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ORIGINAL ARTICLE

A Study on Dispersion and Antibacterial Activity of Functionalizing Multi-walled Carbon Nanotubes with Mixed Surfactant

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Abstract The dispersibility in aqueous phase and antibacterial activity of multi-walled carbon nanotubes (MWNT) with mixed surfactant functionalization has been studied. The ratio of 3:7 between hexadecyltrimethylammonium bromide and octylphenol ethoxylate (TX100) showed the highest dispersing power for MWNT. The use of mixed surfactants formed stable MWNT dispersions at lower total surfactant concentration compared to their concentrations when used alone. UV–Vis spectroscopy, transmission electron microscopy and Fourier transform infrared spectroscopy were employed to characterize the dispersion of MWNT in the aqueous phase. The result indicated that the surfactant molecules had been successfully adsorbed onto the surface of the MWNT. Bacterial toxicity assay showed that the mixed surfactant-functionalized MWNT had a strong antibacterial activity and concentration dependence to *Staphylococcus aureus*. Based on the consideration of the cost and environmental impact, the use of mixed surfactant (CTAB-TX100) should be more favorable for the stable dispersion of MWNT and the improvement of antibacterial activity than that of an individual surfactant. These observations suggested that the mixed surfactant-functionalized MWNT might be a promising antibacterial agent for removal and inactivation of biological contaminants in water treatment applications.

Keywords Multi-walled carbon nanotubes · Mixed surfactant · Dispersibility · *S. aureus* · Antibacterial activity

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Introduction

In the field of water treatment, a great deal of attention has been given to the use of carbon nanotubes (CNT) due to their effectiveness against biological contaminants [1–5]. Kang et al. first reported that highly purified single-walled CNT (SWNT) had strong antibacterial activity against *E. coli K12* [6]. Simultaneously, they found that SWNT were more toxic than MWNT, indicating that the diameter played an important role in the bactericidal effect [7]. In addition, dispersibility of CNT in aqueous solution was reported to have a direct effect on the antibacterial activity because the highly dispersed CNT could provide better surface contact with bacterial cells [8].

In our previous studies, bundled MWNT with an average diameter of 15 nm did not show any obvious antibacterial activity against *Streptococcus mutans* (*S. mutans)* [9, 10]. But through functionalization with surfactants, both the dispersing power of MWNT in aqueous solution and antibacterial activity were enhanced. Indeed, the principle advantage of functionalizing MWNT with surfactants was that it increased the bacterial aggregation potential of MWNT. The bound surfactant molecules on CNT surfaces could effectively interact with bacterial cells, thereby producing an antibacterial effect.

For interaction of surfactants with bacterial cells, cationic surfactants like hexadecyltrimethyl ammonium bromide (CTAB) have been extensively studied. Given the fact that most bacterial cell walls are negatively charged, the cationic head group of CTAB can bind to the oppositely charged bacterial cell wall by electrostatic interaction, thereby destroying the cellular membrane structure and leading to death of the bacteria [11, 12]. Although CTAB itself possess an inherent toxicity to bacteria, its dispersing power for MWNT is limited based on our previous research. Herein, in order to further improve MWNT' dispersibility in aqueous solution and antibacterial activity, simultaneously taking into account reducing the usage amount of surfactants, introduction of mixed surfactants might be a possible solution. In this work, cationic surfactant CTAB was mixed with a non-ionic surfactant, octylphenol ethoxylate (TX 100). The dispersing power for MWNT in aqueous solution was investigated. On the other hand, a systematical study on the antibacterial activity of mixed surfactant-functionalized MWNT was also reported by optical density (OD) growth curve measurements and determination of colony-forming units (CFU) assay.

Experimental Section

Purification of MWNT

MWNT supplied by Chengdu Organic Chemicals Co. Ltd synthesized by the chemical vapor deposition (CVD) method were used in this study. The average diameter was 15 nm. The purification procedure was as follows. First, MWNT were burned at 723 K for 90 min under atmospheric pressure. Then, the burned product was transferred to a flask with 6 M HCl and treated for 12 h to remove the metal catalyst. Subsequently, the acid solution was filtered through a membrane filter and the filter cake was rinsed with deionized water (DW) until the supernatant was pH = 7. Finally, the samples were dried at 333 K for 24 h under vacuum.

Preparation of Surfactant-Functionalized MWNT

CTAB and TX100 were supplied by Sinopharm Chemical Reagent Co., Ltd. Their chemical structures are shown in Fig. 1. For mixed surfactant system, CTAB was mixed with TX 100 at the total surfactant concentration of 0.43 mM. In order to obtain the optimum ratio in the surfactant mixture to produce stable dispersion for MWNT, the process was carried out by changing the surfactant ratio in the mixture. The ratio was 1:9, 3:7, 5:5, 7:3 and 9:1 corresponding to CTAB: TX 100, respectively.

In a typical procedure, 15 mg purified MWNT (p-MWNT) were added to 100 mL of the individual surfactant or surfactant mixture solutions with ultrasonication for 4 h to obtain the surfactant-adsorbed MWNT. The obtained suspension was then centrifuged at 8000 rpm/min for 30 min. For UV–vis characterization, the upper 50 % of the obtained suspension was collected for an examination of absorbance. In other cases, the suspension was filtered using a membrane filter with a pore size of 0.22 μm. The filtered cake was then rinsed two times with DW and dried in a vacuum at 333 K for 24 h prior to use.

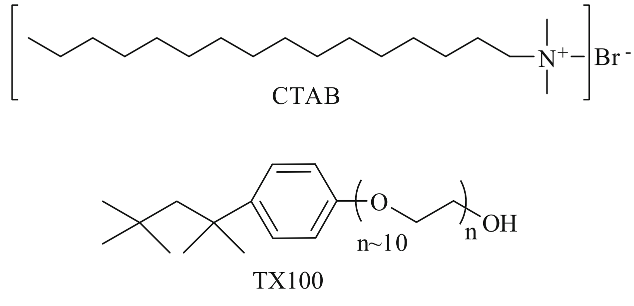

Fig. 1 The chemical structures of the surfactants used in this study

Characterization of Surfactant-Functionalized MWNT

The dispersion of MWNT in the individual surfactant or surfactant mixture solutions was determined using a UV-6300 spectrophotometer (Mapada Co., China) operating from 200 to 800 nm. For TEM (FEI Tecnai G20, USA) observation of the surfactant-adsorbed MWNT, the samples were redispersed in ethanol at a concentration of 0.05 mg/mL. One drop of the above suspension was poured onto a copper grid and examined by TEM. FT-IR measurement was carried out on a Nicolet 870 spectrophotometer (USA) in KBr pellets.

Preparation of Bacterial Cells

Staphylococcus aureus was grown in a 7.5 % NaCl broth. The NaCl broth was produced by adding 3.0 g beef powder (Beijing Aoboxing Bio-tech Co., China), 10.0 g peptone (Beijing Aoboxing Bio-tech Co., China), 75.0 g NaCl (Sigma-Aldrich Co., USA) to 1000 mL DW and controlling the pH value at 7.4. The incubation of bacterial cells was carried out at 310 K under aerobic conditions and harvested in the mid-exponential growth phase. The cells were washed twice and re-suspended in saline solution (0.9 % NaCl) to remove the residual macromolecules and other growth medium constituents.

Treatment of Bacterial Cells by Surfactant-Functionalized MWNT

First, 150 μL of the bacterial cell suspension was transferred into 1.5-mL microcentrifuge tubes, and 50 μL of the surfactant-functionalized MWNT' suspensions at the desired concentration was added to the tubes. 150 μL of the cell suspension and 50 μL of DW were used as controls. In comparison, the unfunctionalized MWNT' suspensions were also evaluated. The tubes were shaken at 170 rpm for 60 min using a universal shaker at room temperature.

Optical Density Growth Curve Measurement

After 60 min, surfactant-functionalized MWNT treatment, the mixture of cells and surfactant-functionalized MWNT was transferred into the NaCl broth, and incubated at 310 K. Cell growth was monitored by measuring the OD at 600 nm every 2 h using a spectrophotometer (Epoch 2, Bio-Tek Co., USA). The growth curves were generated via plotting OD values versus growth time.

Determination of Viable Cell Number by Plating Method

For CFU formation assays of cells treated by the surfactant-functionalized MWNT, a 100-μL sample of each treatment was used. Upon serial dilution, the diluted samples were spread evenly onto solid agar plates for aerobic incubation at 310 K for 48 h, and the colonies were then counted. The solid agar plate was prepared by dissolving 20 g agar powder (Sigma-Aldrich Co., USA) into 1000 mL 7.5 % NaCl broth, then sterilized at 120 °C for 25 min. When the temperature was down to about 50 °C, the sterilized solution was transferred to a 10.0-cm diameter cell culture dish (Sigma-Aldrich Co., USA) and cooled.

Field Emission Scanning Electron Microscopy (FE-SEM) Imaging

The samples were collected on a membrane (0.22 μm in pore size), fixed with 2.5 % glutaraldehyde and post-fixed in 1 % osmium tetroxide. The samples were then dehydrated in a graded series of ethanol (30, 50, 70, 80, 90, 95 and 100 % v/v) and dried at room temperature. The cells were sputtered with gold and viewed by FE-SEM (Quanta FEG 650, USA).

Results and Discussion

Dispersing Power of Surfactant-Functionalized MWNT

It was well-known that pristine CNT, existing mostly in bundles due to van der Waals forces between graphic nanotube surfaces, are insoluble in aqueous media [13, 14]. However, through absorbing surfactant molecules on the tubular surface of CNT, the dispersibility could be significantly improved. The evaluation of the dispersion degree of CNT in water phase could be achieved by measuring the UV–Vis spectrum of the dispersion solution because only individual CNT absorb in the UV–Vis region whereas bundled CNT are not active in this region [15–17]. Based on the fact that higher UV–Vis absorbance is a measure of better dispersion of MWNT in aqueous solution, we compared the UV–Vis absorption spectra of MWNT dispersed in the CTAB-TX 100 surfactant mixture with five different ratios (1:9, 3:7, 5:5, 7:3 and 9:1). The results are shown in Fig. 2. The ratio of 3:7 (curve a) showed a higher dispersing power for MWNT than the other four ratios (curve b–e).

Figure 2 also shows a comparison of MWNT dispersed in mixed and individual surfactant solutions. Curves (f, g, h) are the UV–Vis absorption spectrum of MWNT dispersed in TX100, CTAB surfactant solution and pure water, respectively. It was evident that the dispersibility of MWNT with surfactant is higher than that in the absence of surfactants (Curve h). With the MWNT treated by TX100 and CTAB surfactants, the dispersibility of TX100 surfactant (Curve f) was higher than that of CTAB surfactant (Curve g), which was in accordance with previous reports [9].

It was apparent that the surfactant mixture is more effective at dispersing MWNT than the individual surfactants, indicating that the mixed surfactant system had more significant surface activity than the individual surfactant. This also implied the existence of synergistic interaction for the mixed surfactant CTAB-TX100. It has been reported that for ionic-nonionic mixed surfactant systems, the synergistic nature of the mixture induces a decrease in the CMC owing to the reduction in electrical repulsion between ionic heads of the original ionic surfactant as well as mixed micelle formation between the ionic and nonionic surfactant [18]. The reduction in CMC helps to disperse MWNT at lower total surfactant concentration as compared to the individual surfactants used alone.

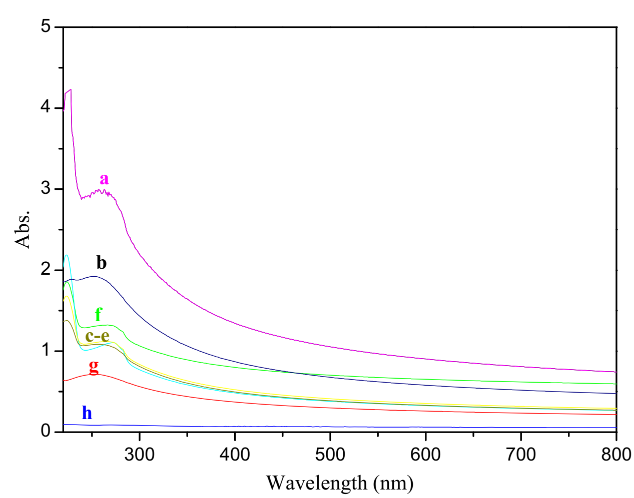

Fig. 2 UV-Vis absorption spectra of MWNT dispersed in the following different surfactant solution: mixed surfactant (CTAB-TX100) with the ratio of (*a*) 3:7, (*b*) 9:1, (*c*) 5:5, (*d*) 7:3, (*e*) 1:9; (*f*) TX100; (*g*) CTAB; (*h*) control sample (without surfactant)

The dispersion stability of CNT in aqueous solution is closely associated with the adsorption ability of the surfactants. The different adsorption capacity of the surfactants on CNT surface could be attributed to differences in chemical structure. It has been reported that surfactant adsorption has a positive correlation with the number of aromatic rings in the surfactants. The $\pi-\pi$ electron donor–acceptor interaction is an important mechanism for the adsorption of aromatic ring-containing chemicals to CNT [19–21]. Therefore, the higher dispersing performance of TX100 was likely due to the presence of an aromatic unit, which enhances the attraction to the MWNT surface.

Figure 3 shows typical TEM images of p-MWNT and surfactant-functionalized MWNT. A high degree of CNT aggregation can be clearly seen, with large aggregates dominating as shown in Fig. 3a. After functionalizing with surfactants, however, individually dispersed MWNT can be observed as shown in Fig. 3b–d. It could be seen from Fig. 3d that the mixed surfactant-functionalized MWNT showed good dispersion compared with the individual surfactant-functionalized system using a higher concentration of CTAB and TX100. This implied that the mixed surfactant system was more efficient at stabilizing MWNT dispersion owing to its synergistic behavior.

FT-IR spectroscopy is a valuable tool for detecting chemical moieties present on the surface of MWNT. Figure 4a shows the FT-IR spectrum of a p-MWNT. There is no clear evidence of functionalized moieties present on the surface of the p-MWNT. In comparison with the spectrum of the p-MWNT, however, Fig. 4b–d shows the characteristic absorption peaks of TX100 and CTAB on MWNT. For instance, the peaks at 2845–2970 cm^{-1} were due to the stretching vibrations of $-CH_2$ and $-CH_3$ groups. The bands between 1050 and 1130 cm^{-1} are associated with the C–X (C–O, C–N) vibrations. This clearly confirms that the functionalized moieties of surfactant molecules adsorb onto the MWNT surfaces, indicating an interfacial interaction between the nanotubes and the surfactants.

Antibacterial Activity of Mixed Surfactant-Functionalized MWNT

Adsorption is one of the simplest techniques that can be used for removal of biological contaminants from water media. Activated carbon is widely used as an adsorbent owing to its porous structure and relatively large surface area [22]. Generally, activated carbon offers only adsorption or capture of bacteria, but does not deactivate bacteria.

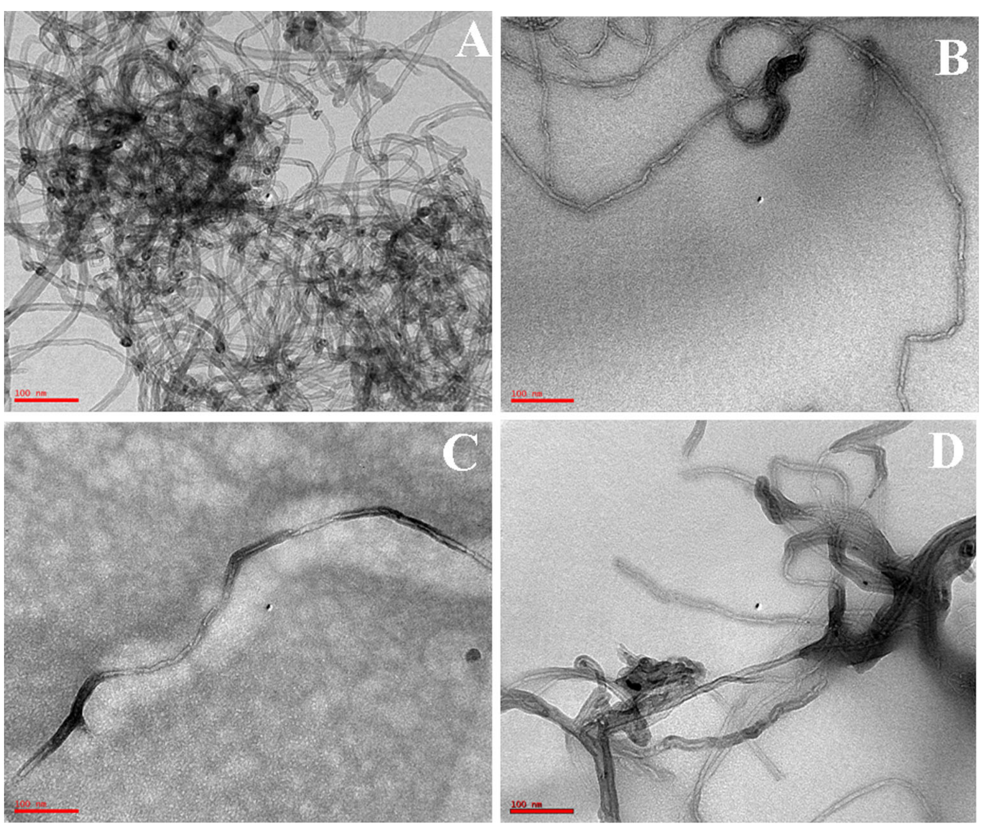

Fig. 3 TEM images of p-MWNT (**a**) and MWNT functionalized by the following different surfactants: **b** TX100, **c** CTAB and **d** mixed CTAB-TX100 (3:7) surfactant. The *scale bar* represents 100 nm

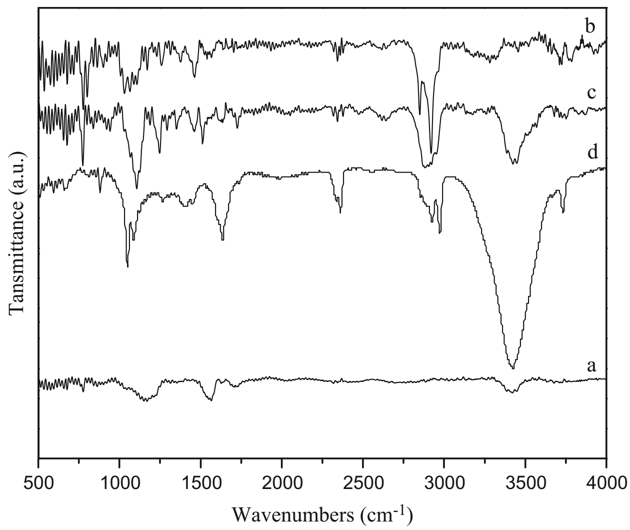

Fig. 4 FT-IR spectra of p-MWNT (*a*) and MWNT functionalized by the following different surfactants: (*b*) CTAB, (*c*) TX100 and (*d*) mixed CTAB-TX100 (3:7) surfactant

In comparison with activated carbon, however, CNT can provide simultaneous capture and deactivation of bacteria. The deactivated efficiency depends on many factors, such as the size and length of the tubes, dispersivity, impurity content and number of layers.

Among the different factors, dispersivity is a crucial parameter because highly dispersed CNT exhibited greater affinity toward bacteria and facilitate bacterial cell contact. The dispersivity of CNT in aqueous solution can be improved with simple surfactant functionalization. As observed in this study, the mixed surfactant system (CTAB-TX100) for functionalization of CNT was more efficient than the individual surfactant systems. The antibacterial activity of mixed surfactant-functionalized MWNT had been investigated by examining their OD growth curves. The result was shown in Fig. 5 where the mixed surfactant-functionalized MWNT exhibited certain antibacterial activity to *S. aureus*. Moreover, the antibacterial activity was concentration-dependent. When the concentrations of mixed surfactant-functionalized MWNT was 1.0 mg/mL, no obvious cell growth was observed within 10 h, suggesting that the high concentration of mixed surfactant-functionalized MWNT could kill almost all of the bacterial cells in the sample. However, with decreasing concentration, the time needed to reach exponential phase growth decreased. A longer delay time is associated with a lower number of surviving cells and stronger antibacterial activity. The growth time of the cells was delayed by approximately 6, 4 and 2 h corresponding to the treatment of mixed surfactant-functionalized MWNT at concentration of 0.5, 0.25 and 0.125 mg/mL, respectively.

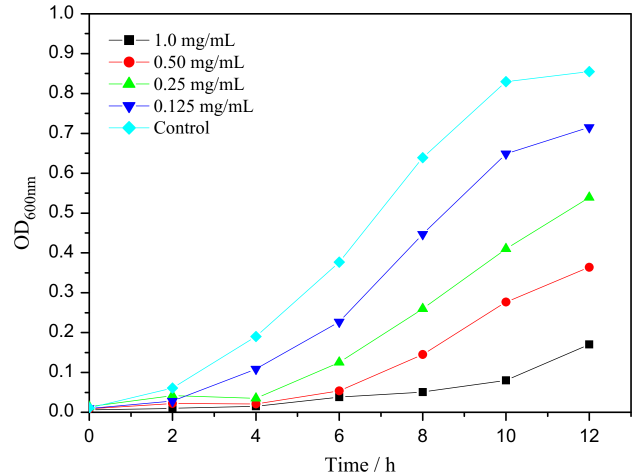

Fig. 5 OD growth curves of *S. aureus* after being treated with the mixed CTAB-TX100 (3:7) surfactant -functionalized MWNT at different concentrations. Cells treated with DW served as a control

Figure 6 shows a comparison of the OD growth curves of *S. aureus* treated by p-MWNT, individual surfactant-functionalized MWNT, mixed surfactant- functionalized MWNT and surfactant solution alone at a concentration of 0.5 mg/mL for 60 min prior to their growth in NaCl broth. It can be observed that the unfunctionalized MWNT show similar exponential growth as the control sample, implying that MWNT without surfactant functionalization did not have any significant antibacterial activity.

This result is consistent with the CFU reduction assay, as shown in Fig. 7 where the viable cell number did not show any significant reduction. With treatment by individual surfactant-functionalized MWNT, CTAB-functionalized MWNT exhibit a higher inactivation to *S. aureus* than TX 100-functionalized MWNT, indicating that the

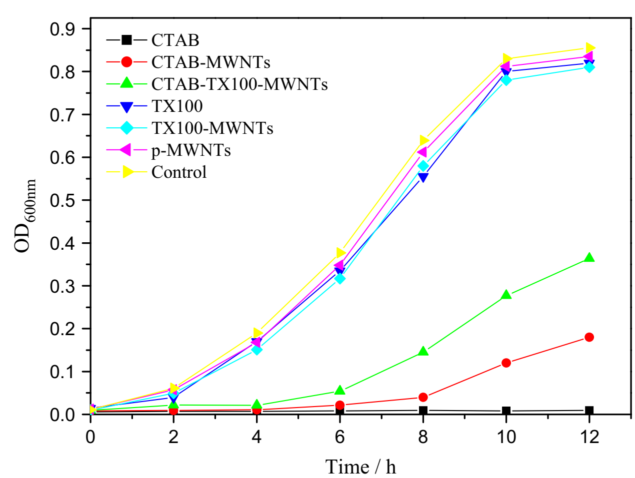

Fig. 6 OD growth curves of *S. aureus* after being treated with the different agents at a concentration of 0.5 mg/mL for 60 min. Cells treated with DW served as a control

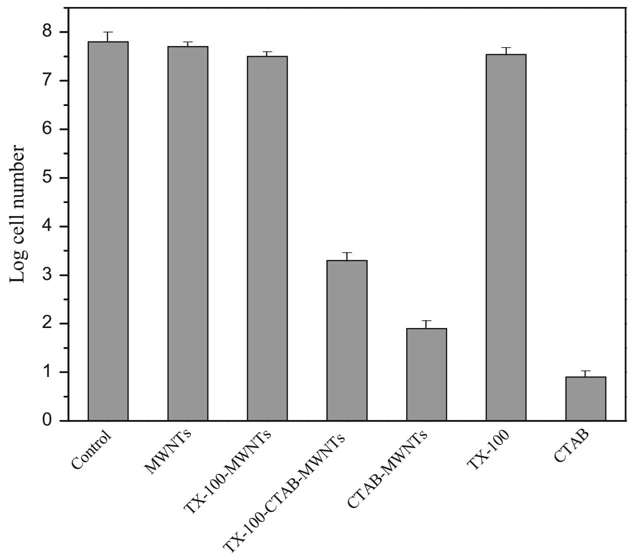

Fig. 7 Logarithmic value of the number of viable cells after being treated with the different agents at a concentration of 0.5 mg/mL for 60 min. Cells treated with DW served as a control

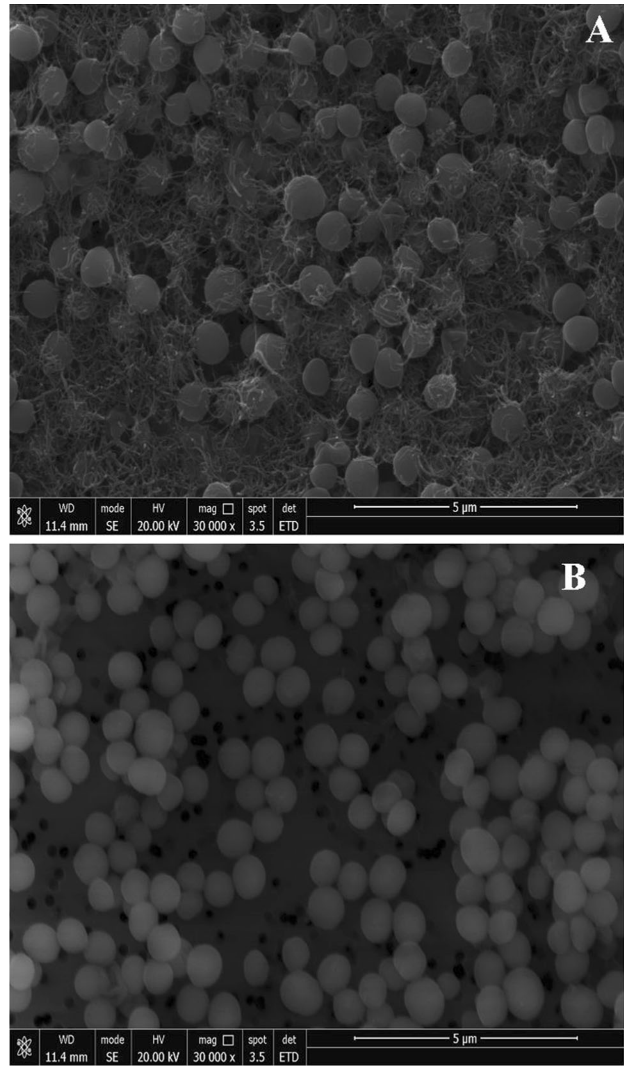

Fig. 8 FE-SEM images of the bacterial cells treated with mixed CTAB-TX100 (3:7) surfactant (**a**) and non-treated bacterial cells (**b**)

antibacterial efficiency of surfactant-functionalized MWNT was closely related to that of adsorbed surfactant molecules. According to published literature, CTAB, being a cationic surfactant/quaternary ammonium compound (QAC), could cause cell membrane rupture by chemical reactions and electrostatic effects [23]. The antibacterial action of QAC is attributed to its positive charge, which allows for formation of electrostatic bonds with negatively charged sites on the bacterial cell walls. These electrostatic bonds created stress in the cell wall, leading to cell lysis and death. Whereas TX100, a non-ionic surfactant, is widely used for cell membrane solubilization and shows no pronounced toxicity toward bacterial cells, which is consistent with our results presented in Figs. 6 and 7. After 60 min treatment with 0.5 mg/mL CTAB surfactant solution, no obvious cell growth was observed within 12 h and the number of viable cells was reduced by 6.9 log. While in the case of the TX100 surfactant's treatment, the cells acted very similar to the control sample and only 0.26 log reduction was achieved, suggesting that TX100 surfactant itself had no significant antibacterial activity to *S. aureus*.

As mentioned previously, the mixed surfactant-functionalized MWNT not only form a stable dispersion in aqueous phase, but also show a strong inactivation to bacterial cells. The result was also shown in Figs. 6 and 7 where the cells treated with 0.5 mg/mL mixed-surfactant functionalized MWNT delayed their growth time for about 6 h and achieved 4.5 log reduction in viable cell number. Although the antibacterial activity of mixed surfactant-functionalized MWNT was slightly lower than that of individual CTAB-functionalized MWNT at the same concentration, the dispersing power of mixed surfactant-functionalized MWNT in aqueous solution is significantly higher than that of individual surfactant-functionalized MWNT.

From an economic point of view, the use cost of mixed CTAB-TX100 surfactant is lower than that of individual CTAB surfactant because of the relatively higher price of CTAB. The difference in CTAB and TX100 price is at least 3 times. The high dispersibility could provide more opportunity for MWNT to contact/capture bacterial cells, forming MWNT-cell aggregates which precipitate out of solution, which undoubtedly plays a role in purifying the water phase, namely removing bacteria. When the surfactant-functionalized MWNT come in contact with cells, the bound surfactant molecules (CTAB) on the surface of MWNT can interact with the cell membrane, ultimately causing cell lysis and death, which actually plays a disinfecting role, namely killing bacteria. These results were further confirmed by FE-SEM images. Figure 8a shows

FE-SEM images of *S. aureus* interacting with the mixed surfactant-functionalized MWNT. It can be seen that the bacteria are entangled by the CNT in a spider weblike morphology. Furthermore, some cellular integrity is destroyed after treatment with the mixed surfactant-functionalized MWNT at 0.5 mg/mL for 60 min. As a comparison, the morphology of cells without MWNT treatment is shown in Fig. 8b where the integrity of the membrane structure was maintained.

Conclusions

The mixed system of cationic surfactant CTAB and nonionic surfactant TX100 was found to form stable MWNT dispersions. The ratio of 3:7 (CTAB: TX100) showed the highest dispersing power for MWNT owing to synergistic interactions. UV–Vis and FT-IR spectra showed that the surfactant molecules had successfully absorbed on the surface of the MWNT, indicating the simplicity and efficiency of employing surfactants for dispersing MWNT. The determination of the OD growth curves and viable cell number showed that the mixed surfactant-functionalized MWNT had strong antibacterial activity to *S. aureus*. Based on cost and environmental impact considerations, the use of mixed surfactant (CTAB-TX100) is more favorable for forming stable MWNT dispersions and improvement of antibacterial activity than that of an individual surfactant. These results suggested that the mixed surfactant-functionalized MWNT might be a promising antibacterial agent for removal and inactivation of biological contaminants in water treatment applications.

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