2012

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Recommended Citation

Dong, Lifeng, Alex Henderson, and Christopher Field. "Antimicrobial activity of single-walled carbon nanotubes suspended in different surfactants." Journal of Nanotechnology (2012).

Hindawi Publishing Corporation
Journal of Nanotechnology
Volume 2012, Article ID 928924, 7 pages
doi:10.1155/2012/928924

Research Article

Antimicrobial Activity of Single-Walled Carbon Nanotubes Suspended in Different Surfactants

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Received 29 June 2011; Accepted 16 August 2011

Academic Editor: Michael M. Craig

We investigated the antibacterial activity of single-walled carbon nanotubes (SWCNTs) dispersed in surfactant solutions of sodium cholate, sodium dodecylbenzene sulfonate, and sodium dodecyl sulfate. Among the three surfactants, sodium cholate demonstrated the weakest antibacterial activity against *Salmonella enterica*, *Escherichia coli*, and *Enterococcus faecium* and thereby was used to disperse bundled SWCNTs in order to study nanotube antibiotic activity. SWCNTs exhibited antibacterial characteristics for both *S. enterica* and *E. coli*. With the increase of nanotube concentrations from 0.3 mg/mL to 1.5 mg/mL, the growth curves had plateaus at lower absorbance values whereas the absorbance value was not obviously affected by the incubation ranging from 5 min to 2 h. Our findings indicate that carbon nanotubes could become an effective alternative to antibiotics in dealing with drug-resistant and multidrug-resistant bacterial strains because of the physical mode of bactericidal action that SWCNTs display.

1. Introduction

Due to their unique chemical and physical properties, single-walled carbon nanotubes (SWCNTs) have been extensively investigated as the building blocks for nanoscale electronic devices [1–3] and the catalyst supports for direct ethanol/methanol fuel cells [4–6]. For these applications, the bundled nanotubes usually need to be dispersed into individual nanotubes through surfactant stabilization of the hydrophobic nanotube surfaces. Several surfactants, such as sodium dodecyl sulfate (SDS), sodium dodecylbenzene sulfonate (SDBS), and sodium cholate (SC), were reported to efficiently disperse bundled nanotubes into suspensions of individual nanotubes [7, 8]. With the increasing production of SWCNTs and their broad applications, it is critical to evaluate the biomedical implications of nanotubes in terms of antibacterial activities and human health impacts. In our previous study, both SDS and SDBS and their conjugates with SWCNTs demonstrated toxicity to 1321N1 human astrocytoma cells even as low as 0.05 mg/mL for 30 min. On the other hand, the proliferation and viability of the cells were not affected by SWCNTs alone or by conjugates of SWCNTs with various concentrations of SC [9, 10]. In this study, we investigated further the antimicrobial activity of the same SWCNTs and their conjugates with SDS, SDBS, and SC. The utilization of the same solutions of SWCNTs and their surfactant conjugates provides comparative results of the effects of the SWCNTs on bacterial and mammalian cells. As reported in the literature, SWCNTs have given different and sometimes contradictory toxicity results, likely due to the heterogeneous nanotube samples consisting of metal catalysts, catalyst supports, amorphous carbon, and carbon nanoparticles [11, 12].

The antimicrobial activity of SWCNTs has been reported to be related to a number of factors. Yang et al. tested three different lengths of SWCNTs (<1 μm, 1–5 μm, and ~5 μm). At the same weight concentrations, longer nanotubes exhibited stronger antimicrobial activity [13]. Arias and Yang et al. also demonstrated that SWCNTs having surface groups of –OH and –COOH exhibited extremely strong antimicrobial activity to both Gram-positive and Gram-negative bacterial cells, whereas SWCNTs-NH_2 demonstrated little toxicity [14]. Vecitis et al. reported that electronic structure is an important factor regulating SWCNT antimicrobial activity [15]. Kang et al. reported that the size (diameter) of nano-tubes is a key factor governing their antibacterial effects [16]. In this

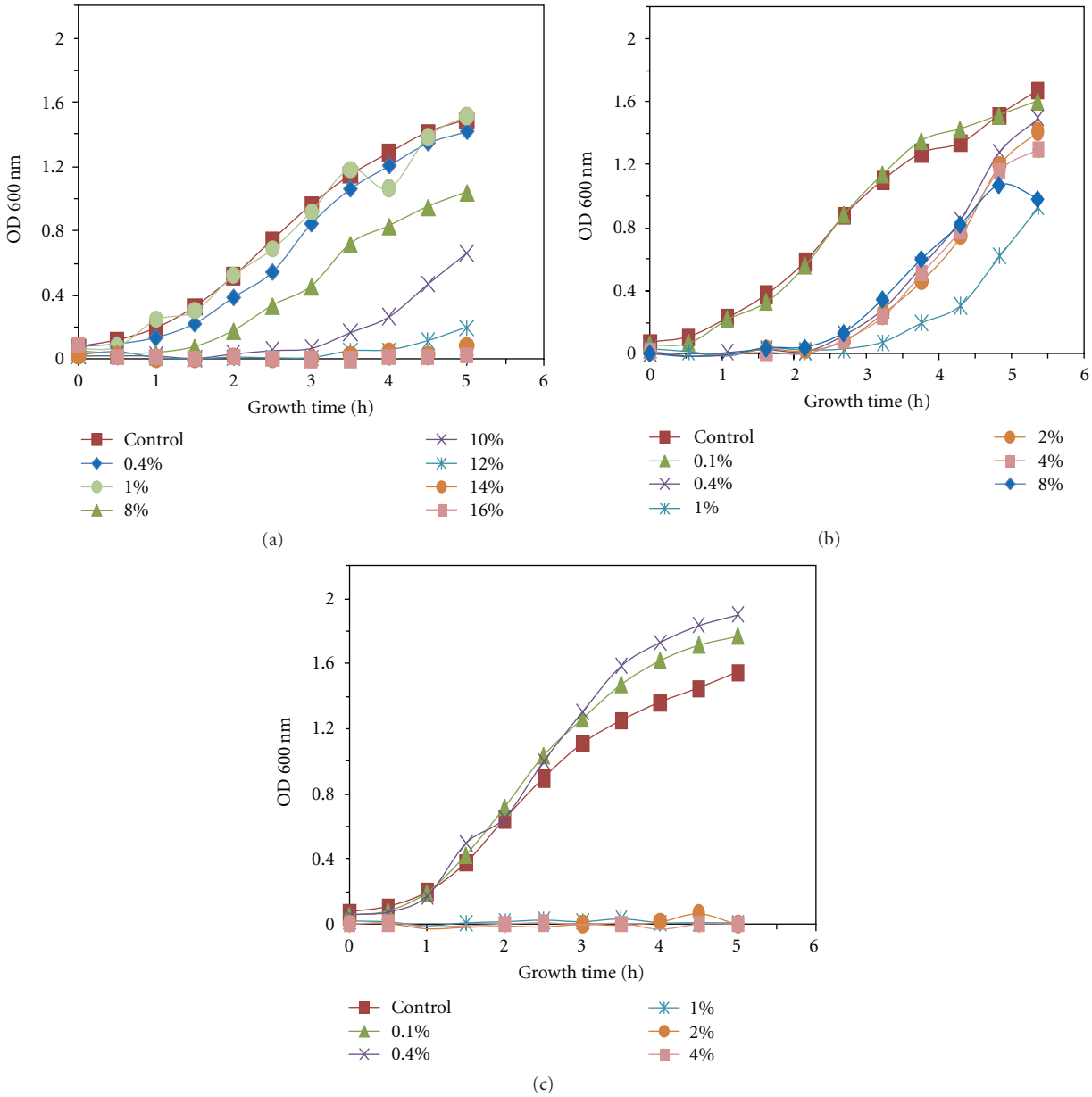

FIGURE 1: OD growth curves of *S. enterica* in BHI broth at 37°C after treatment with surfactant-only solutions and incubation for 1 h: (a) treated with 0.4, 1, 8, 10, 12, 14, and 16% SC; (b) treated with 0.1, 0.4, 1, 2, 4, and 8% SDS, and (c) treated with 0.1, 0.4, 1, 2, and 4% SDBS. Controls were cells without surfactant treatments. A blank was used before each reading. Blanks were samples without cells or surfactant.

study, the antimicrobial activity of SWCNTs suspended in different surfactants was evaluated by the appearance of the exponential bacterial growth phase. The effects of SWCNTs' concentration and treatment time on their antimicrobial activity were also tested.

2. Materials and Methods

2.1. Chemicals. brain heart infusion (BHI) was purchased from Becton, Dickinson, and Company (Sparks, MD). SDS, SC, and SDBS were purchased from Sigma-Aldrich (St. Louis, MO). Solution concentrations were made by diluting a stock surfactant solution to the specified concentration using sterile Milli-Q (mQ) water. Carbon nanotubes were purchased from BuckyUSA (Houston, TX).

2.2. Bacterial Cultures. The cultures were prepared by inoculating BHI broth in a test tube with bacteria transferred from a plate to the test tube using a cotton swab. The cultures grown were *Escherichia coli* (*E. coli*) (ATCC #11303), *Salmonella enterica* (*S. enterica*) (ATCC #19585), and *Enterococcus faecium* (*E. faecium*) (ATCC #19634). The culture to be studied the next day was incubated in a 37°C shaker with constant agitation at 200 rpm overnight. Incubation time

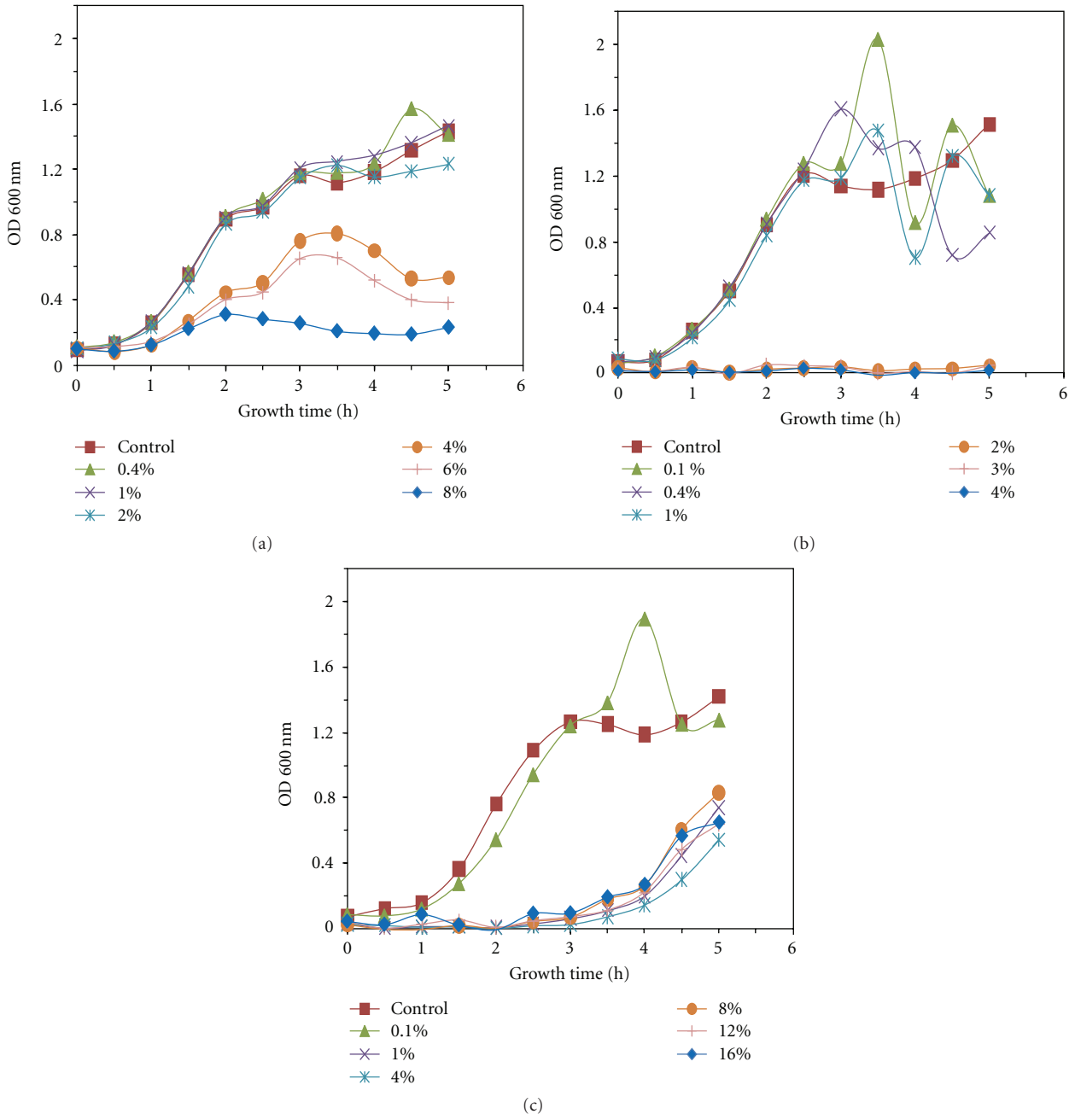

FIGURE 2: OD growth curves of *E. coli* after treatment with surfactant-only solutions and incubation for 1 h: (a) treated with 0.4, 1, 2, 4, 6, and 8% SC; (b) treated with 0.1, 0.4, 1, 2, 3, and 4% SDBS, and (c) treated with 0.1, 1, 4, 8, 12, and 16% SDS. Other conditions are the same as in Figure 1.

was approximately 18–20 h. One milliliter of the incubated culture was centrifuged at 3300 g for 2 min. The supernatant was removed, and the remaining pellet was washed with 1 mL mQ water three times. The bacterial cells were resuspended in 1 mL mQ water.

2.3. Treatment of Bacterial Cells with SWCNTs. Fifty microliters of the cell suspension were diluted in 500 μL of surfactant or SWCNT/surfactant solution and allowed to incubate at 37°C and 200 rpm for 1 h or for a designed treatment time. The blank solution contained 550 μL of mQ water, and the control solution contained 500 μL of mQ water and 50 μL of cell solution. After the incubation, 1.45 mL of BHI broth was added to each solution for a final volume of 2 mL.

2.4. Optical Density (OD) Growth Curve Measurements. After the addition of BHI, 100 μL aliquots were taken from the solutions every 30 min for the next 5 h and tested for optical density. The remaining solutions continued to incubate in the shaker at 37°C and 200 rpm. Cell growth

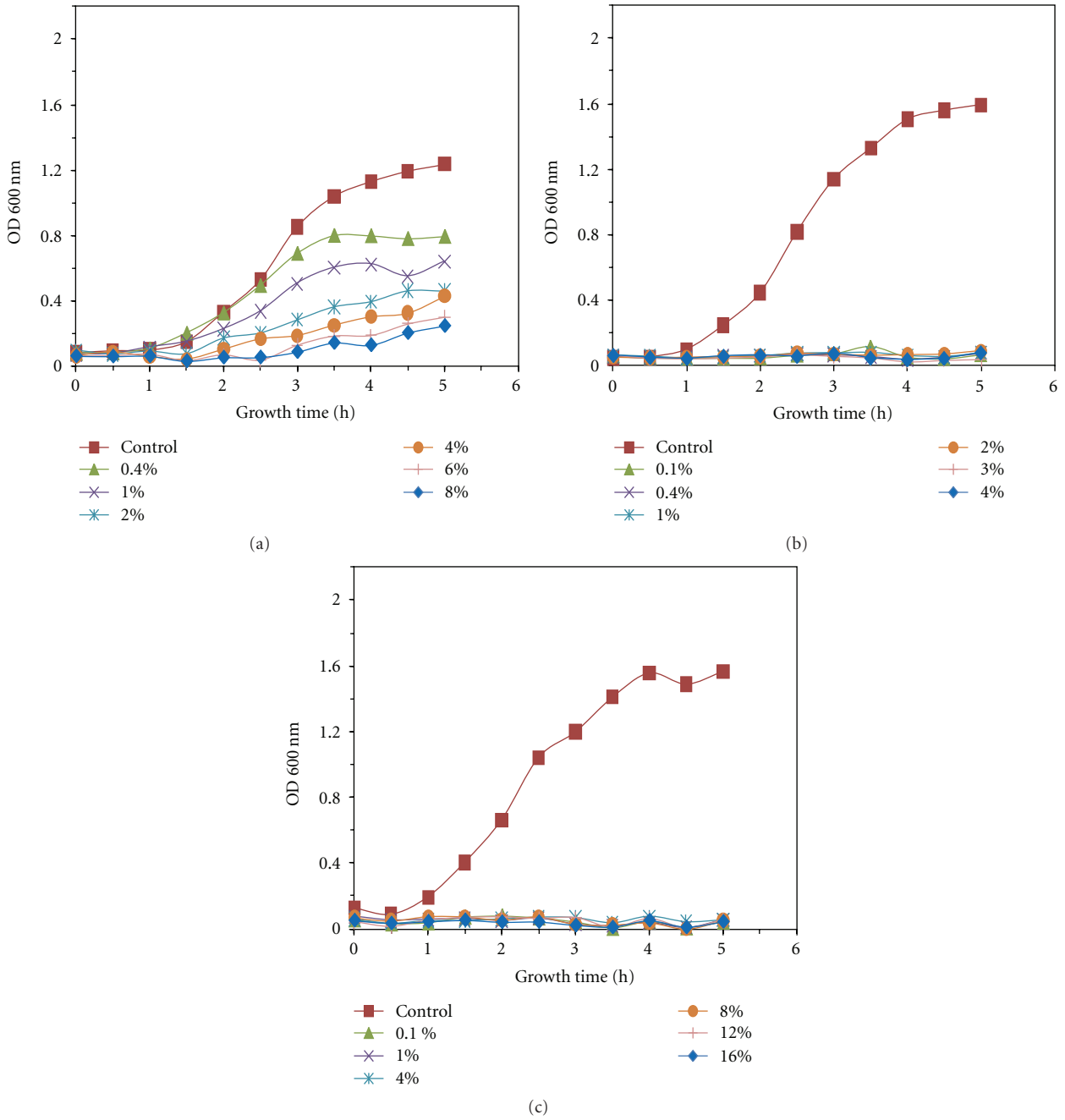

FIGURE 3: OD growth curves of *E. faecium* after treatment with surfactant-only solutions and incubation for 1 h: (a) treated with 0.4, 1, 2, 4, 6, and 8% SC; (b) treated with 0.1, 0.4, 1, 2, 3, and 4% SDBS, and (c) treated with 0.1, 1, 4, 8, 12, and 16% SDS. Other conditions are the same as in Figure 1.

was measured using a Beckman Coulter DU 520 spectrophotometer at 600 nm. Growth curves were created by plotting OD values versus time. The SWCNT-containing graphs were created by subtracting a blank containing the same SWCNT concentration as the experimental sample in order to create values consistent with the control that did not contain SWCNTs. After subtracting the SWCNT blank, absorbance was related to the quantity of cells. The time delay of exponential growth directly results from the initial viable bacterial cell number. Therefore, a delay in growth time indicates a lower initial viable cell number. This result means that a negative deviation from the control growth curve indicates antibacterial activity. All experiments were executed in triplicate.

3. Results and Discussion

3.1. Antibacterial Effects of Various Surfactants. Prior to the investigation on nanotube interactions with bacteria cells, we studied how various surfactants interacted with

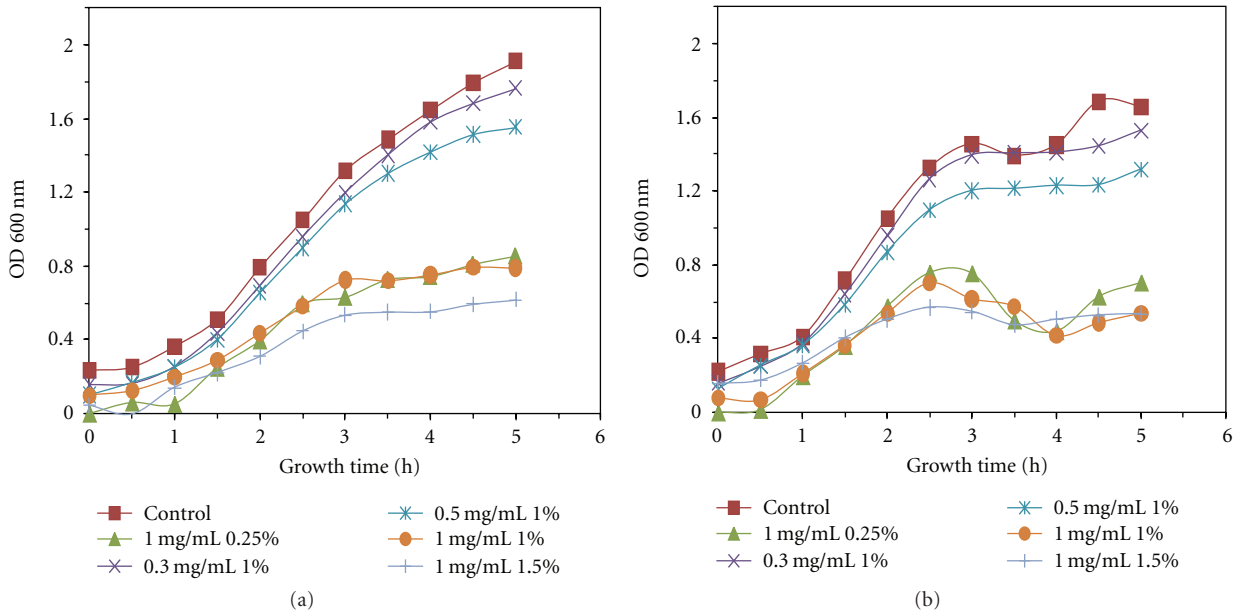

Figure 4: OD growth curves of (a) *S. enterica* and (b) *E. coli* after treatment with SWCNT + surfactant solutions and incubation for 1 h. The legend shows SWCNT concentration in mg/mL and SC surfactant concentration in %. A blank containing the same SWCNT concentration as the experimental sample was subtracted from each of the SWCNT-containing data sets in order to measure absorbance created by cells. Other conditions are the same as in Figure 1.

the *S. enterica, E. coli, and E. faecium* in the absence of SWCNTs. Figure 1(a) shows SC interaction with *S. enterica*. Sodium cholate displayed nearly complete killing of *S. enterica* at treatment concentrations of 12% and greater. Ten percent SC treatment delayed exponential growth for approximately 3 h. Eight percent SC delayed growth for approximately 1.5 h. Sodium cholate did not inhibit growth at 0.4% and 1% treatments. Figure 1(b) displays SDS interaction with *S. enterica*. All concentrations greater than 0.4% showed similar results, delaying growth for approximately 2 h. Interestingly, 1% SDS generated a slightly enhanced antibacterial effect compared to other concentrations. *S. enterica* in SDBS is displayed in Figure 1(c). One percent and greater SDBS treatments demonstrated complete bacterial killing. Levels lower than 1% did not deviate much from the control.

Figure 2(a) shows the SC effect on *E. coli*. Eight percent SC treatments demonstrated the strongest antibacterial activity while concentrations of 2% and smaller showed minimal antibacterial effects. *E. coli* in SDBS is given in Figure 2(b). Treatments with concentrations of 2% and greater provided complete killing, yet concentrations of 1% and lower caused minimal deviation from the control. Sodium dodecyl sulfate had similar effects on *E. coli* (Figure 2(c)) as it did on *S. enterica*. Each concentration, other than 0.1%, demonstrated comparable results, delaying exponential growth for approximately 3 hours.

Cultured *E. faecium* was much more sensitive to the surfactants than either *S. enterica* or *E. coli*. In SC (Figure 3(a)), the growth curves decreased in order of increasing surfactant concentration. Sodium dodecylbenzene sulfonate, shown in Figure 3(b), demonstrated complete effectiveness at all tested concentrations, and SDS also showed complete effectiveness at all tested concentrations (Figure 3(c)). Due to its vulnerability with our tested surfactants, no further experiments were conducted on *E. faecium*.

3.2. Antibacterial Effects of SWCNTs. Based on the results above, SC proved to be the best candidate to investigate antibacterial effects of SWCNTs since it contained the highest surfactant concentration without inhibiting bacterial growth. A treatment concentration of 1% was selected due to its ability to disperse SWCNTs effectively yet not inhibit bacterial cell growth. This indicates that all or almost all of the growth inhibition created by SC + SWCNT solutions is due to carbon nanotube activity. In addition to using 1% SC solutions, we also included a 0.25% SC trial in order to verify how different surfactant concentrations affect SWCNT activity through increased or decreased dispersion. Figure 4(a) displays *S. enterica* tested in SC solutions with varying SWCNT concentrations. The growth curves decreased in order of increasing SWCNT concentration. Interestingly, the solutions of 1 mg/mL SWCNTs + 0.25% SC and 1 mg/mL SWCNTs + 1% SC showed similar curves. These results suggest that concentrations of 0.25% SC were able to disperse 1 mg/mL SWCNTs as well as 1% SC. Figure 4(b) shows *E. coli* tested in SC solutions with varying concentrations of SWCNTs. The growth curves decreased in order of increasing SWCNT concentration and generated a curve similar to that of *S. enterica* in the solutions containing 1 mg/mL SWCNTs + 0.25% SC and 1 mg/mL SWCNTs + 1% SC. A plateau effect is seen in these trials in which higher concentrations of SWCNTs cause the growth curve to plateau at lower absorbance values. This observation suggests that SWCNTs limit cell growth via a concentration-dependent mechanism.

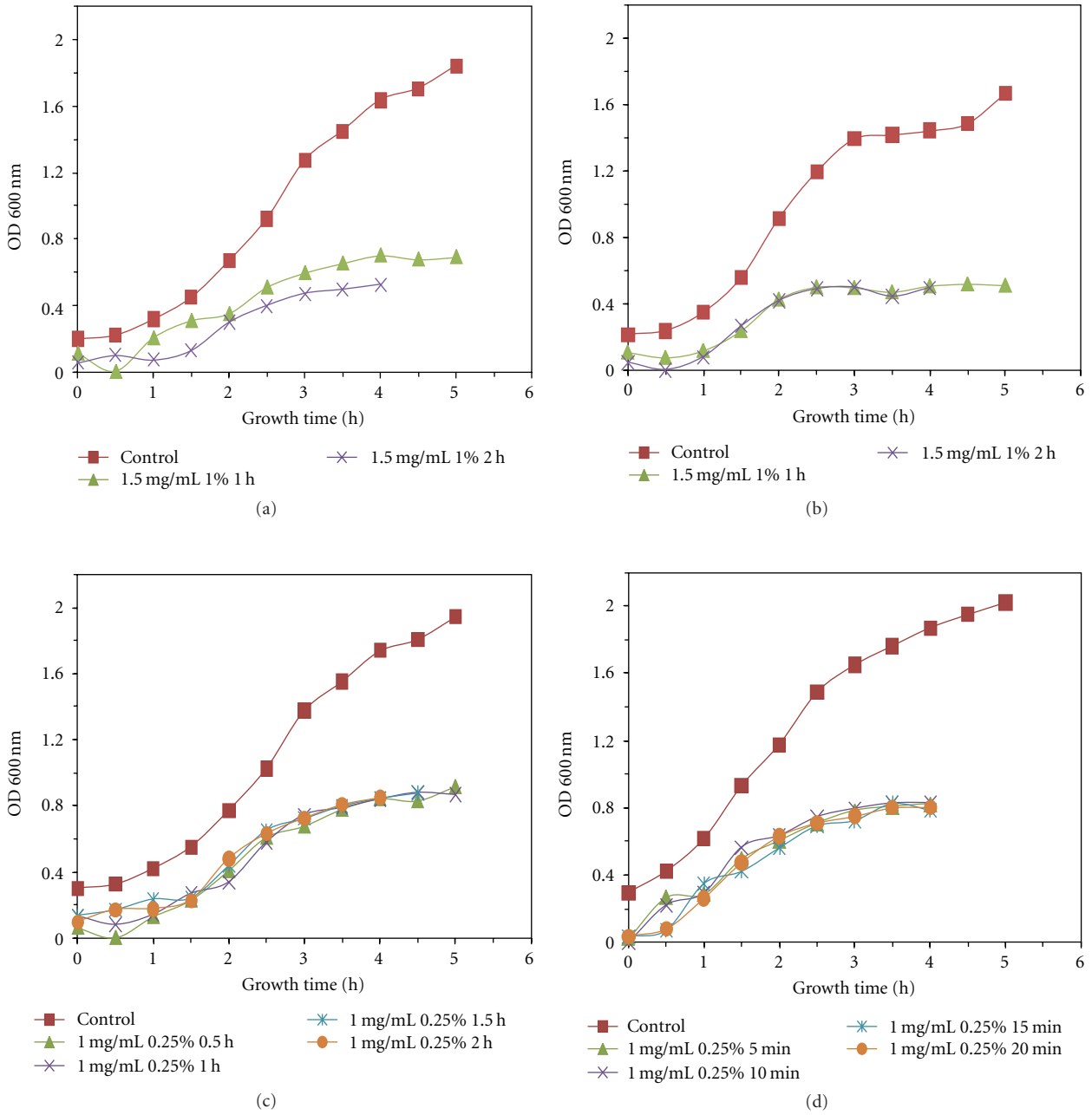

Figure 5: OD growth curves of bacterial cells after treatment with SWCNT + surfactant solutions. The legend shows SWCNT concentration in mg/mL, SC surfactant concentrations in %, and incubation times in h or min: (a) *S. enterica* and (b) *E. coli* treated with 1.5 mg/mL and 1% for 1 h and 2 h, (c) *S. enterica* treated with 1 mg/mL and 0.25% for 0.5 h, 1 h, 1.5 h, and 2 h, and (d) *S. enterica* treated with 1 mg/mL and 0.25% for 5 min, 10 min, 15 min, and 20 min. Nanotube blanks were created as in Figure 4. Other conditions are the same as in Figure 1.

Figures 5(a) and 5(b) show *S. enterica* and *E. coli* in solutions of 1.5 mg/mL SWCNTs + 1% SC with incubation times of 1 h and 2 h, respectively. There was no discernible difference in cell growth between the two incubation times. Figure 5(c) shows *S. enterica* in solutions of 1 mg/mL SWCNTs + 0.25% SC with incubation times of 0.5 h, 1 h, 1.5 h, and 2 h. These differences in incubation time had no effect on cell growth. Because 0.5 h incubation produced no difference in growth rate, a test was executed using identical SWCNT and SC concentrations with incubation times of 5 min, 10 min, and 15 min (Figure 5(d)). These results also show that incubation time is not a factor at the times tested, thus providing indication that SWCNTs produce an antibacterial effect quickly (<5 min). Growth curves in Figures 5(a)–5(d) also exhibit a lowered plateau as seen in Figures 4(a)-4(b), providing additional evidence that SWCNT concentration is the primary factor in producing the antibacterial effect.

4. Conclusions

Sodium cholate proved to be a desired surfactant with which we examine SWCNT antibacterial activity because it displayed the weakest inhibitory activity among broadly used surfactants. Sodium cholate did not provide complete bactericidal effects on *S. enterica* until the bacterium was treated with 12% SC in solution. By contrast, SDS and SDBS demonstrated total or nearly total effectiveness at 1% concentrations. Similar findings with *E. coli* indicated that SC can be used to disperse bundled SWCNTs into individual nanotubes and thereby examine SWCNT antibiotic ability. On the other hand, *E. faecium* is too sensitive to the surfactants to examine SWCNT implications. Results from the SWCNT tests indicate that nanotube concentration is the deciding factor in antibacterial effect. Incubation times ranging from 5 min to 2 h did not produce different results. It is promising to see the strong antibacterial effect of SWCNTs in solution with SC, because this same combination of materials proved to have low toxicity for 1321N1 human astrocytoma cells in our previous studies. Low toxicity to humans and high antibiotic effect make SWCNT-surfactant solutions relevant in biomedical applications and problems surrounding drug-resistant and multidrug-resistant bacterial strains. Further studies are required to test the legitimacy of a SWCNT-SC mixture and understand the mechanisms which could explain both low human toxicity and high antibacterial effect.

Acknowledgments

This work was partially supported by a Summer Faculty Fellowship from Missouri State University, the Cottrell College Science Award from Research Corporation for Science Advancement, and the American Chemical Society Petroleum Research Fund (47532-GB10). The authors thank Dr. Michael M. Craig for helpful discussions and editing.

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