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Influence of carbon nanotube bundling on their layer-by-layer assembly and antimicrobial activity

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Antimicrobial surfaces are potentially useful for a variety of health care related applications. Single walled carbon nanotubes (SWNT) have shown promise as antimicrobial agents, but important questions persist concerning the effects of tube
15 bundling, a common phenomenon owing to strong hydrophobicity. We investigate here the influence of bundling on the layer-by-layer (LbL) assembly of SWNT with charged polymers, and on the antimicrobial properties of the resultant films. We employ a poly(ethylene glycol) functionalized phospholipid (PL-PEG) to disperse SWNT in aqueous solution, and consider cases where SWNT are dispersed i) as
20 essentially isolated objects and ii) as small bundles. Quartz crystal microgravimetry with dissipation (QCMD) and ellipsometry measurements show the bundled SWNT system to adsorb in an unusually strong fashion – with layers twice (when hydrated) and three times (when dried) as thick as those of isolated SWNT. Molecular dynamics simulation reveals a lower PL-PEG density and degree of solution extension on
25 bundled versus isolated SWNT. These, and especially the lower polymer density results in a lower degree of steric repulsion, which together with stronger van der Waals attraction, may explain the thicker adsorbed layers. Scanning electron micrographs reveal *Escherichia coli* on films with bundled SWNT to be essentially engulfed by the nanotubes, whereas the bacteria rest upon films with isolated SWNT.
30 While both systems inactivate 90% of bacteria in 24 h, the bundled SWNT system is “fast-acting,” reaching this inactivation rate in 1 h. This study demonstrates the significant impact of SWNT bundling on LbL assembly, explores its microscopic origins, and illustrates its use toward bacteria-engulfing, fast-acting, antimicrobial coatings.

Introduction

The impressive physical, mechanical, chemical, electronic, and optical properties of carbon nanotubes (CNT) have established them as a primary research focus since their discovery by Iijima.¹ Research has focussed on developing applications in energy production and storage systems,² reinforcements for high-performance composites,³ sensing materials,^{4, 5} drug delivery vehicles,^{6, 7} cancer therapeutics,^{8, 9} and antimicrobial agents.^{10, 11} Although discovered only recently, the antimicrobial activity of CNT has attracted significant attention.¹¹⁻¹⁷

Antimicrobial materials are designed to kill bacteria, and could be used to prevent the infection of medical devices. There are approximately 2 million cases of nosocomial infections in the US annually, and half are associated with implantable devices.¹⁸ Thus far, the focus has been primarily on bio-inspired antimicrobial surfaces such as antimicrobial peptides, bacteriolytic enzymes and essential oils, antimicrobial polymers with protonated amine groups, and various antibiotics.¹⁹⁻²¹

CNT possess certain advantages over other antimicrobial agents: they are highly stable, do not leach over time, are easy to functionalize, and are proving to be compatible with human cell lines.¹¹ A thin film coating arrangement is most ideal: the base material is potentially rendered antimicrobial with a minimum number of nanotubes. Layer-by-layer (LbL) assembly with charged polymers offers a facile approach toward uniform, conformal films, of nanoscale thickness, using purely aqueous processing (which is environmentally friendly and appropriate for many biocompatible polymers).

A key challenge in CNT LbL assembly is rendering the highly hydrophobic CNT aqueous soluble. Two possibilities are covalent functionalization with hydrophilic moieties, and non-covalent functionalization via amphiphilic species.^{22, 23} In both cases, CNT bundling is an important issue, as the aqueous dispersion is usually only partial. Although a common phenomenon, the influence of bundling on both the LbL assembly and the properties of the assembled film is not well understood.²⁴

In this study, we disperse single walled CNT (SWNT) with an amphiphilic polymer, poly(ethylene glycol) functionalized phospholipid (PL-PEG), such that SWNT exist i) as essentially isolated objects, and ii) as small bundles. We then investigate the LbL assembly of isolated and bundled SWNT with charged polymers, and the antimicrobial properties of the resultant films. We employ molecular simulation to investigate the microscopic origins of the LbL assembly behavior. Our key findings are i) an unusually strong adsorption of bundled SWNT during LbL assembly, ii) a microscopic explanation in terms of the PL-PEG coating on bundled versus isolated SWNT, and iii) a very rapid antimicrobial action in bundled SWNT films, possibly owing to enhanced SWNT-bacterial contact.

Experimental

SWNT synthesis and solubilization

SWNT were synthesized via CO decomposition at 1073 K and 6 atm using a Co-MCM-41 catalyst. Details of the synthesis are provided elsewhere.¹¹ The diameter of our SWNT is 0.8 to 1.2 nm and the length is 300 ± 100 nm. Previous studies showed SWNT within this length range to be more antimicrobial than longer SWNT (3-5 μ m).¹¹ SWNT were dispersed in HEPES buffer (pH 7.4) at 0.1% w/v. PL-PEG (Sunbright DSPE-050-PA, NOF Corp.) at 0.2% w/v was employed to disperse SWNT in an aqueous environment. Isolated and bundled SWNT were dispersed with PL-PEG in HEPES buffer via a probe sonicator at a power level of 60 W for one hour and 5 minutes, respectively.

SWNT solution characterization

A Zeta Potential Analyzer & Particle Sizer (ZetaPALS, Brookhaven Instruments) was employed to measure the electrophoretic mobility and effective length of SWNT. Electrophoretic mobility measurements and sizing were done by phase analysis light scattering and dynamic light scattering, respectively. The laser wavelength was 658 nm. Absorbance spectra measurements of SWNT-PL-PEG solutions were performed by employing NIR absorption spectroscopy at 580 nm (NanoSpectralyzer, Applied NanoFluorescence, LLC).

Film fabrication and characterization

Microscope cover glass (for antimicrobial assays, UV-vis absorbance), Si wafer (for SEM, ellipsometry, and Raman measurements) or silica coated QCMD sensor chips (see below) served as substrates to SWNT-polyelectrolyte films. Surface cleaning was carried out by exposure to UV-ozone for 10 minutes, followed by washing with 2% Hellmanex® and deionized water.

The substrates were coated via alternate immersion into, or alternate introduction via flow of (during QCMD experiments), polymer (5 minute adsorption steps followed by three separate 1 minute rinsing steps) and SWNT solutions (30 minute and 2 hour adsorption steps for isolated and bundled SWNT solutions, respectively, followed by three separate 5 minute rinsing steps). Poly(L-lysine) (PLL) (molecular weight 70,000-150,000, Sigma) and poly(L-glutamic acid) (PGA) (molecular weight 50,000-100,000, Sigma) solutions were 0.01% w/v in HEPES buffer. The pure polymer (control) film was (PLL/PGA)₄, and the SWNT containing samples were (PLL/SWNT-PL-PEG/PGA)₄ with either isolated or bundled SWNT-PL-PEG solutions and (PLL/SWNT-PL-PEG/PGA)₁₀ with isolated SWNT-PL-PEG solutions.

Quartz Crystal Microgravimetry with Dissipation Monitoring (QCMD) (D300, Q-Sense, Sweden) was used to characterize the adsorption kinetics of LbL assembly of SWNT/PLL/PGA. Silica coated sensor chips (QSX 303, Q-Sense) were employed. Scanning Electron Microscopy (SEM) (Hitachi SU-70) was used to study film morphology. SEM images were obtained at 10 kV. UV-vis absorption spectra of the (PLL/SWNT-PL-PEG_{bundled}/PGA)_x with increasing layer numbers (1-30) were taken on Cary-100 spectrophotometer at 25 °C. Horiba Jobin-Yvon T64000 spectrometer, using 633 nm He-Ne laser excitation was employed to obtain Raman spectra measurement. A PhE-101 Discrete Wavelength Ellipsometer (Angstrom Advanced Technologies) was employed on polyelectrolyte films formed on Si wafers to measure the thickness of our samples.

Antimicrobial assay

Bacterial viability was quantified via LIVE/DEAD® Cell Viability Assay. Films were incubated for 1 and 24 hours with bacteria at concentration 1×10^7 cells/mL in HEPES buffer (pH 7.4) at 37 °C. Films were then exposed to propidium iodide (Invitrogen, LIVE/DEAD® BacLight™ Bacterial Viability Kit) for 15 minutes to stain the dead cells with red fluorescence (owing to compromised membrane permeability). To stain the live cells with green fluorescence, films were incubated with SYTO®-9 nucleic acid stain (Invitrogen, LIVE/DEAD® BacLight™ Bacterial Viability Kit) for 5 min. Live and dead cells were enumerated through fluorescence microscopy (Olympus BX41 Model U-LH100HG, Japan). Statistical analysis was performed by employing single factor analysis of variance (ANOVA) in Excel Statistic 2003 Program, with three samples for each assay.

Simulation

SWNT-PL-PEG in aqueous solution was described within the framework of the MARTINI coarse-grained (CG) force-field.^{25, 26} Although originally developed for lipids and detergents,^{25, 26} this representation has been successfully applied to proteins,²⁷ lipoproteins,²⁸ bio- and synthetic polymers,^{29, 30} and nanostructures such as CNT,³¹ fullerenes,^{32, 33} and graphene.³⁴ A PL-PEG concentration of 0.19 v/w (300 PL-PEG molecules) was realized in the presence of a single SWNT (diameter ~0.8 nm) or a hexagonal bundle of 7

SWNT (diameter ~ 3.2 nm). Due to computational cost, the polymeric LbL components were omitted from the simulations. The CG models correspond to atomistic (10, 0) SWNT and DSPE-PEG-NH₂ with a PEG length of 45 monomeric units (PL-PEG molecular weight of 3000). The 20 nm long SWNT spanned a periodic simulation box of size (20, 20, 20) nm³ filled with CG MARTINI water beads.

All simulations were performed using the GROMACS 4.5.5 simulation package.^{35, 36} The stochastic velocity rescaling thermostat of Bussi *et al.*³⁷ was employed with temperature $T = 298$ K. A pressure of 1 bar was maintained with the Parrinello-Rahman pressure control³⁸ using $\tau_p = 4$ ps semi-isotropically, with the SWNT axial direction pressure controlled separately. A cut-off of 1.2 nm was employed for the Lennard-Jones (LJ) potential and electrostatic interactions. The LJ interactions were shifted to zero smoothly between 0.9 nm and 1.2 nm, and the Coulombic interactions between 0 nm and 1.2 nm. A time step of 8 ns was used to be consistent with the PL-PEG simulations of Lee *et al.*³⁹⁻⁴¹

Simulations lasted 2.24-2.4 μ s, with the first 400 ns discarded. Reported simulation times have been scaled by a factor of 4, corresponding to an effective speed-up factor in the diffusion rates of, *e.g.*, water⁴² and lipids^{25, 26} in the MARTINI CG model. Additional details of the simulation models, system construction, protocols, and analysis methods are provided in Supporting Material.

Results

Aqueous solutions of SWNT solubilised with PL-PEG are used throughout. We introduce the terminology “isolated” and “bundled” to refer to SWNT-PL-PEG samples sonicated (at 60 W) for 5 and 60 minutes, respectively. It should be acknowledged that a certain degree of bundling may be present in the “isolated” sample.

We begin by characterizing, via Raman spectroscopy, the diameter and chemical defect extent of the SWNT employed here. In **Figure 1**, we show the spectra of bundled versus isolated SWNT samples, adsorbed to Si wafers, around the radial breathing mode (RBM) (frequencies below 400 cm⁻¹) and D and G bands (1200-1700 cm⁻¹). The RBM occurs due to vibration of carbon atoms in the nanotube’s radial direction, and provides information on diameter, aggregation level, and deformation.^{43, 44} Absorbance frequency (ω , in cm⁻¹) can be related to SWNT diameter (d , in nm) by the following equation: $\omega = 223.5/d + 25.5$.⁴⁴ Our results suggest an individual SWNT diameter in the range of 0.8-1.3 nm, confirming our previous results obtained by transmission electron microscopy (TEM) and Raman spectroscopy.¹¹ Absorbance peaks in both isolated and bundled systems occur at the same frequencies, confirming the SWNT in both systems to be of the same diameter (the more pronounced peaks in the bundled system is due to the larger number of tubes per aggregate).^{45, 46} In **Figure 1b**, we show the Raman spectra for frequencies between 1200 and 1700 cm⁻¹ where D (around 1300 cm⁻¹) and G (1590 cm⁻¹) bands appear. The intensity (I_G/I_D) ratio is known to be inversely proportional to density of surface defects.⁴⁷ We observe intensity ratios of 5.9 and 8.7 for bundled and isolated SWNT systems, respectively, suggesting bundled SWNT to possess a higher density of defects.

In **Table 1**, we report on the characterization of isolated and bundled SWNT in aqueous dispersion. The electrophoretic mobility — defined as migration velocity divided by electric field strength — of isolated SWNT is significantly higher than that of bundled SWNT: 0.55×10^{-8} m²/Vs versus 0.15×10^{-8} m²/Vs. Assuming a rod-like geometry, the effective lengths associated with these mobilities differ by a factor of three, indicating bundled SWNT to be significantly longer (and possibly more branched) than isolated SWNT. Absorbance at 580 nm generally correlates with degree of dispersion;⁴⁸ as expected, we note a significantly higher absorbance of our isolated versus bundled SWNT system.

In **Figure 2**, we show quartz crystal microgravimetry with dissipation (QCMD) sensograms demonstrating LbL assembly of poly(L-lysine) (PLL), poly(L-glutamic acid) (PGA), and SWNT-PL-PEG. In **Figure 2A**, we observe isolated SWNT to adsorb rapidly (90% saturation within 5 min) and yield a QCMD frequency decrease about five times that of the initial PLL layer. No desorption is observed upon replacing the SWNT-PL-PEG solution with pure buffer. Interestingly, a subsequent PGA layer yields a frequency increase, probably associated with layer compaction and release of water (QCMD frequency shift is sensitive to the mass of adsorbed macromolecules and any trapped solvent).^{49, 50} The next PLL layer

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yields a large frequency decrease, comparable to that of the SWNT layer, indicating strong adsorption and a more swollen film. Subsequent layers yield results similar to those described above. Some significant differences are present in the QCMD sensogram with bundled SWNT (**Figure 2B**). We observe adsorption of bundled SWNT to saturate quite slowly (90% saturation requires about 3 h), and to yield a frequency decrease about twenty times that of the initial PLL layer. However, the bundled SWNT layer is not fully stable to a buffer rinse: about 1/3 of the above-described frequency decrease is “lost” during exposure to pure buffer. Including losses during rinsing, a roughly two-fold difference in frequency shift exists between bundled and isolated SWNT, suggesting a much thicker layer in the former case, possibly related to the greater length / degree of branching suggested by the mobility measurements in **Table 1**. Subsequent PGA and PLL adsorption yields modest frequency decreases, of about the same magnitude as that of the initial PLL layer. The frequency shifts associated with subsequent SWNT layers are somewhat smaller than that of the initial layer, but tend to be less sensitive to a buffer rinse, so the net frequency shifts (including the rinse) are about the same as for the initial layer. QCMD sensograms also provide the dissipation values (provided in Supplementary Material) which are informative in terms of sample viscosities. Bundled SWNT system presents higher dissipation values than isolated system suggesting the bundled system to be more viscous than the isolated system. The significantly enhanced SWNT deposition with the bundled system offers the possibility of nanoscale films of large and controllable SWNT content in a minimum number of deposition steps.

In **Table 2**, we present ellipsometry measurements of the thickness of various SWNT-polyelectrolyte films. In the case of isolated SWNT, film thickness is observed to increase by about 30 nm per SWNT layer. In contrast, the thickness per SWNT layer in the bundled system is about 100 nm. This three-fold difference in film thickness exceeds the two-fold difference in QCMD frequency shift (**Figure 2**), suggesting the isolated system to collapse to a greater degree upon dehydration.

To further investigate the relation of SWNT content with layer number, we show in **Figure 3** absorbance spectra of the bundled system. (In fact, only isolated SWNT absorb in this range,⁵¹ but since isolated SWNT are expected to mix with bundled SWNT in equal proportions for the various layer numbers, their absorbance gives a good estimate of overall film SWNT content.) We observe the absorbance spectra to increase approximately linearly with layer number, and conclude the bundled SWNT content to similarly exhibit linear scaling.

To investigate surface topography, we consider scanning electron micrographs (SEM) of LbL assembled SWNT-containing films (**Figure 4**). Consistent with our QCMD and ellipsometry results, we observe bundled SWNT (**Figure 4a**) to adsorb to a greater extent than the isolated SWNT (**Figure 4b**). In **Figures 4c** and **4d**, we show SEM images of *Escherichia coli* incubated in bundled and isolated SWNT, respectively. In the bundled SWNT system, most of *Escherichia coli* (shown in black) are engulfed by SWNT. In the isolated SWNT system, the SWNT come into contact with *Escherichia coli* (as expected), but the bacteria rest upon the film, and are not engulfed. These images suggest the bacteria to be intact and the cell membrane to be undamaged.

In **Figure 5**, we show the inactivation rate of *Escherichia coli* following one and twenty-four hour incubations for various SWNT/polyelectrolyte films. A pure polyelectrolyte (control) film is fairly nontoxic, exhibiting less than 20% inactivation. Isolated SWNT with 4 and 10 layers are not very effective in the first hour of incubation, exhibiting inactivation rates of less than 20%, but become much more toxic following 24 hours, where they inactivate 90% of the bacteria. Bundled SWNT are very effective for both a 1 hour and a 24 hour period of incubation, in both cases exhibiting an inactivation rate of about 90%. Therefore, we conclude that the bundled SWNT system offers significant antimicrobial activity, even at short time scales, with fewer deposition steps required compared to the isolated system. Asterisks indicate statistical significance to $p < 0.001$ compared to the control film.

To investigate the molecular-level origins of the observed differences in the bundled versus isolated SWNT-PL-PEG LbL assembly, we turn to molecular simulation. In **Figure 6**, we show simulation snapshots and observe rather distinctive differences in the PL-PEG coating of isolated versus bundled SWNT. What appear to be a full micelle and a partial micelle form around an isolated SWNT, whereas a sparser monolayer of PL covers the bundle. In both cases the PEG chains extend outward toward the water.

The micelle-like structures are able to form around the isolated, but not bundled, SWNT for steric reasons: an isolated SWNT (diameter 0.8 nm) fits nicely within the hydrophobic core of a PL-PEG micelle (average hydrophobic core diameter $\sim 3.0 \pm 0.2$ nm) without significantly perturbing its structure, whereas the bundle (diameter ~ 3.2 nm) cannot do so. Simulations at a lower concentration (0.12 w/v) and with longer chain length (120 monomers) reveal similar findings (data not shown). We find a higher PL-PEG density (1.0 versus 0.25 molecules/nm²) and a higher PEG chain radius of gyration (1.86 ± 0.03 nm versus 1.66 ± 0.04 nm, indicating greater PEG extension away from the surface) for isolated versus bundled SWNT systems. Decreased PL-PEG density and PEG chain extension imply a lesser degree of steric repulsion among bundled versus isolated SWNT and, as we discuss further below, lead to thicker layers in LbL assembly and enhanced bacterial contact and inactivation.

Discussion

We investigate here the influence of degree of nanotube bundling on the LbL assembly of PL-PEG functionalized SWNT, and on the antimicrobial properties of the resultant films. We find the degree of bundling to significantly affect film morphology, the extent of SWNT loading within the film, and ultimately the antimicrobial activity of the films. Bundled SWNT result in films with 2-fold thicker layers, and with a more confluent lateral distribution of nanotubes. In addition, bacteria situated on films containing bundled (but not isolated) SWNT become engulfed by the nanotubes, and are inactivated more rapidly than those on films containing isolated SWNT. These results suggest nanoscale films of significant and controllable SWNT content can be achieved via bundled SWNT in a minimum number of deposition steps (although at present, the steps tend to be slow).

Molecular simulation provides significant insight into these experimental observations. In particular, we find a lower PL-PEG density, and a lower degree of PEG extension into solution, for bundled versus isolated SWNT, suggesting a lesser degree of steric repulsion between bundled SWNT. We believe this suppressed repulsion, perhaps together with a larger van der Waals attraction,^{52, 53} to result in a greater extent of adsorption and thicker adsorbed layers. The high SWNT content, and perhaps the less compact arrangement, of the bundled films lead to a very interesting observation: bacteria becoming engulfed with nanotubes. The lower PL-PEG density may also increase the degree of SWNT exposure to contacting bacteria. Increased SWNT content, enhanced direct contact, and the possibility of engulfment likely act together to yield the faster inactivation rate observed here for *Escherichia Coli*. In work on related systems, Szleifer *et al.*,⁵³ Shvartzman-Cohen *et al.*,⁵⁴ and Angelikopoulos and Bock⁵⁵⁻⁵⁷ have shown, via molecular theory and simulation, the repulsive barrier against aggregation to increase with adsorbed polymer density.

These simulation results additionally point to the importance of the size of the PL-PEG micelle hydrophobic core relative to the SWNT diameter. When the former is larger than the latter, micelle-like structures may form around the SWNT, resulting in a high PL-PEG density and enhanced steric repulsion. However, when the sizes become comparable, the micelle-like structures cannot form, and a sparser layer of PL-PEG results. The ratio of these length scales is likely an important variable governing film assembly. In particular, decreasing the PL size could result in a sparser coverage of isolated SWNT, and possibly enhanced film assembly and enhanced rate of microbial inactivation.

Carbon nanotubes are very hydrophobic, and can only be dispersed in aqueous solvent through functionalization. Covalent functionalization is widely reported in the literature, and involves the attachment of chemical groups such as amine and carboxyl to the carbon surface.^{22, 23} A drawback is that

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covalent functionalization may alter the intrinsic properties of SWNT.⁵⁸ Non-covalent functionalization involves assembly with amphiphilic molecules such as surfactants, polymers, and biomacromolecules, and is considered to be an attractive route to disperse SWNT without disturbing its intrinsic properties.⁵⁹ Phospholipid-PEG is an amphiphilic molecule developed to disperse SWNT for biological applications.^{58, 60} A high rate of solubility is achieved via hydrophobic interactions between phospholipid and SWNT sidewalls, and hydrophilic interactions between the poly(ethylene glycol) (PEG) chains and the aqueous phase. The PEG chains also confer a degree of stability within a biological environment. Availability of functional groups for further bioconjugation is another attractive characteristic of PL-PEG. SWNT-PL-PEG systems have been studied by several other groups in the context of biological applications.^{61, 62} Dai and co-workers employed SWNT-PL-PEG for imaging and drug delivery, and reported a blood circulation time of 22.1 hours, exceeding the previous SWNT record of 5.4 hours.⁶¹ Ou *et al.* functionalized PL-PEG/SWNT with protein A and attached an alpha(v)beta(3) monoclonal antibody for cancer targeting therapy, and observed a high targeting efficiency with low cellular toxicity.⁶² Our choice of the polymer amphiphile PL-PEG is motivated by its high dispersion capability, potential for biofunctionalization, and wide range of potential biomedical applications.

As a method of assembling functional thin film nanomaterials, the layer-by-layer (LbL) technique offers many attractive features.⁶³ LbL assembly involves the consecutive adsorption of oppositely charged macromolecules onto a substrate, and its benefits include facile fabrication, and the possibility to coat a variety of geometries, to embed bioactive species into films, and to control morphology/composition through numerous control variables such as polymer type, solution salt, and pH.^{64, 65} Several studies have addressed the LbL assembly of carbon nanotubes.⁶⁶⁻⁷³ These and other studies show LbL to yield a very high SWNT content, up to 50 wt%. In contrast, other common methods to load nanotubes within a polymer matrix (*e.g.* in situ polymerization, blending, extrusion) generally result in loadings of 1-15%.⁶⁶ However, previous studies have not addressed the issue of SWNT bundling on LbL assembly.

Several groups have investigated the antimicrobial nature of carbon nanotubes,¹¹⁻¹⁷ and some have focused on the influence of nanotube functionalization and/or degree of bundling. Ahmed *et al.* demonstrated that improved dispersion rate of SWNT in the presence of an electroactive polymer, polyvinyl-N-carbazole (PVK), result in a higher toxicity level of SWNT.¹² They associated increased toxicity level to increased dispersion rate; however, as explained, they did not investigate the other elements in the system, such as morphological modifications, electronic interactions, and charge transfer effects associated with PVK introduction. (In contrast, our study investigates the effect of SWNT bundling degree while keeping all other compositional variables constant.). Pasquini *et al.* studied the effect of functionalization on antimicrobial activity of SWNT and found surface functionalization to have indirect effect through the impact of aggregation state.⁷⁴ They showed less compact SWNT with larger polydispersity to exhibit higher inactivation rates of bacteria. Liu *et al.* considered other forms of carbon, namely graphite, graphite oxide, graphene oxide, and reduced graphene oxide, and found the aggregation rate of graphene-based materials to correlate with the bacteria inactivation level, smaller size aggregates inactivating more bacteria.⁷⁵ Wick *et al.* studied the effect of agglomeration of SWNT on their cytotoxicity on human mesothelioma cell line MSTO 211H, and found more aggregated SWNT samples to induce more cytotoxicity.⁷⁶

Overall, the very few studies on the bundling effect of SWNT on antimicrobial activity seem to be inconsistent and incomplete and this study is one of the first to establish the correlation between bundling effect of SWNT and their antimicrobial activity.

Conclusion

We present the aqueous dispersion of SWNT via the biocompatible amphiphilic polymer PL-PEG, the LbL assembly of SWNT dispersed as isolated objects and small bundles, molecular simulation of PL-PEG coated isolated and bundled SWNT, and the antimicrobial properties of the resultant films. We observe bundled SWNT to adsorb very strongly during LbL assembly – 2-3 times greater than isolated SWNT – offering the possibility of nanoscale films of significant and controllable nanotube content in a minimum number of deposition steps. Simulations suggest dense micelle-like structures, and sparser monolayers, of PL-PEG to form on isolated and bundled SWNT, respectively. The decreased PL-PEG density and solution extension on the bundled system may lead to a lower steric repulsion, which together with larger van der Waals attraction, serve to explain the large extent of adsorption. We find films containing isolated SWNT to inactivate 90% of *Escherichia coli* in 24 h, whereas films containing bundled SWNT reach this level in only 1 h, suggesting a fast-acting mechanism possibly related to enhanced SWNT content and/or bacterial contact. This study demonstrates how SWNT bundling can act as a powerful control variable influencing LbL assembly and antimicrobial ability.

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† Electronic Supplementary Information (ESI) available: Molecular structure of PL-PEG additional materials and methods, and details of the simulations. See DOI: 10.1039/c2sm27444b

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Figure 1: Raman spectra of bundled and isolated SWNT-PL-PEG samples with polyelectrolyte films formed on Si wafers. Fig 1a shows the RBM mode and Fig 1b describes the D and G bands.

Figure 2: Quartz crystal microgravimetry with dissipation (QCMD) sensograms of A) (PLL/SWNT-PL-PEG_{isolated}/PGA)₄ and B) (PLL/SWNT-PL-PEG_{bundled}/PGA)₄.

Figure 3: Absorbance spectra of $(\text{PLL}/\text{SWNT-PL-PEG}_{\text{bundled}}/\text{PGA})_x$ with increasing layer numbers (1-30). Increase in absorbance spectra is evident of the SWNT-PL-PEG adsorption.

Figure 4: Scanning electron microscopy (SEM) images of a) (PLL/SWNT-PL-PEG_{bundled}/PGA)₄ and b) (PLL/SWNT-PL-PEG_{isolated}/PGA)₄ c) sample (a) after 24 hr *Escherichia coli* incubation d) sample (b) after 24 hr *Escherichia coli* incubation. Red arrows point to some of the SWNT present in the images. Bundled SWNT-PL-PEG adsorbs very strongly on the polymer film covering the full surface with layers of nanotubes (a and c) whereas isolated SWNT-PL-PEG reveal individual or small bundled SWNT that are scattered on the polymer film (b and d). *Escherichia coli* are clearly visible in black in (c) and (d). In both images, bacteria seem to be intact. Most of the surface of the bacteria is engulfed by the bundled SWNT-PL-PEG in (c) whereas in (d) bacteria membrane is surrounded by dispersed SWNT-PL-PEG.

Figure 5: Percent inactivation of *Escherichia coli* (K12) at 1 hr and 24 hr on various substrates, as determined by LIVE/DEAD assay. Control polymer films (PLL/PGA)₄ do not induce significant toxicity. Isolated SWNT-PL-PEG polymer samples are not as effective as the bundled SWNT-PL-PEG film within the first hour of incubation. After 24 hour incubation, all SWNT-PL-PEG containing samples (bundled and isolated) inactivate most of the bacteria (~90%). Asterisks are placed on each bar that are statistically significant to $p < 0.001$ compared to the control film.

Figure 6: Representative visualizations of the PL-PEG aggregation (45 monomer PEG) on a single SWNT and a bundle of SWNT with 0.19 v/w concentration (300 PL-PEG molecules). a) The cross-section view of the PL-PEG coating of an isolated SWNT. b) The corresponding side-view with just the PL part of the bound PL-PEGs. The formed micelle is clearly visible. c) The cross-section view of PL-PEG coating of the 7 SWNT bundle. d) The corresponding side-view with just the PL part of the bound PL-PEGs. e) A snapshot showing the simulation box, the PL-PEG micelles, and aggregation on the SWNT. All the snapshots are at time 600 ns. In the visualizations a)-d), only the surface bound PL-PEG, or their PL parts, and the SWNTs are shown but e) shows also the PL-PEG in solution forming micelles. In all the snapshots, the SWNTs are colored in silver, the aliphatic tails of the PL part of the PL-PEG is in cyan, the phosphate in blue, the glycerols in pink, and the PEG chain is red. Water and sodium counterions are omitted in the visualizations for clarity.

	Mobility ($10^{-8} \text{ m}^2/\text{Vs}$)	Effective Length (nm)	Absorbance (@ 580 nm)
SWNT-PL-PEG (isolated)	0.55 ± 0.02	400 ± 100	4.1
SWNT-PL-PEG (bundled)	0.16 ± 0.08	1200 ± 100	1.8

Table 1: Electrophoretic mobility, effective diameter, and absorbance values of isolated and bundled SWNT-PL-PEG systems.

Film description	Thickness (nm)
(PLL/SWNT-PL-PEG _{isolated} /PGA) ₄	120 ± 20
(PLL/SWNT-PL-PEG _{isolated} /PGA) ₁₀	350 ± 20
(PLL/SWNT-PL-PEG _{bundled} /PGA) ₄	400 ± 10

Table 2: Ellipsometry measurements of various films containing SWNT-PL-PEG.