

Antimicrobial Applications of Electroactive PVK-SWNT Nanocomposites

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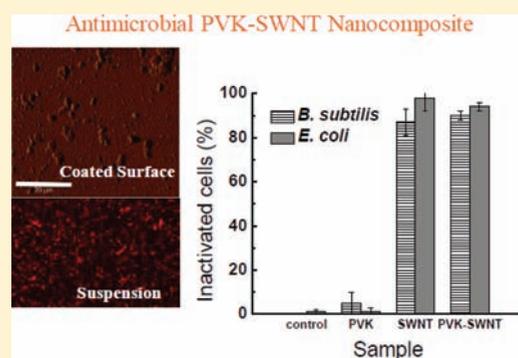
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S Supporting Information

ABSTRACT: The antibacterial properties of a nanocomposite containing an electroactive polymer, polyvinyl-*N*-carbazole (PVK) (97 wt %), and single-walled carbon nanotubes (SWNT) (3 wt %) was investigated as suspensions in water and as thin film coatings. The toxic effects of four different PVK-SWNT (97:3 wt %) nanocomposite concentrations (1, 0.5, 0.05, and 0.01 mg/mL) containing 0.03, 0.015, 0.0015, and 0.0003 mg/mL of SWNT, respectively, were determined for planktonic cells and biofilms of *Escherichia coli* (*E. coli*) and *Bacillus subtilis* (*B. subtilis*). The results showed that the nanocomposite PVK-SWNT had antibacterial activity on planktonic cells and biofilms at all concentration levels. Higher bacterial inactivation (94% for *E. coli* and 90% for *B. subtilis*) were achieved in planktonic cells at a PVK-SWNT concentration of 1 mg/mL. Atomic force microscopy (AFM) imaging showed significant reduction of biofilm growth on PVK-SWNT coated surfaces. This study established for the first time that the improved dispersion of SWNTs in aqueous solutions in the presence of PVK enhances the antimicrobial effects of SWNTs at very low concentrations. Furthermore, PVK-SWNT can be used as an effective thin film coating material to resist biofilm formation.



INTRODUCTION

Materials used in aquatic environments and medical devices have high potential for biofilm formation.¹ Biofilms are complex aggregations of microorganisms surrounded by an extracellular matrix and have been reported to grow on conducting and exposed surfaces of biomedical devices, marine and industrial instruments, and pipes. Biofilm growth has led to several health and economic problems. The problems include antibiotic-resistant infections, increased energy consumption, excessive operational expenditures, and accelerated corrosion problems.² To solve these problems, different types of coatings, that can protect the surface from biofilm formation, have been developed, such as polyamide and polypropylene with silver,^{3,4} antibiotics,^{4–7} quaternary ammonium salts,⁸ cationic peptides,⁹ and metal ions.¹⁰ However, the synthesis of biofilm resistant surfaces tends to be complex and expensive, and often the surfaces loses effectiveness due to leaching or depletion of the antimicrobial agents.^{5–7}

Recently, several studies have shown that single-walled-carbon nanotubes (SWNTs) have antimicrobial properties against diverse groups of microorganisms, like bacteria (both Gram-positive and Gram-negative), protozoa, and viruses.^{8–12} SWNT-coated surfaces have also been shown to significantly inhibit *E. coli* biofilm formation.⁷ However, the use of SWNTs as antimicrobial agent is still limited by its poor dispersibility in

most solvents as well as its high cost.^{5,13,14} Alternatively, SWNTs combined (as a filler component) with polymers provide better dispersion and can potentially increase or maintain the same antimicrobial properties of SWNT materials, while providing a broad range of structural, mechanical, and degradation properties.^{1,5,15} Unfortunately, there have only been a handful of studies about antibacterial effects of polymer-SWNT nanocomposites. None of them have explored the possibility of using these composites as robust coating materials to resist biofilm formation. Electroactive polymers are an excellent choice for such nanocomposites, because of their anticorrosion properties and facile surface application (via electrodeposition).^{16,17} Among the available electroactive polymers, polyvinyl-*N*-carbazole (PVK) is an excellent candidate due to its good thermal and mechanical properties, and its ability to form robust thin films (i.e., conducting polymer network (CPN)) on any conducting surface.^{18,19} Furthermore, PVK contains the aromatic *N*-carbazole group that facilitates π - π stacking as well as donor-acceptor

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interactions making it a more compatible polymer for carbon-based nanomaterials like SWNTs.^{20,21}

In this study, we investigated the PVK-SWNT nanocomposite antibacterial properties to planktonic cells (i.e., cells in suspension prior to biofilm formation) and biofilms. The bacterial toxicity of different concentrations of PVK-SWNT dispersed in water were investigated against Gram-positive (*B. subtilis*) and Gram-negative (*E. coli*) bacteria, as well as the potential inhibition properties of biofilm formation on coated surfaces with the PVK-SWNT nanocomposite. The results showed for the first time that, by improving dispersibility of SWNT in solution, higher bacterial toxicity of SWNT can be achieved, even in concentrations as low as 0.0003 mg/mL of SWNT. Furthermore, PVK-SWNT coated surfaces with only 3% of SWNTs significantly inhibited biofilm formation. This result shows that coated surfaces for antimicrobial purposes can be made with reduced concentrations of SWNT.

MATERIALS AND METHODS

Single-Walled Carbon Nanotubes (SWNTs) Characterization and Preparation. Single-walled carbon nanotubes (SWNTs) were purchased from Cheap Tubes Inc. (Vermont). The characterization of these nanomaterials can be found in the Supporting Information (SI, Table S1, Table S2, and Figure S1). The SWNTs were further purified by heating at 200 °C for 6 h prior to use. The SWNT suspension (1 mg/mL) was prepared as described in our previous work.^{22,23} Briefly, SWNTs were dispersed in DI water through 3 cycles of sonication for 1 h immediately before use for the antimicrobial assays.

PVK-SWNT Nanocomposite Solutions. The poly(*N*-vinyl carbazole) (PVK) was purchased from Sigma-Aldrich Chemicals (ca. MW = 25 000–50 000 g/mol). All solvents used for the PVK-SWNT preparation were purchased from Sigma Aldrich and were of analytical grade. The PVK-SWNT (97/3 wt % ratio PVK/SWNT) was prepared according to previously reported procedure.¹⁶ The PVK/SWNT ratio of 97/3 (wt %) was selected on the basis of the high dispersibility and stability of SWNT for long periods of time (several months) as described in our previous work.¹⁶ Briefly, a 97/3 wt/vol % ratio of PVK/SWNT was prepared in *N*-cyclohexyl-2-pyrrolidone (CHP). The purified SWNT was first dissolved in CHP and sonicated for 4 h. Then, in a separate vial, the PVK was dissolved in CHP and sonicated for 30 min. The PVK solution was then slowly mixed with the SWNT solution and followed by sonication for 1 h. After this, the PVK-SWNT dispersion was centrifuged (4400 rpm, 1 h), and the black precipitate was removed. The remaining solution of PVK-SWNT dispersion was then treated with methanol (5 mL) and again centrifuged (4400 rpm) for 30 min. The black precipitate was collected and redispersed in water followed by 20 min of ultrasonication. This procedure furnished a stable and well dispersed PVK-SWNTs solution. For the bacterial measurements, different PVK-SWNT concentrations (1.0, 0.5, 0.05, and 0.01 mg/mL) dispersed in water were used. The SWNT concentrations (mg/mL) from the prepared PVK-SWNT (97/3 wt %) dispersions are provided (Table S3). The characterization of PVK-SWNT nanocomposite and preparation of PVK-SWNT nanocomposite conducting polymer network (CPN) films can be found in the Supporting Information; the results are presented in Figures S2 and S3.

Bacterial Culture and Antimicrobial Activity of SWNT and PVK-SWNT. Single isolated colonies of *E. coli* MG 1655 and

B. subtilis 102 were inoculated and incubated in 5 mL of Tryptic Soya Broth (TSB) (Oxoid, England) overnight at 35 °C and 200 rpm. The bacterial culture was centrifuged at 3000 rpm for 10 min. The cells were washed and resuspended in phosphate buffer solution (PBS, 0.01 M, pH = 7.4) (Fisher Scientific). The bacterial suspension was adjusted to give an optical density (OD) of 0.5 at 600 nm, which corresponds to a concentration of 10⁷ colony forming units (CFU)/mL. For the antimicrobial activity assay, bacterial cultures were exposed for 3 h to the different nanomaterials. Briefly, aliquots of 180 μ L of bacterial suspensions (10⁷ CFU/ml) in PBS and noninoculated PBS buffer with bacteria (used as blanks) were pipetted in a 96-well flat bottom plate (Costar 3370, Corning, NY) containing triplicates of 20 μ L of the following samples suspended in DI water: (1) SWNT at concentration of 1.0 mg/mL; (2) PVK-SWNT nanocomposite at concentrations of 1.0, 0.5, 0.05, and 0.01 mg/mL; and (3) 1 mg/mL of PVK. The control samples contained 20 μ L of DI water only with 180 μ L of bacterial suspensions. To account for the absorbance of SWNT and PVK-SWNT nanomaterials suspended in the bacterial samples, 20 μ L of each concentration of SWNT and PVK-SWNT were added to 180 μ L of PBS only and later used as blanks to subtract from the original samples. The plates were then incubated at 37 °C at 50 rpm for 3 h. After 3 h, 20 μ L of the bacteria exposed to the different materials, the negative controls, and the blank samples were transferred into 96 well-plates containing 200 μ L TSB. The samples were then incubated at 37 °C at 50 rpm, and the bacterial growth was monitored using Synergy MX Microtiter plate reader (BioTek, VT) by measuring the OD₆₀₀ every hour until the bacteria reached stationary phase (Figure 1 a and b). The results for *E. coli* and *B. subtilis* growth after exposure to the nanomaterials were reported at their midlog phases, i.e., after 3 and 5 h, respectively (Figure 1c). Final OD values for each bacterial solution exposed to the different nanomaterial samples were determined by subtracting the OD values acquired from their respective blanks. The results are reported as average OD values with standard deviations of the triplicate samples from all three performed experiments. Statistical analyses (two-sided t-test, 95% confidence interval) were performed to determine whether the OD values of the samples with SWNT or PVK-SWNT were significantly different from the control. The same statistical analysis was also performed between OD values from SWNT and PVK-SWNT samples. The antimicrobial activity was also measured using the “live/dead assay” as previously described.²⁴ The experimental methods are presented in the Supporting Information.

Biofilm Formation Assay with OD Measurement. In this assay, we measured the total biofilm growth under exposure of different concentrations of nanomaterials as previously described.⁷ Briefly, biofilm growth was measured by using 96-well flat bottom plate (Costar 3370, Corning, NY). The concentrations of nanomaterials used in this assay were (1) SWNT at concentration of 1.0 mg/mL; (2) PVK-SWNT nanocomposite at concentrations of 1.0, 0.5, 0.05, and 0.01 mg/mL; and (3) 1 mg/mL of PVK. The control samples contained only DI water instead of nanomaterials. The 96-well plate was prepared with bacteria and nanocomposites following the same procedure as described in the Bacterial Culture and Antimicrobial Activity of SWNT and PVK-SWNT section. For both *E. coli* and *B. subtilis*, plates were prepared in triplicate. After inoculation of bacteria with the nanomaterials, the 96-well plates were incubated at 35 °C for 48 h and then stained according to crystal violet staining method for biofilm quantification described elsewhere.²⁵ Briefly,

supernatant from the wells in the plate was poured out, and the plate was washed three times. For staining, 300 μ L of 0.1% crystal violet was added in each well and incubated for 20 min in room temperature. After incubation, the staining solution was poured out and washed three times. In each well 300 μ L of ethanol solution in acetone (80% vol/vol) was added, and the plate was read at OD₅₄₀. The results are expressed as average OD values with standard deviations using all triplicates. Statistical analyses were performed as described in the Bacterial Culture and Antimicrobial Activity of SWNT and PVK-SWNT section.

Biofilm Formation Measurements on SWNT and PVK-SWNT Nanocomposite Coated Surfaces. Inhibition of biofilm growth was determined on coated ITO (indium tin oxide) surfaces. Unmodified ITO, electrodeposited PVK-SWNT (97/3 wt % PVK/SWNT), electrodeposited PVK, and spin coated SWNT-modified films on ITO were individually placed in a 12-well plate (FalconBD). Each well of the 12-well plate, containing TSB, were inoculated with 300 μ L of bacterial cells at OD of 0.5 and incubated at 37 °C for 48 h. After incubation, the ITO surfaces were taken out and gently rinsed with sterile DI water. Biofilm fixation was done according to cell fixation method previously described.²⁶ Briefly, the ITO surfaces were incubated with 2% glutaraldehyde and subsequently dehydrated with increasing concentrations of ethanol (25%, 50%, 75%, 95%, and 100%). The surfaces were vacuum-dried overnight prior to AFM measurements. AFM topography measurements were done on the ITO substrates under ambient conditions with a PicoSPM II (PicoPlus, Molecular Imaging-Agilent Technologies) in the intermittent contact mode. Images obtained were processed using Gwyddion software (2.13). To determine any toxic effects of residual cleaning agent (TBAH and acetonitrile) present on ITO surfaces after preparation of the surfaces, a control slide was rinsed with TBAH and acetonitrile and vacuum-dried overnight. A bare glass slide (without the cleaning agents) was used as control. These glass slides were incubated with *E. coli* for 5 h, and then, viability assay (Live/Dead) was performed. Each slide was tested in duplicate. The results showed no visible toxicity toward *E. coli*. Results can be seen in the Supporting Information (Figure S4).

In addition to the AFM analysis, bacterial regrowth potential test after attachment to the modified ITO surface was tested through the well established method of the plate agar test.²⁵ The brief description of the methodology used is presented in the Supporting Information.

RESULTS AND DISCUSSION

PVK-SWNT Characterization. The dispersion of PVK-SWNT (97–3 wt %) nanocomposites were characterized using FT-IR and UV–vis. FT-IR measurements confirmed the functional groups present in the nanocomposite (Figure S2). As controls, IR measurements of PVK and SWNT were also acquired. As expected, no distinctive IR peaks were observed for the pure SWNTs. The PVK-SWNT nanocomposite showed peaks similar to those of pure PVK. In particular, the peak at 1255 cm⁻¹, due to the C–N stretching of vinyl carbazole, was observed in both PVK and PVK-SWNT nanocomposite.

UV–vis spectra of the PVK-SWNT dispersion were acquired to measure interfacial interaction of SWNT and PVK. Results are shown in Figure S2b. On the basis of the results, no absorption peaks at the visible region were observed for pure SWNTs. The pure PVK, however, showed two distinct peaks at 330 and 343 nm, which can be attributed to the transitions of the pendant

carbazole moieties of PVK.²⁷ Similar absorption peaks were observed for the PVK-SWNT nanocomposite with a slight decrease in intensity and a red-shift by \sim 10 nm due to the incorporation of SWNT.

Electrodeposited PVK-SWNT coated surfaces were characterized using XPS to determine elemental composition on the surface. Figure S3a,b shows the narrow scans in the N1s and C1s of the electrodeposited PVK-SWNT and PVK surfaces. To estimate the amount of SWNT after electrocrosslinking, N/C ratios of PVK-SWNT and PVK were acquired. For PVK-SWNT, a calculated N/C ratio value of 9.4 was obtained while for PVK, the N/C ratio was calculated as 9.7. Using the obtained N/C ratios, the amount of PVK and SWNT on the film was 97% and 3%, respectively.

UV–vis spectra after electrodeposition of the PVK-SWNT, Figure S3c, showed the disappearance of the well-defined peaks at 342 and 352 nm that were initially found for the PVK-SWNT dispersion (Figure S2b). A new broad band centered at 450 nm was depicted after the electrodeposition process, attributed to the electrochemical cross-linking of the carbazole pendants in PVK.^{28,29} These results correlate well with our previous studies on electropolymerized PVK and carbazole-containing precursors.^{27,29}

Antibacterial Effects of Nanocomposites on Planktonic Cells. The toxic effects of PVK-SWNT, PVK, and SWNT solutions to *E. coli* and *B. subtilis* were evaluated by OD₆₀₀ measurements of the total bacterial growth after exposure to the nanomaterials (Figure 1a,b). The results show that maximum toxicity levels were achieved for both *E. coli* and *B. subtilis* at a concentration of 1 mg/mL of PVK-SWNT. Furthermore, pure SWNTs (1 mg/mL) were less toxic than the PVK-SWNT composite (1 mg/mL), with only 0.03 mg/mL of SWNTs. Cell exposure for 3 h to SWNT (1 mg/mL) and PVK-SWNT (1 mg/mL) led to growth inhibition of \sim 60% and \sim 64% for *E. coli*, and \sim 57% and \sim 63% for *B. subtilis*, respectively (Figure 1c). Exposure to 1 mg/mL of SWNTs and PVK-SWNT nanocomposites for 1 h did not present any apparent toxic effects on *B. subtilis* (data not shown). From these observations, it is possible that 1 h exposure of *B. subtilis* to the nanomaterial was too short for effective inactivation of the bacterial cells. This time dependency of SWNT toxic effects on *B. subtilis* was also observed in another study.³⁰ In this study, 1 h exposure of *B. subtilis* to carbon nanotubes showed no significant growth inhibition, while 4 h exposure resulted in significant growth inhibition of *B. subtilis*.³⁰ The possible reason for *B. subtilis* resistance to carbon nanotube at shorter exposure time can be explained by the presence of a thicker peptidoglycan layer present in *B. subtilis*.³⁰ Additionally, Figure 1c also demonstrated that the levels of tolerance of *E. coli* and *B. subtilis* to SWNTs and PVK-SWNT were not the same. These findings were similar to other studies.^{5,31,32} The different levels of tolerance of different microorganisms to carbon-based nanomaterials are still a matter of continuing research. Several hypotheses for the different toxicity levels consider differences in cell wall structure, the protective effect of the outer membrane surface properties, and ability to form spores and/or unique repair mechanisms of different microorganisms.³⁰ Hence, from these results, it can be inferred that toxic effects of SWNTs and PVK-SWNT on both *E. coli* and *B. subtilis* are both time and concentration dependent.

The comparable toxicity of 100% SWNTs (1 mg/mL) with PVK-SWNT nanocomposites (1 mg/mL) containing 0.03 mg/mL of SWNT can be explained by a better dispersion of the SWNTs

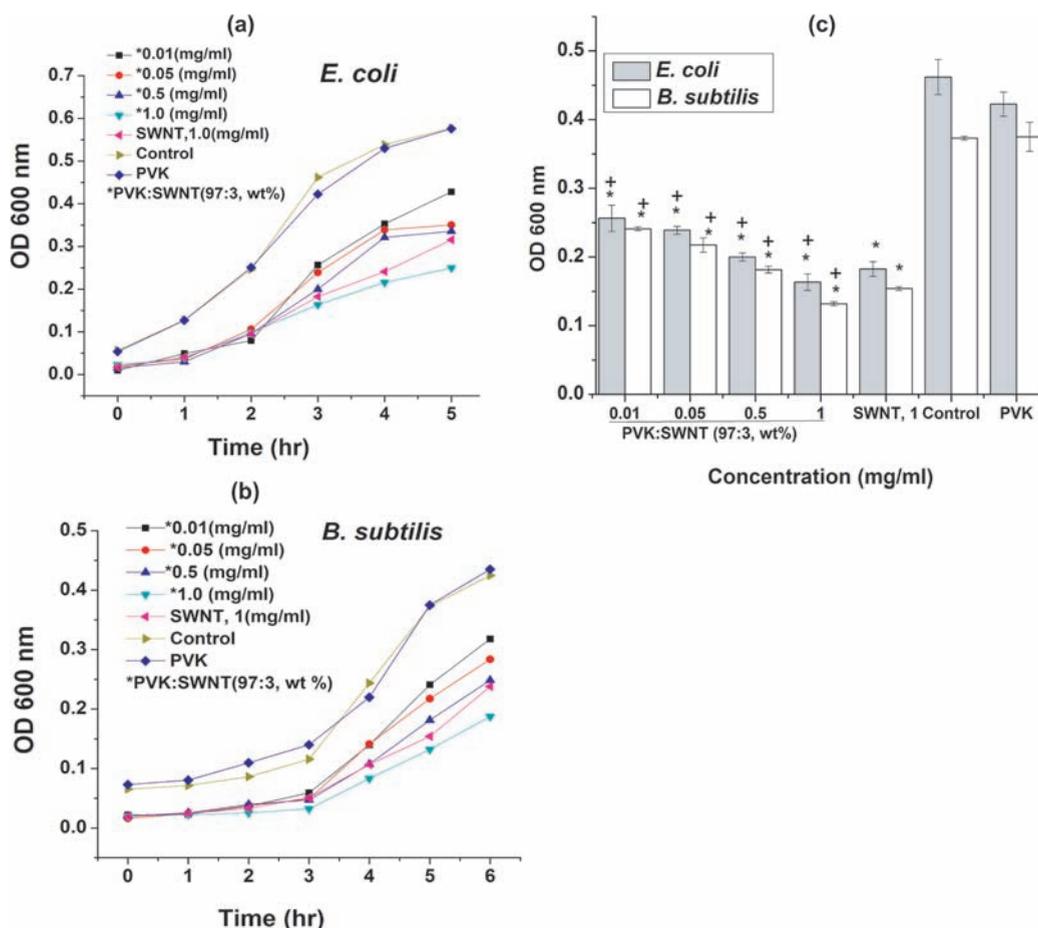


Figure 1. Bacterial growth curves on TSB after 3 h exposure to PVK (1 mg/mL), SWNT (1 mg/mL), and PVK – SWNT: (a) *E. coli*, (b) *B. subtilis*. (c) OD measurements of the bacterial growth at midlog phase for *E. coli* and *B. subtilis* after 3 h exposure to nanomaterials. Midlog phase was determined to be 3 h for *E. coli* and 5 h for *B. subtilis* for the present experimental conditions. The symbols * and + correspond to statistically different results between the control and the different SWNT samples, respectively.

in aqueous solution in the presence of PVK, as previously demonstrated.¹⁶ This better dispersion of the SWNTs particles in aqueous media is because of the effective π – π stacking and donor–acceptor interactions between the carbazole group and the SWNT. In the case of SWNT toxicity toward bacteria, dispersion is an important parameter and highly dispersed SWNTs cause greater cell contact and can potentially increase cell damage.^{9,13} In this study, only the dispersion effect of SWNT on PVK was investigated. Although synergistic effects of the two constituents in the system (i.e., morphological modifications, electronic interactions, charge transfers, or a combination of these effects) could also be responsible for the enhanced antimicrobial activity in the nanocomposite, they were not investigated in this study.

The live/dead assay was performed to determine the viability of the bacterial cells after interaction with nanomaterials (Figure 2). Fluorescence microscopy was used to assess the loss of bacterial viability after incubation. Figure 2 shows representative fluorescence images for the bacterial solutions incubated with the nanocomposite PVK-SWNT and the control. Results show that, in the absence of the nanomaterials, all cells were alive (Figure 2a). While cellular damage was observed in ~94% and ~98% of the *E. coli* cells exposed to PVK-SWNT and SWNTs, respectively. For *B. subtilis*, ~90% and ~87% of the cells were

damaged after exposure to PVK-SWNT and SWNTs, respectively. The two most hypothesized mechanism of SWNT toxicity to bacteria are physical disruption of bacterial membrane and oxidative stress.^{5,7,24,33,34} From this study, we can say that the addition of PVK did not prevent one of these two mechanisms to happen since most of the cells exposed to PVK-SWNT were red-stained cells, which indicated that the PI dye could penetrate inside the damaged cells.

Biofilm Growth Inhibition in the Presence of Nanocomposite. Although short-term toxicity of SWNTs on microbes has been extensively investigated by many researchers, there are only a handful of studies on long-term toxicity effects of SWNT and SWNT nanocomposites.²³ In this study, we investigated toxic effects of PVK-SWNT and SWNTs on biofilm formation for both *B. subtilis* and *E. coli* through the crystal violet methodology.²⁵ The results showed (Figure 3) that less biofilm was formed after 48 h exposure of *E. coli* and *B. subtilis* to PVK-SWNT and SWNTs than the control. For *E. coli*, PVK-SWNT samples showed inhibition of biofilm growth by as much as ~13% relative to the control; while for the SWNT samples, only ~5% inhibition was observed. Similarly, for *B. subtilis*, both PVK-SWNT (1 mg/mL) and SWNT (1 mg/mL) samples showed the least biofilm growth, ~28%, relative to the control. In Figure 3, it is also noticeable that, for *E. coli*, differences in PVK-SWNT concentration did not result into

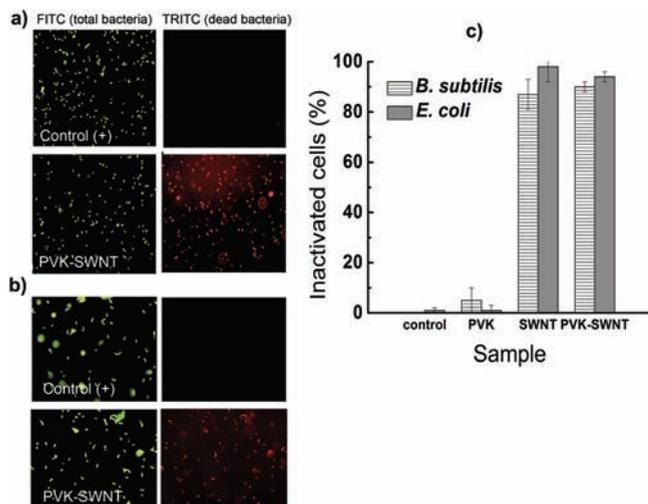


Figure 2. Viability assay for the bacteria exposed to nanocomposite. (a) Representative digital images after live and dead cell staining of *E. coli* exposed to PVK-SWNT and *E. coli* without the nanomaterial (control). (b) Representative digital images after live and dead cell staining of *B. subtilis* exposed to PVK-SWNT and *B. subtilis* without the nanomaterial (control). (c) Correlation of the % of nonviable *E. coli* and *B. subtilis* (inactivated cells %) after exposure to PVK-SWNT, SWNT (1 mg/mL), and PVK.

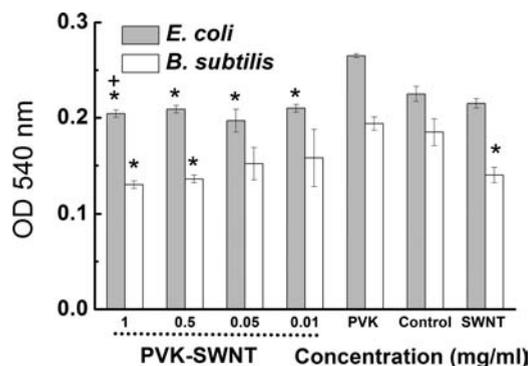


Figure 3. OD measurements obtained after biofilm test for the bacteria exposed to PVK-SWNT (97/3 wt %) at different concentrations. The symbols * and + correspond to results statistically different from the control and SWNT (1 mg/mL) samples, respectively.

different toxic effects on biofilm formation. This could be attributed to two reasons. First, based on *E. coli* and *B. subtilis* growth curves, the generation time of *E. coli* in this media is much faster than that of *B. subtilis*. Therefore, the *E. coli* cells that survived after initial contact with SWNTs will replicate faster and potentially outnumber the available SWNTs that could inactivate them. Second, other studies showed that *E. coli* cells can endure and recover from membrane damages and oxidative stresses after contact with carbon nanotubes.²⁴

Antimicrobial Effects of PVK-SWNT Nanocomposite Immobilized on Surfaces. To demonstrate the efficiency of PVK-SWNT and pristine SWNTs as potential coating materials to prevent bacterial deposition and biofilm formation, the agar printing assay was performed with *E. coli* and *B. subtilis*. For this measurement, electrodeposited PVK-SWNT and spin-coated SWNTs onto ITO surfaces were used. The nanocomposite-modified film contained 3% SWNT and 97% PVK (Figure S3). The results of PVK-SWNT were compared against electro-cross-linked PVK, spin-coated SWNT on ITO surfaces, and unmodified

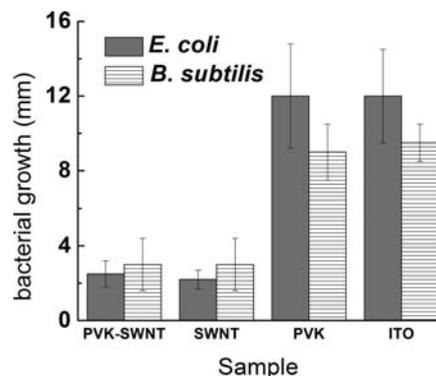


Figure 4. Agar printing assay to determine the survival of bacteria deposited onto ITO surfaces containing electrodeposited PVK-SWNT (97/3 wt % PVK/SWNT), spin coated SWNT (1 mg/mL), and electrodeposited PVK. Bare ITO surfaces were used as control. The amount of growth around the ITO coated and noncoated surfaces were determined using a caliper micrometer.

ITO surfaces as a control. The results showed that the percent bacterial inactivation on the coated PVK-SWNT surfaces compared to the unmodified ITO surfaces were 67% and 80% for *B. subtilis* and *E. coli*, respectively (Figure 4). The PVK-coated surfaces did not present any antimicrobial property for either *E. coli* or *B. subtilis*, which suggests that the toxicity observed with the PVK-SWNT nanocomposite was due to the presence of SWNT only. Furthermore, these results show that antimicrobial activity for PVK-SWNT nanocomposite solutions were maintained even after electrodeposition.

Even though antibacterial properties of SWNT-coated surfaces were described in other studies, these studies used either pure SWNT or other nanocomposite materials than PVK for short incubation time.^{7,24,34} However, this study is the first one to demonstrate that very low concentrations of SWNTs can be embedded in nanocomposites without losing the antimicrobial properties after prolonged exposure to bacteria (i.e., 48 h). In this study we embedded only 3% of SWNT in PVK-SWNT, which achieved almost similar inhibitory effects as 100% SWNT (Figure 4). Furthermore, this study shows that the use of PVK improves dispersibility of SWNT in aqueous solution, achieving a more homogeneous deposition of SWNTs onto surfaces¹⁶ and at the same time maintaining the antimicrobial property of SWNT.

To investigate the long-term bacterial toxicity of the electropolymerized PVK-SWNT films, biofilms were allowed to grow for 48 h on modified ITO surfaces. The biofilm growth and area covered by microbial growth on the surface were determined by AFM. As control, AFM images of the electropolymerized PVK, spin-coated SWNT, and the unmodified ITO substrate were also taken. The results show that *E. coli* (Figure 5) and *B. subtilis* (Figure S5) biofilms were able to form on unmodified ITO and PVK films. However, on electrodeposited PVK-SWNT and SWNT films, just a few cells, but not a biofilm, were observed on the surface after 48 h. These observations demonstrate that the nanocomposite-modified surface can effectively prevent biofilm growth the same way as pure SWNT films.⁷ Live/dead staining and imaging of the bottom layer of the biofilm in direct contact with SWNT and PVK-SWNT surfaces showed that ~80–90% of the cells were dead for both *E. coli* and *B. subtilis*, whereas only ~3–10% bacterial cells were dead on bare ITO surfaces (Figure S6). These results are in agreement with

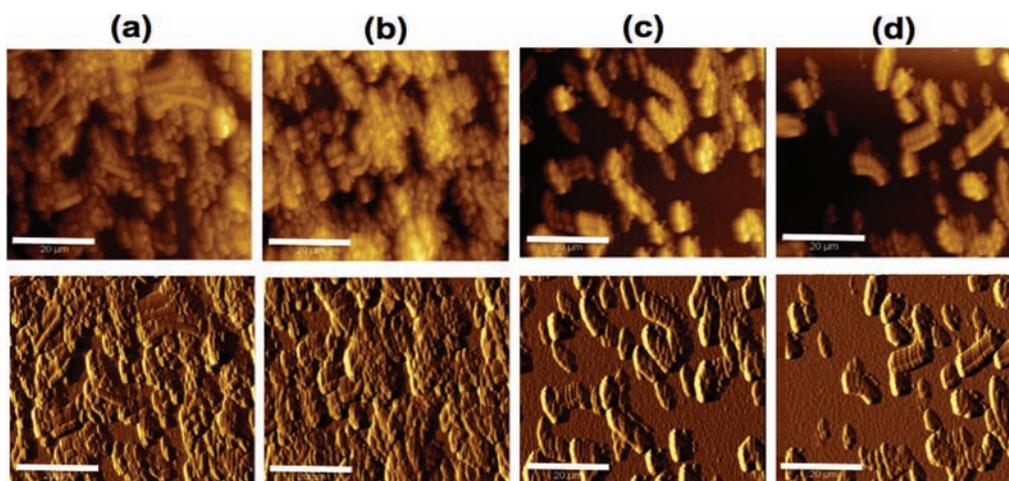


Figure 5. AFM (top) topography and (bottom) amplitude images of biofilm formation of *E. coli* on (a) ITO, (b) electrodeposited PVK, (c) spin coated SWNT (1 mg/mL), and (d) electrodeposited PVK-SWNT (1 mg/mL) coated surfaces (scale: 20 μm).

previous studies where small amounts of incorporated SWNT into polylactic-*co*-glycolic acid (PLGA) as polysulfonate (PSF) exhibited almost equivalent toxicity as 100 wt % SWNT coated surfaces.^{7,34} The mechanism of SWNT nanocomposites on bacterial colonization inhibition has been suggested as the direct contact of bacteria with SWNT ends and bundles that extend from the nanocomposite.³⁴ It is possible that our system (PVK-SWNT films) follows a similar toxicity mechanism. It is worth mentioning that the PVK-SWNT nanocomposite can be electrodeposited onto any conducting surface, which in terms of cost and ease of application is significantly better than 100% SWNT coatings.

Overall, this study shows that SWNTs can be embedded into the electroactive polymer PVK to form stable PVK-SWNT nanocomposite dispersions and films. This mixture increased the dispersion and effective bacterial toxicity of SWNT into aqueous media and led to a more homogeneous coating of PVK-SWNT on ITO surfaces via electrodeposition. In both suspension and coated form, PVK-SWNT exhibited stronger antibacterial effects to *E. coli* and *B. subtilis* when compared to SWNT and PVK alone. PVK-SWNT, with only 3% SWNTs (0.03 mg/mL of SWNT), exhibited similar or stronger antibacterial effects as compared with 100% SWNTs (1 mg/mL of SWNTs). Our study demonstrated for the first time that, by improving dispersibility of SWNT in solution, higher bacterial toxicity of SWNTs can be achieved. These results also demonstrated that it is possible to obtain more economical SWNT antimicrobial coated surfaces by significantly reducing the need of higher loads of SWNTs when embedding the SWNTs in the polymer PVK.

■ ASSOCIATED CONTENT

S Supporting Information. Supplementary data. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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