹ Cell study with neutrophils had also been conducted for oxidized CNTs with evidence of degradation.^{18,19}

While the enzymatic oxidation is efficient at degrading carbon nanomaterials with high defect densities, its effect on pristine SWCNTs has not been fully studied. The MPO-catalyzed reaction could oxidize the sidewalls of SWCNTs, generating defect sites which quench the luminescence of SWCNTs needed in NIR imaging. In this work, we analyzed the initial MPO-catalyzed oxidation of SWCNTs coated with common surfactants such as sodium cholate (SC) and sodium deoxycholate (SDC) as well as cytosine—phosphate—guanine (CpG) oligonucleotides, bovine serum albumin (BSA), phosphatidylserine (PS), and a branched phospholipid-polyethylene glycol (PL-PEG).

Experimental

High-Pressure CO Conversion (HiPco) synthesized SWCNTs were purchased from NanoIntegris, Inc. (Skokie, IL). Sodium cholate (SC), sodium deoxycholate (SDC), sodium hypochlorite (NaOCl), and bovine serum albumin (BSA) were purchased from Sigma Aldrich. SWCNTs were dispersed in 1% wt. SC or SDC at concentrations of 0.1 mg/mL. The solutions were sonicated for 2 hours. BSA-SWCNTs were prepared by dispersing SWCNTs with BSA in water using bath sonication, at a SWCNT:BSA ratio of 1:10 for a final SWCNT concentration of 0.1 mg/mL.²⁶ Branched PL-PEG was synthesized by an amide coupling with (Methyl-PEG₁₂)₃-PEG-NHS ester (Thermo Scientific) and N-(aminopropyl)polyethyleneglycol carbamylidisteaoyl phosphatidylethanolamine (DSPE-050PA, NOF Corporation) in anhydrous dichloromethane.²⁷ N,N-Dicyclohexylcarbodiimide (DMAP) and 4-dimethylaminopyridine were added after 12 hours. The resulting PL-PEG has a molecular mass of ~8k Da.²⁷ PL-PEG was stirred for 24 hours and collected by vacuum filtration. PL-PEG-SWCNTs were prepared by sonicating SWCNTs with PL-PEG at a ratio of 1:10 for a final SWCNT concentration of 0.1 mg/mL. CpG oligonucleotides (5'-TCGACGTTTTGACGTTTTGACGTTTT-3') were purchased from Integrated DNA Technologies. CpG DNA-SWCNTs were prepared by sonicating SWCNTs with CpG DNA at SWCNT:CpG ratio of 1:5 for a final SWCNT concentration of 0.1 mg/mL.¹⁷ Phosphatidylserine (Fisher, NC9474115) SWCNT solutions were prepared at a SWCNT:phosphatidylserine mass ratio of 1:5. All SWCNT solutions were centrifuged at 3500 rpm for 15 minutes. The top 90% of the supernatant solutions were transferred and used as stock solutions for later experiments.

Lyophilized purified native human MPO was purchased from Athens Research and Technology, Inc. (Athens, GA) and reconstituted with 350 μL of nanopure water to give a final concentration of 2.0 μM . For the MPO reactions, 150 μL of the dispersed SWCNTs stock solution were mixed with 500 μL of distilled water, 12 μL of MPO solution and 20 μL of 5 M NaCl (EM Science, Germany). The solutions were incubated for 1 hour before the first EE map was obtained. The reaction was initiated by the addition of 4 μL of 18.75 mM

H₂O₂ (Fisher), rested for 30 minutes before sequential EE mapping. A total of five H₂O₂ additions were applied unless stated otherwise. H₂O₂ test strips (Quantofix®) were used to monitor the consumption of H₂O₂ and to verify the activity of the enzyme. In the NaOCl control, MPO solutions were not added and 4 µL H₂O₂ was replaced by 4 µL 18.75 nM NaOCl.

For the dialysis experiments, 300 µL of the surfactant protected SWCNTs were diluted with 1 mL of distilled water for the initial EE map. The solutions were then dialyzed in 1 L of water with Float-A-Lyzer G2 dialysis device overnight before EE mapping. For dialysis in the presence of MPO, 300 µL of SWCNTs were diluted with 1 mL of distilled water. MPO solution (24 µL) were added and incubated for 3 hours before dialysis. The solution was EE-mapped before and after the dialysis.

Neutrophils were isolated from human buffy coat (Central blood bank, Greentree, PA) by density gradient centrifugation utilizing Histopaque (1.077 g/mL) (Sigma, St. Louis, MO). The pellet containing neutrophils was collected, and contaminated erythrocytes were removed using RBC (Red Blood Cells) lysis buffer. Neutrophils were washed twice with calcium and magnesium free PBS, and suspended in RPMI-1640 (no phenol red; GLICO) medium, with a concentration of 5×10^6 cells/mL. Neutrophil suspensions were incubated with SWCNTs at a ratio of 1 million cells/1 µg of SWCNTs. The dilution resulted with a 0.01 wt. % of the corresponding SWCNT coatings. In a separate control experiment, neutrophils were subjected to 0.01 wt. % of SDC to examine their survivability. Living cells can be observed after 3 hours. Neutrophils were activated by the addition of N-formyl-methionyl-leucyl-phenylalanine (fMLP) and cytochalasin B (CyB) at final concentration of 100 nM and 5 µg/mL, respectively. The solutions were incubated for 3 hours, with SWCNT emissions monitored every 30 mins. Mouse embryonic fibroblasts (MEF) cells were used as a control with RPMI (no phenol red; GLICO) medium.

Photoluminescence excitation-emission (EE) maps were obtained using a Fluorolog 322 spectrofluorometer (HORIBA Jobin Yvon, Kyoto, Japan) equipped with a DSS-IGA020 L detector (Electro-Optical Systems, Phoenixville, PA). The excitation wavelength was scanned from 580 to 800 nm in 5 nm increments, and the emission was detected from 900 to 1300 nm in 2 nm increments. A second spectrofluorometer (Nanolog, Horiba Jobin Yvon) was also used with excitation wavelength scanned from 300 nm to 800 nm and emission detected between 820 nm to 1580 nm with 1.5 nm increments.

Raman measurements were performed using a Renishaw InVia Raman microscope (Wotton-under-Edge, UK) with a 633 nm laser. Samples were drop-casted on a glass slide and allowed to dry under ambient conditions overnight. Scans were carried out at a laser power of 1.7 mW with an accumulation time of 10 seconds over the range from 100 to 3200 cm⁻¹. Spectra were acquired from multiple locations, and were normalized to the most intense peak and averaged.

TEM images were obtained using a Morgagni transmission electron microscope (FEI, Hillsboro, OR) with an 80 keV electron beam. Sample (10 µL) was diluted by a factor of 100 and sonicated for 10 mins. 10 µL of the diluted sample was drop-casted onto carbon-

coated lacey copper grids (Pacific Grid-Tech, San Francisco, CA), and allowed to dry overnight at ambient conditions.

Results and discussion

Figure 2 shows the excitation-emission maps (EE map) of sodium cholate protected SWCNTs (SC-SWCNTs) before and after oxidation with MPO (Figure 2a,b). SWCNTs chiralities on the EE maps were assigned according to literature.²⁸ For controls, various reagents, including MPO (Figure 2c), H₂O₂ (Figure S1a,b) or both (Figure S1c,d), had been taken out or replaced with equal volumes of water. Hydrogen peroxide (H₂O₂) concentrations were tested by colorimetric test strips. With MPO/H₂O₂/NaCl (Figure 2b), the H₂O₂ was undetectable 30 mins after each addition, suggesting that MPO was actively consuming H₂O₂. In the absence of MPO (Figure 2c), H₂O₂ concentration increased with the additions.

Figure 2b depicts the EE map of SC-SWCNTs after five additions of H₂O₂ into the reaction mixture (MPO/NaCl). After these additions, PL intensities of SWCNTs have significantly decreased. Interestingly, the emission of (6,5) SWCNTs remained of a higher intensity relative to (9,4) and (7,6). The SWCNT distribution changes in EE map were not observed in any of the controls. In the absence of MPO halogenation cycle, EE maps exhibit similar chirality distributions, i.e., no changes of emission intensities relative to (6,5) CNT, revealing that the ratio between different SWCNTs remains constant (Figure S1). Doorn and co-workers demonstrated similar diameter-dependent changes in the contour plots with SDS-SWCNTs by the addition of NaCl salt.²⁹ Our control experiments in Figure 2 and S1 reveal that salt was not the cause as all samples contained the same NaCl concentrations.

A more detailed progress of the myeloperoxidase oxidation of SC-SWCNTs over a total of 14 H₂O₂ additions is summarized as a time-lapse figure in the supporting information (Figure S2). The bandgap emission of the SWCNTs was quenched in diameter-dependent order. Figure S2d summarizes the changes in emission for different nanotube chiralities in comparison to the (6,5) SWCNTs. It reveals the order of quenching in the sequence of (9,4), (7,6), (8,4), (7,5), and (6,5) SWCNTs, which have diameters of 0.916, 0.895, 0.840, 0.829 and 0.757 nm, respectively.³⁰ The data from Figure S2 are further fitted to different rate law equations (Figure S2e-f). Reaction is verified to be first order to SWCNTs. Rate constant values for different CNT chiralities were extrapolated by applying first order rate equation to the data (Figure S2h). The result are presented in Figure 2d and show that larger diameter SWCNTs have a higher rate constant than smaller diameter ones.

To test if the SWCNTs were oxidized in the process, SWCNTs samples were subjected to Raman spectroscopy, and their D/G ratio was analyzed (Figure 3). The changes in Raman D/G signals at various reaction stages were not significant. We attribute this to the fact that photoluminescence spectroscopy is much more sensitive to low defect levels than Raman spectroscopy.³¹⁻³³ Our observation here is in agreement with the reported photoluminescence quenching in diazonium ion reaction, where severe quenching was observed with slight changes (+0.03) in Raman D/G ratio.⁹

Sodium cholate is often compared to other bile salt derivatives such as sodium deoxycholate and sodium taurodeoxycholate for their similarity in chemical structure.^{9,34} When sodium cholate was replaced by sodium deoxycholate, SWCNTs peaks on the EE maps were intense before and after the MPO/H₂O₂/NaCl reaction (Figure S3), suggesting that SDC-SWCNTs were well dispersed and well protected from any quenching or oxidation. In comparison to SC, SDC provided a better protection for SWCNTs under the same oxidative environment, and could be the result of a thicker coating layer formed by SDC than SC.³⁴

The observed changes in SWCNT emissions during MPO/H₂O₂/NaCl incubation could be rationalized by either nanotube oxidation or removal of the surfactant coating. In the previous work, “stripping” of PEG coating from the SWCNT surface was observed when PEG-modified SWCNTs were exposed to activated neutrophils.¹³ Surfactant desorption could reduce the solubility of SWCNTs, which reduces the PL emission intensity by SWCNT bundling.³⁵ To stimulate surfactant desorption, removal of surfactant from SC-SWCNTs and SDC-SWCNTs was implemented by dialysis.^{36,37} Figure S4 shows the EE maps before and after dialysis. After overnight dialysis, the emission intensities were quenched by 24% in SC-SWCNTs (Figure S4a and S4b) and by 17% in SDC-SWCNTs (Figure S4d and S4e). The quenching was uniform for all nanotubes, suggesting that the diameter-dependence observed in MPO oxidation was not a direct result of surfactant desorption. Another dialysis experiment was performed in the presence of MPO. Other proteins, such as Concanavalin-A³⁷ and horseradish peroxidase,³⁶ had been adsorbed to SWCNTs using a surfactant exchange method developed by Graff et al.,³⁷ in which the SWCNTs were first suspended in a solution of sodium cholate and subsequently dialyzed in the presence of protein to remove the surfactant and form a protein-SWCNT complex. We used the dialysis membrane with a molecular weight cut-off at ~5 kDa. The pores of this membrane are big enough for SC or SDC micelles, but not for MPO enzyme molecules. SC-SWCNT and SDC-SWCNT samples were incubated with MPO for 3 hours and dialyzed overnight. EE maps from samples dialyzed with MPO are depicted in Figure S4c and S4f. In the presence of MPO, the quenching was more severe. We attribute this dialysis result to MPO binding onto SWCNT surface. As the surfactants were removed by dialysis, SWCNT surface became more accessible to MPO causing further quenching. It is worth noting that the quenching with MPO present was less severe in SDC-SWCNTs (36%) than in SC-SWCNTs (47%), pointing to the same conclusion that SDC provides better protection to SWCNTs than SC. More importantly, the observed quenching was not diameter dependent as in the case of the MPO/H₂O₂/NaCl oxidation.

The same diameter-dependence quenching from Figure 2b can be observed when the MPO/H₂O₂/NaCl oxidation components are replaced by the halogenation product, hypochlorite (OCl⁻). In Figure 4, SC-SWCNTs were exposed to sodium hypochlorite at 0.81 M, a quantity equivalent to 1080 additions of H₂O₂ and perfect efficiency in the MPO halogenation cycle, to illustrate that early stage oxidation by the MPO/H₂O₂/NaCl system in Figure 2 will eventually lead to complete degradation. EE maps on Figure 4b show similar diameter-dependence comparing to those produced by MPO/H₂O₂/NaCl system. As the reaction progressed (Figure 4c), all SWCNT peaks disappeared and a new broad emission band near 900 nm appeared. The origin of this new peak is unclear, but is expected to be related to light-scattering or luminescence by the newly-formed by-products. The sample in

Figure 4c was also investigated by Raman spectroscopy and transmission electron microscopy (TEM), from which the disappearance of D and G bands on Raman spectrum and the loss of tubular structures on TEM images indicate the complete degradation of SWCNTs.^{19,21,38} In Figure S5, SC-SWCNTs were exposed to the equivalent amount of OCI^- produced by H_2O_2 in Figure 2b. Together, Figure 4 and S5 provide evidence that the chirality-dependent quenching was caused by OCI^- , and that given a high OCI^- concentration or longer reaction time, early oxidation altering the PL spectra will be followed by complete degradation of SWCNTs shown by Raman and TEM.

Our results with SC-SWCNTs and SDC-SWCNTs indicate that surfactant/protecting micelle affects the biodegradation of SWCNTs. We extended our work to other previously investigated biomolecule-based coatings such as DNA strands, phospholipid-polyethylene glycol, bovine serum albumin, and phosphatidylserine. CpG oligonucleotides are selected for this work due to their immunostimulatory properties.³⁹ Although CpG DNA does not activate neutrophils directly, it can induce an enhanced in-flux of neutrophil to site of infection, increasing the production of reactive oxygen species and overall effectiveness.³⁹ CpG oligonucleotides have been coupled with SWCNTs (CpG DNA-SWCNTs) and have been demonstrated to have a photo-hyperthermic effect for cancer treatment in mice.¹⁷ Branched PL-PEG coated SWCNTs (PL-PEG-SWCNTs) have been utilized for circulation studies in mice.⁴⁰ BSA coated SWCNTs (BSA-SWCNTs) and phosphatidylserine coated SWCNTs (PS-SWCNTs) were analyzed for cellular uptake.^{26,41} Solutions of CpG DNA-SWCNTs, PL-PEG-SWCNTs, BSA-SWCNTs, and PS-SWCNTs were prepared according to the literature procedures through sonication and centrifugation.^{17,26,27,41} The samples were subjected to MPO-catalyzed oxidation and their EE maps are presented in Figure 5. Changes in intensity values and control experiment are available in Figure S6 and S7.

CpG DNA-SWCNTs after the MPO reaction showed a dominant (6,5) signal upon MPO oxidation comparable to SC-SWCNTs (Figure 5a). BSA-SWCNTs also shared the diameter dependent trend. SWCNTs of (7,6) and (8,4) chiralities were about 1.2 times brighter than (6,5) SWCNTs before, and became ~70% of the brightness of (6,5) SWCNTs after the MPO oxidation (Figure 5b). Not all tested biological coatings shared the same diameter dependence quenching behaviour. PL-PEG-SWCNTs showed excellent protection like those observed with SDC-SWCNTs. The intensity of the PL-PEG-SWCNTs was not quenched by the MPO reaction (Figure 5c). Finally, PS-SWCNTs showed some degree of quenching, but not diameter dependent. Over the course of the MPO oxidation, all PS-SWCNTs emissions were quenched and resembled the same distribution as the initial EE map (Figure 5d). These results demonstrate that the diameter-dependence of MPO-catalyzed oxidation of SWCNTs is not restricted to surfactants and can also occur with DNA or protein coatings and can potentially extend to the biodegradation of opsonized SWCNTs in vivo, where SWCNTs are coated with different biomolecules (coronas) such as proteins and lipids.⁴²

With these results on MPO-catalyzed oxidation of protected SWCNTs, neutrophil oxidation of coated SWCNTs was attempted. Upon activation by N-formyl-methionyl-leucyl-phenylalanine (fMLP) and cytochalasin B (CyB), neutrophils had been shown to degrade oxidized SWCNTs through the MPO oxidation pathway.¹⁹ Here, we incubated the SDC-SWCNTs, CpG DNA-SWCNTs, and BSA-SWCNTs with activated and non-activated

neutrophils. Incubations with mouse embryonic fibroblast (MEF) cells were also performed as non-oxidative controls. Figure 6 summarizes our results with the normalized emission intensities of (7,6) SWCNTs. EE maps of these results are available in Figure S8.

SDC-SWCNTs were chosen for their resistance towards OCl^- from our MPO experiments. The different results from active neutrophils, non-active neutrophils and EMF cells indicate that the spectral changes were not due to surfactant displacement by cell media. NIR emission from SDC-SWCNTs was 93% of the initial intensity after MEF incubation, showing resistance towards MEF cells and cell media. When incubated with neutrophils, however, the emission dropped to 30-40% regardless of whether the neutrophils were activated or not. It is possible that SWCNTs triggered the neutrophils activation without the specific agents (fMLP and CyB), which could explain the analogous results in activated vs non-activated neutrophils.⁴³ As mentioned above, SDC-SWCNTs can prevent the MPO oxidation, implying that the observed quenching with neutrophils might be due to other non-MPO pathways. The effects of activated neutrophils are realized via three major synergistically interacting mechanisms: i) oxidative burst, ii) release of granules and iii) formation of neutrophil extracellular traps (NETs). While MPO is the major contributor to SWCNT modification through oxidative reactions of MPO, the other factors such as antibacterial serine proteases (neutrophil elastase, proteinase 3 and cathepsin G of the azurophilic granules), plasma membrane and cytosolic proteins and lipids as well as DNA are also likely candidates for the SWCNT modification.⁴⁴ These secreted from the activated neutrophils factors – acting together - can potentially affect the SWCNT wrapping, facilitate its displacement from the SWCNT surface and enhance the MPO-driven oxidative degradation process thus explaining more efficient SWCNT degradation by neutrophils as compared to MPO alone.⁴⁵

CpG DNA- and BSA- were chosen for their diameter dependence shown in MPO oxidation. It is important to note that such diameter dependence was not observed when incubated with either neutrophils or MEF cells (Figure S8). CpG DNA-SWCNTs fluorescence was quenched to 33% of the initial intensity in both activated and non-activated neutrophils and to 70% in MEF cells. As CpG DNA does not activate neutrophils, the similarity between activated and non-activated neutrophils suggests that the observed spectral changes were not a result from oxidative burst.³⁹ The reduction in emission might be due to interactions between CpG DNA-SWCNTs with neutrophils and, to a lesser extent, MEF cells. Incidentally, BSA-SWCNTs were not affected by non-activated neutrophils, had 90% emission with MEF cells and 69% emission with activated neutrophils, making BSA-SWCNTs the least affected among the three coatings tested in the cell experiments.

We and others have previously suggested diameter dependence in MPO-catalyzed oxidation by analyzing radial breathing modes (RBM) in SWCNT Raman spectra.^{18,22} While Raman RBM modes can be correlated to nanotube diameters,³⁰ our new PL data reveal the relative changes in nanotube chiralities. Our data shows the selective oxidation beginning from the larger diameter SWCNTs, as the quenching of SC-SWCNT progressed from the top right corner of the EE map to the bottom left, corresponding to nanotube with (9,4) chirality (0.916 nm) to (6,5) (0.757 nm).³⁰ Our data with chirality precision conformed that MPO-catalyzed oxidation can be selective in SWCNTs.

The impact of the observed PL selectivity in the MPO-catalyzed oxidation of SWCNTs is two-fold. From a materials chemistry standpoint, our results suggest that (6,5) SWCNTs are more resistant to oxidation in comparison to nanotubes of other chiralities as in the case for SC-SWCNTs, CpG DNA-SWCNTs, and BSA-SWCNTs. Since the emission signal from (6,5) SWCNTs was less effected by oxidation than the other nanotubes, it would be reasonable to track the emission of (6,5) SWCNTs for NIR imaging purpose. However, from a biological imaging standpoint, (6,5) SWCNTs absorb light at 566 nm which is outside the range of “biological window”. Within our sample pool, (7,6) SWCNTs have the closest match to the biological window, with excitation at 642 nm and emission at 1115 nm. We have demonstrated sodium deoxycholate is excellent in protecting this band from MPO oxidation. Among the biological coatings we tested, PL-PEG-SWCNTs appeared to be the most efficient in protecting SWCNTs from the MPO-catalyzed oxidation.

We have attributed the diameter dependent spectral changes in fluorescence to the MPO halogenation cycle. Non-activated MPO ($-H_2O_2$) showed no reduction in SWCNTs emission and dialysis experiments showed quenching with no diameter dependence. Therefore, we concluded that our observation was not the result of MPO binding to SWCNTs. In fact, we identified OCI^- oxidation to be the reason, as we have demonstrated in experiments with sodium hypochlorite (Figure 4).

The observed differences in the effects of coatings might be related to OCI^- ion diffusion to the coated nanotubes through the surfactant layers. SC-SWCNT layers were calculated by Fagan et al. to be ~ 1 nm thinner than those of the SDC-SWCNTs.³⁴ Our results here also indicate that SC-SWCNTs are worse at excluding quenchers than SDC. Diameter dependence with SC was illustrated by Hilmer et al. in diazonium reactions.⁹ SDC showed more resistance to a given concentration of diazonium ions than SC,⁹ just as we observe in this enzymatic oxidation.

The OCI^- diffusion argument can also be applied to DNA-coated SWCNTs. Taylor and co-workers had modelled DNA wrapping on SWCNTs. They showed an average 0.34 nm between DNA and SWCNTs, a typical π - π stacking distance, which was independent of the CNT chirality and the DNA sequence.⁴⁶ Furthermore, they indicated that larger diameter SWCNTs have a longer helical wrapping periodic distance along the tube. With wrapping period of 3.2 nm for (6,5) SWCNTs and 4.2 nm for larger (11,10) SWCNTs, it implies that all DNA wrapped SWCNTs have a gap that can be attacked by OCI^- . Moreover, larger diameter CNTs have a wider gap between helical wrapped DNA. In fact, this matches with our observed diameter dependence from CpG DNA-SWCNTs. CpG DNA was selected for this work because of its biological relevance.³⁹ As Zheng et al. demonstrated, the length and the sequence of DNA can affect its binding to SWCNTs, leading to SWCNTs separation by chirality using ion exchange chromatography.⁴⁷ It was hypothesized that each specific sequence of DNA can form an ordered DNA barrel structure only on one particular (n,m) CNT.⁴⁷ Such specific interaction would result in the protection of SWCNTs of particular chirality. The interactions between specific ssDNA and their corresponding SWCNTs were demonstrated by Strano et al. in their study of fluorescence modulation of DNA-wrapped SWCNT to multiple biomolecules.⁴⁸

Our results illustrate that the choice of coating is the primary factor in the observed diameter dependence of the MPO oxidation. However, it is also possible that larger diameter SWCNTs have a higher reactivity in OCl^- oxidation. We have previously suggested diameter reactivity based on redox potentials.²¹ The electrochemical potentials of SWCNTs have been previously determined, and the bandgaps of SWCNTs were shown to increase as the nanotube diameter decreases.²¹ In this model, electron transfer takes place from the top of the valence band of SWCNT to the oxidizing species. Since the potential difference between hypochlorite and SWCNTs is larger with larger diameter SWCNTs, reaction between large diameter SWCNTs and OCl^- should be more energetically favorable.²¹

Previous studies have shown that oxidized SWCNTs can be degraded by both halogenation cycle and peroxidase cycle of the MPO. Without NaCl, MPO undergoes only peroxidase cycle, and is markedly less effective in degrading oxidized SWCNTs.¹⁹ It was suggested that the carboxyl sites resulted from OCl^- oxidation would lead to better binding of MPO and subsequent biodegradation through both peroxidase and halogenation cycles.¹⁹ As the fluorescence emission required pristine SWCNTs, our nanotubes had a low density of functional groups for MPO to bind to. Our study here focused on the early stages of SWCNT oxidation by MPO catalysis and therefore we focused on the OCl^- pathway. Although we showed OCl^- can degrade SWCNTs using Raman spectroscopy and TEM, also previously demonstrated by Vlasova et al.,²⁰ the debate on the efficiency of a single-pathway degradation (halogenation only) versus a dual-pathway degradation (using both peroxidase and halogenation cycles) is beyond the scope of this paper.

It is worth noting that sodium cholate used in this work is only physisorbed to the nanotube surfaces. The physisorbed surfactants are much more likely to be stripped off in comparison to the covalently attached molecules, as was recently demonstrated for PEG functionalized SWCNTs by Bhattacharya et al.¹³ Our dialysis data showed universal quenching of nanotubes with all chiralities, suggesting that stripping or desorption is not the cause of the diameter dependence in MPO-catalyzed oxidation. However, these results do not rule out the possibility that the diameter-dependent quenching is a two-step process, where surfactants detach from the SWCNTs first, followed by OCl^- attack on the exposed surface.

Our understanding from experiments with surfactant protected SWCNTs can be extended to other biologically-relevant coatings, as demonstrated with CpG DNA, BSA, PL-PEG and PS. Diameter-dependent quenching was observed from CpG DNA-SWCNTs and BSA-SWCNTs upon MPO catalyzed oxidation, but not from PL-PEG-SWCNTs or PS-SWCNTs. These results indicate that coatings can alter the biological behavior of the SWCNTs.

For our neutrophil experiments, it appears that there are other factors contributing to the nanotube fluorescence quenching in addition to MPO halogenation. Particularly, SDC-SWCNTs and CpG DNA-SWCNTs showed some unexpected results. The resistance of SDC-SWCNTs towards MPO oxidation was not observed with neutrophils. Diameter dependence with CpG DNA-SWCNTs in MPO-containing system was also not observed as emission signals were quenched uniformly by neutrophils. As the reduction of signals were detected for both activated and non-activated neutrophils, the cause of quenching could be related to the stripping of coatings by neutrophils and the activation of neutrophils by

SWCNTs. Controlled incubations with MEF cells indicated that the observed spectral changes were not a result of the cell culture media.

Conclusions

In this work, we demonstrated the MPO-catalyzed oxidation of surfactant-protected SWCNTs. Photoluminescence data show that at the early stages of oxidation, there is a diameter selectivity, where larger diameter SWCNTs undergo oxidation first, similar to results previously reported using Raman spectroscopy.^{18,22} We also show that surfactant desorption and MPO binding was not the cause, but OCl^- produced in MPO halogenation cycle oxidizes SWCNTs resulting in diameter-dependent quenching. The difference between SC-SWCNTs and SDC-SWCNTs suggests that surfactants can strongly influence the reactivity of SWCNTs. Similar diameter dependence was also observed with CpG DNA-SWCNTs and BSA-SWCNTs, but not with PL-PEG-SWCNTs or PS-SWCNTs. These findings underline how coatings bring an extra degree of complexity to the nanomaterials and how biodegradation or other reactions would be affected. This work provides an understanding on the enzymatic degradation of pristine but protected SWCNTs, and can be applied and extended to other carbon nanomaterials and nano-composites.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

The project described herein was supported by the National Institute of Environmental Health Sciences (Award R01ES019304). The authors thank Tom Harper and the Department of Biological Sciences of the University of Pittsburgh for provision of access to instruments.

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Figure 1.

a) Crystal structure of MPO (image adapted from ref ⁴⁹). b) Peroxidase and halogenation cycles of MPO adapted from ref ³⁸. c) Molecule structures of the surfactants, sodium cholate (SC) and deoxycholate (SDC), used in this study. d) Chiralities of SWCNTs investigated in this work are highlighted in yellow.

Figure 2.

The excitation-emission (EE) maps of SC-SWCNTs a) before activation of the halogenation cycle of MPO and b) after five additions of H₂O₂ into the reaction mixture (MPO/NaCl). c) EE map for SC-SWCNTs treated with H₂O₂/NaCl (without MPO). d) Calculated rate constants for different SWCNTs versus their diameters.

Figure 3. Raman spectra of SWCNT before and after the reaction. Inset shows the change of D/G ratio over H₂O₂ additions.

Figure 4.

Complete degradation of SC-SWCNTs by NaOCl. EE maps of SC-SWCNTs a) before the addition of NaOCl, b) 1 day, and c) 3 days after. d) Raman spectra and TEM images of SC-SWCNTs e) before and f) after reaction.

Figure 5. EE maps of a) CpG DNA-SWCNTs, b) BSA-SWCNTs, c) PL-PEG-SWCNTs, and d) PS-SWCNTs after oxidation with MPO/H₂O₂/NaCl. Inserts are EE maps before the reactions.

Figure 6.
Normalized intensity of (7,6) SWCNTs after incubation with MPO, neutrophils and MEF.