Title: Efficient Surface Modification of Carbon Nanotubes for Fabricating High Performance CNT Based Hybrid Nanostructures

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Abstract:

Carbon nanotubes (CNTs) were chemically modified to achieve strong binding strength with the attached functional components as well as good dispersability and nanoparticle size-uniformity. An efficient multi-oxidation process was developed to create porous out layer with many nanoscale defects on the surface of CNTs for metallic nanoparticle close attachment and bond sufficient oxygen-containing groups, which assisted the dispersion of CNTs in the aqueous solution. The surface modified CNTs have advantages of strong binding capability, large surface area, high mechanical strength and good dispersability, which show great potential as building blocks for hybrid nanomaterials. Monodispersed silver nanoparticles with an average size of 3 nm were formed from inside the created nanoscale defects on the surface of CNTs without any obvious agglomerations. The fabricated hybrid exhibited much enhanced anti-bacterial performance compared to commercial silver nanoparticles due to the combined anti-bacterial effects of CNTs and silver nanoparticles. With these superior properties, the developed surface modification process could be widely

used for improving the performances of many CNT based hybrid nanomaterials in diverse applications.

1. Introduction

Novel hybrid nanomaterials with desirable structures and properties have attracted considerable attention in many fields ranging from energy storage to catalysis, sensing, and medical treatments due to their unlimited possible combinations of distinct properties of inorganic, organic, or even bioactive materials [1–8]. Among all the alternatives, carbon nanotubes (CNTs), based on their special one-dimensional structures, large surface areas, and excellent physical and chemical properties, are believed to be promising building blocks for constructing hybrid nanomaterials [9,10]. So far, a vast number of CNT based hybrid nanomaterials with multiple functions have been developed and applied in many fields. For example, CNTs coated with highly conductive metal nanoparticles for improving the electrical and mechanical properties of electrode materials [11]. CNTs deposited with catalytic nanoparticles, such as Pt, Pd and Ru, as high-performance catalysts for applications in fuel cell electrodes, Li-ion batteries and supercapacitors [12–14]. Silver nanoparticle decorated CNT hybrid nanomaterials can improve the antibacterial activity of textile fibers [15]. Those successful applications indicated the great potential of CNT based hybrid nanomaterials as novel functional agents to improve material's performance. However, there are some serious challenges that limit the performance of CNT based hybrid nanomaterials, including uneven structures, agglomerations, short lifetime and poor reliability [16–19]. Importantly, the short lifetime and poor reliability are mainly caused by the weak bonding strength between CNTs and attached functional components. Due to the strong chemically inert graphitic surface of CNTs, functional components can be easily detached from the surface of CNTs under small structural deformations and lead to the failure of hybrid nanomaterials [11,20]. Therefore, to address these challenges and improve the performance of CNT based hybrid nanomaterials, it is essential to improve the bonding strength between CNTs and attached functional components

In this work, we developed a multi-oxidation process to modify the original chemically inert graphitic surface of CNTs for close bonding with silver nanoparticles. The key benefit of the multi-oxidation process lies in its abilities to create a porous out layer with abundant nanoscale defects and also bond large amount of oxygen-containing groups on the surface of CNTs. These surface modifications of CNTs facilitate the penetration of silver cations into the deep layer of CNTs and form silver nanoparticles that closely bonded with the CNT surface.

Moreover, the oxygen containing groups can offer excellent dispersability and stability to the final hybrid. According to different material characterization results, the multi-oxidation process also demonstrated much higher activation efficiency than traditional acid etching processes [21,22]. The growth of silver nanoparticles was carried out in a silver complex solution which can maintain a stable reaction speed within a certain period. Morphology characterization has shown that silver nanoparticles with an average size of 3 nm are uniformly grown around the surface of CNTs without any obvious agglomerations. The fabricated hybrid exhibited much enhanced anti-bacterial properties compared to commercial silver nanoparticles due to the combined anti-bacterial effects of hybrid nanostructures as well as good reliability.

2. Experimental

2.1. Materials

CNTs with a mean diameter of 100 nm and an average length of 9 μm were obtained from Sigma Aldrich and rinsed in both acetone and de-ionized water before use. Unless otherwise stated, CNTs in this work refer to multi-walled carbon nanotubes. Silver nanoparticles (Ag NPs) with an average diameter of 5 nm were purchased from nanoComposix. Sodium nitrate (99%), potassium permanganate (99%), sulfuric acid (98%), hydrogen peroxide (30%), tin(II) chloride (98%), silver nitrate (99%), ammonium hydroxide solution (28%), ammonium sulfate (99%), ethylenediamine (EN) (99%), ethylenediaminetetraacetic acid (EDTA) (99%) and cobalt(II) sulfate heptahydrate (99%) were purchased from Sigma Aldrich. Luria-Bertani (LB) medium (10g/L tryptone, 10g/L NaCl and 5g/L yeast extract) is used for *E.coli* bacteria growth. Other reagents and solvents were used as received and without further purification.

2.2. Fabrication of M-CNTs and A-CNTs

Initially, 0.5 g of CNTs was mixed with 0.375 g of NaNO_3 , 1.5 g of KMnO_4 and 23 mL of concentrated H_2SO_4 in a container placed into an ice-water bath. Immediately, the reactor containing the above solution was transferred into an ultrasonic bath. The surface modification of the CNTs was performed in an ultrasonication bath for 30 min, and the temperature in the bath was kept in the range of 30-40°C. After that, the solids were separated from the solution by using centrifugation at 5000 rpm for 2 min, and then washed several times both with 30% of hydrogen peroxide and de-ionized water until the pH value of filtrate was close to 7.0. The remainder of the multi-oxidation solution was stored for future use. The CNTs modified by the multi-oxidation process are described as M-CNTs. A-CNTs refer to pristine CNTs treated in a mixed nitric acid and sulfuric acid (1:3, v/v) solution under

vigorous stirring for 4 hours at 80°C. Thereafter, A-CNTs were separated from the solution by using centrifugation and then washed several times with de-ionized water until the PH value of filtrate was close to 7.0.

2.3. Silver nanoparticle growth on the surface of M-CNTs

The silver nanoparticle growth solution consisted of two separate solutions, the aqueous silver ion source solution (9 mM AgNO₃, 5.9 M NH₄OH, 0.9 M (NH₄)₂SO₄, 13 mM EN, 14 mM EDTA) and the aqueous reducing agent solution (120 mM CoSO₄·7H₂O, 2.3 M NH₄OH, 165 mM EN, 80 mM EDTA), mixed in equal amounts prior to reaction. The reaction was carried out at room temperature with a mild stirring and the growth time ranged from 0.5 h to 24 h in order to determine the silver mass ratio as the function of reaction time. Silver coated M-CNT hybrids (M-CNT/Ag) were collected from the solution through vacuum filtration. To avoid oxidation of the cobalt complexes, the dissolved oxygen in de-ionized water was removed by an ultrasonication treatment, and deposition was performed in a closed vessel under a nitrogen atmosphere.

2.4. Anti-bacterial activity of M-CNT/Ag and Ag NPs

The anti-bacterial activity of M-CNT/Ag was compared to commercial silver nanoparticles (5 nm). Bacteriostatic and bactericidal activity of M-CNT/Ag and Ag NPs were analyzed by monitoring the growth of bacteria and time killing assay respectively. In order to examine the effect of M-CNT/Ag and Ag NPs on growth of bacteria, overnight-grown *E.coli* culture were sub-inoculated into fresh LB medium containing control samples, and samples of 12.5 µg/ml, 25 µg/ml and 50 µg/ml of M-CNT/Ag and Ag NPs respectively. The sub-inoculated *E.coli* culture was incubated at 37°C with continuous agitation and growth of bacteria was monitored by measuring the optical density at 600 nm at 1 h time intervals. For time killing assay, 2×10⁷-5×10⁷ inoculum from the overnight growth of *E.coli* was inoculated into fresh LB medium containing control samples, and samples of 12.5 µg/ml, 25 µg/ml and 50 µg/ml of M-CNT/Ag and Ag NPs respectively and were incubated at 37°C with continuous agitation. 100 µl were taken from the samples for the determination of viability of bacterial cells at time points of 0, 1, 2, 4, 8 and 24 h. Serial dilutions (10¹ to 10⁵) were prepared in 0.89% saline. The diluted sample (100 µL) was plated onto LB agar plates. The plates were incubated at 37 °C for 24 h, and the number of colonies was counted.

The anti-bacterial activity of M-CNT/Ag and Ag NPs on biofilm bacteria was also evaluated. *E.coli* UTI89 biofilm was formed on a 13mm tissue culture on a plastic coverslip (Sarstedt, Inc. Newton, USA). Briefly, plastic discs were placed in 24 well plates containing

LB broth with 2×10^6 - 5×10^6 cfu/ml of bacterial inoculum and incubated at 37°C for 24 h to allow the formation of biofilms. After 24 h of undisturbed biofilm formation, the old culture medium was replaced with fresh LB broth containing control samples, and samples of 12.5 µg/ml, 25 µg/ml and 50 µg/ml of M-CNT/Ag and Ag NPs respectively and incubated at 37°C for 24 h. The 24 h treated biofilms were dip washed and collected in 0.89% NaCl and homogenized by using a sonication probe for 10 seconds. The homogenized suspensions were diluted serially and plated on LB agar plate to count the colonies. For SEM, biofilms treated for 24 h were sequentially dip washed and fixed with 3% glutaraldehyde and dehydrated using series of graded ethanol (30, 40, 50, 60, 70, 80, 90 and 100%) for 15 min each. The dried biofilm were then sputter coated with gold before SEM imaging.

2.5. Characterization

Fourier transform infrared spectroscopy (FT-IR, PerkinElmer, UATR Two) and X-ray photoelectron spectroscopy (XPS, PerkinElmer, 5500) were used to identify the surface functionalization of CNTs. Raman microscopy (Raman, Horiba, XploRA) and X-ray diffraction (XRD, Philips X'Pert Materials Research Diffractometer) were used to identify the structural changes after surface activation. Surface area measurements were performed using the Brunauer-Emmett-Teller (BET) nitrogen absorption method on a TriStar 3000 V6.04 A surface area and pore analyzer. Pore size distribution was calculated by the Barrett-Joyner-Halenda (BJH) method using a desorption isotherm. The microstructures of CNTs were examined using transmission electron microscopy (TEM, FEI Tecnai, T20). Thermal gravimetric analysis (TGA, PerkinElmer, Pyris 1) was employed to investigate the impact of surface modification on the decomposition temperature of CNTs and to test silver mass ratio changes. All samples were heated from 50°C to 90°C in a dry air flow with a heating rate of 20°C/min. The dispersion properties of M-CNT and M-CNT/Ag were characterized by UV-Visible spectroscopy (Ultra violet-visible spectrometer, PerkinElmer, Lambda 900) with scanning wavelengths ranging from 200 to 800 nm, and the intensity at a wavelength of 417 nm was chosen for measuring concentration variation of the M-CNT/Ag dispersion. The morphology change of *E.coli* bacteria was characterized by SEM (JEOL JSM-6301F).

3. Results and discussions

3.1. Surface activation of CNTs

Fig.1 illustrates the activation mechanism of the CNT surface by a treatment with the multi-oxidation solution and ultrasonication as well as the following silver growth on CNTs. As shown, the surface modification of CNTs was carried out in a multi-oxidant solution

consisting of both an intercalating agent (H_2SO_4) and oxidants (KMnO_4 , NaNO_3) under low-power ultrasonication. Initially, the oxidation process started from the edge and surface defects of the pristine CNTs. These positions are more vulnerable to attack from the oxidants due to their incomplete sp^2 carbon structures. Consequently, many oxygen-containing groups, such as phenolic, ether, carboxyl and carbonyl groups, were generated at these places. With expansion of the defect area, sulfuric acid, as the intercalating agent, started to penetrate into the inner graphitic layers of CNTs and caused deep oxidation, which generated the porous out layer with large amounts of nanoscale defects. Besides the oxidation treatment, a low-power ultrasonication was also maintained during the whole modification process to increase the surface roughness of the M-CNTs.

Fig.1. Schematic of the multi-oxidation process of CNTs (multi-walled carbon nanotubes) and the following silver nanoparticle growth.

The multi-oxidation process was much more efficient at modifying the CNTs than traditional acid etching processes. To make a comparison, pristine CNTs were also treated by following the typical acid etching procedure reported in many publications [23,24]. Fourier-transform infrared spectroscopy (FTIR) analysis was employed to study the functional group changes on the M-CNT surface and compare the activation efficiency of different processes. As shown in Fig.2a, the spectrum of pristine CNTs shows some inherent characteristic peaks corresponding to oxygen-containing functional groups, such as epoxy, carbonyl and carboxyl, which most likely resulted from the production process used by the material supplier. By comparison, the spectrum of M-CNTs shows significant increases in oxygen-containing groups on M-CNT surfaces. For instance, the characteristic peaks at 1220 cm^{-1} and 1700 cm^{-1} corresponding to the breathing vibration of C-O and the stretching vibration of C=O from carbonyl and carboxyl groups became much more intense. A new strong and broad peak appeared in the range of $1400\sim 1536\text{ cm}^{-1}$, which represented the stretching vibration of C-O. In addition, two more characteristic peaks appeared at 2870 cm^{-1} and 2960 cm^{-1} , due to the stretching vibration of C-H. These results indicate the successful bonding of the oxygen-containing groups on the M-CNT surface. For A-CNTs, it shows a limited increase of oxygen-containing groups compared to the pristine CNTs, such as C-O and C=O groups at

1220 cm^{-1} and 1700 cm^{-1} . However, the intensities of these peaks are much smaller than that of M-CNTs, indicating that the traditional acid etching process only has a weak activation effect. In addition, the multi-oxidation process has a shorter treatment time (30 min) and lower temperature (30~40°C) compared to the acid etching process, demonstrating the high modification efficiency of the multi-oxidation process.

Fig.2. (a) FTIR spectra of pristine CNTs, A-CNTs and M-CNTs. (b) XPS curves of pristine CNTs, A-CNTs and M-CNTs. (c) TGA curves of pristine CNTs, A-CNTs and M-CNTs. (d) Raman spectroscopy of pristine CNTs, A-CNTs and M-CNTs. (e) XRD spectra of pristine CNTs, A-CNTs and M-CNTs. (f) Pore volume distribution of pristine A-CNTs and M-CNTs.

X-ray photoelectron spectroscopy (XPS) was used to quantitatively characterize the oxidation degree and carbon/oxygen ratios (C/O) in M-CNTs and A-CNTs. As shown in Fig.2b, pristine CNTs show a high purity without any metal atoms detected from the XPS spectra. The C/O ratio of pristine CNTs is about 98:2, indicating the existence of small amount of oxygen and defects. Compared to pristine CNTs, O1s peaks increased significantly in both M-CNTs and A-CNTs. The M-CNTs, in particular, showed the highest O1s peak intensity, and also the C1s peak of the M-CNTs became smaller and broader compared to the other two samples, indicating the bonding of sufficient oxygen functional groups. Furthermore, the C/O ratios in different samples are 98:2 (pristine CNTs), 90:10 (A-CNTs) and 70:30 (M-CNTs). Such a high ratio of oxygen in M-CNTs is essential for the homogeneous dispersion of the final hybrids due to the formation of hydrogen bonds between oxygen groups and water molecules. Thermal gravimetric analysis (TGA) was performed to show the effect of oxygen groups on the property change of CNTs. As shown in Fig.2c, all

samples were heated from 50 to 900°C in a dry air flow with a heating rate of 20 °C/min. It has been found that the decomposition temperatures of M-CNTs and A-CNTs were decreased from 800 to 330°C and 630°C respectively when compared with pristine CNTs. The significant decrease of decomposition temperature of M-CNTs is due to the deep oxidation caused by the multi-oxidation process. Defects and oxygen-containing groups make the CNT structure less stable during heating. Moreover, as a result of burning, there were no remnants detected in all three samples, indicating that the three samples were high pure, and a high purity of the carrier materials is desirable for ensuring the best possible performance of the silver nanoparticles.

The created defects on the surface of M-CNTs act as nucleation sites and are essential to ensuring the size and uniformity of the silver nanoparticles. Therefore, it is important to analyze the size and amount of defects. Raman spectroscopy was employed to examine the change of the defect area on CNTs before and after surface modification (Fig.2d). The spectrum of the pristine CNTs presents a strong G peak at 1580 cm⁻¹ and a weak D peak around 1335 cm⁻¹ representing the high crystallinity of the CNT's structure and the low number of defects on CNTs respectively [25]. Compared to that, the ratios of D and G band intensities (I_D/I_G) of M-CNTs and A-CNTs increased significantly after surface modification, and M-CNTs showed the highest I_D/I_G ratio. This result means that the defect area on the surface of M-CNTs is greatly expanded due to the intercalation and deep oxidation of multi-oxidants. X-ray diffraction (XRD) analysis shows consistent results with Raman spectroscopy. As shown in Fig.2e, a distinct diffraction peak appeared at 26° is indexed as the (002) reflection of the hexagonal graphitic structure [26]. After surface activation, M-CNTs show a significantly decreased and widened graphitic peak compared to that of pristine CNTs and A-CNTs, representing a breakdown in the graphitic lattice structure in M-CNTs induced by the activation treatment. To analyze the average size of those defects, Barrett-Joyner-Halenda (BJH) pore volume and size distribution tests were carried out, and the result is shown in Fig.2f. Compared to A-CNTs, M-CNTs present a strong peak between 3 and 4 nm in the pore distribution curve, implying the formation of large amount of nanoscale defects with a narrow size distribution on M-CNTs. Moreover, Brunauer-Emmett-Teller (BET) surface area analysis show that the surface area of M-CNTs (33.5 m²/g) is over twice that of A-CNTs (16.3 m²/g) due to the created nanoscale defects.

Fig.3. (a-b) SEM image of A-CNTs (a) and M-CNTs (b) dispersed on the silicon chip, and the inset shows the zoom out SEM images of each sample. (c) UV-visible spectra of A-CNT and M-CNT aqueous colloids. (d) Optical images of fresh A-CNT and M-CNT aqueous colloids (left) and one week after preparation (right). (e) Optical images of deposited A-CNT and M-CNT thin films on polystyrene petri dishes.

Based on the results above, it can be concluded that the multi-oxidation process efficiently creates abundant nanoscale defects and also form large amounts of oxygen-containing groups onto the surface of CNTs. These surface modifications also result in M-CNTs having superior dispersability. Fig.3a-b shows the SEM images of A-CNTs and M-CNTs dispersed on the silicon chip surface respectively. It can be found that A-CNTs show a poor dispersion property and form large size bundles due to the strong Van der Waals force. As a comparison, M-CNTs show a much homogeneous dispersion on silicon surface due to the strong hydrogen bonds formed between the M-CNTs surface and the substrate surface. Fig.3c exhibits the UV-visible spectra of the aqueous colloids of M-CNTs and A-CNTs. The UV-visible spectrum of M-CNTs shows a maximum at 240 nm and gradually decreased from UV to near IR. The characteristic peak at 240 nm indicates the acquisition of monodispersed M-CNT colloids [27,28]. As a control, A-CNT colloids have a very low absorbance at 324 nm in their UV-visible spectrum because of the existing CNT bundles which are less active at wavelengths between 200 and 800 nm [28]. Fig.3d shows the colloid stability of A-CNTs and M-CNTs. It has been found that the M-CNT colloid exhibited good stability while a precipitation occurred in the A-CNT colloid over a period of one week after preparation. Such a good stability of M-CNT colloids is attributed to the electrostatic stabilization effect of abundant oxygen functional groups on each individual M-CNT surface. In addition, the

oxygen functional groups play a crucial role in determining the morphology of the deposited CNT thin films. Fig.3e shows the deposited A-CNT film and M-CNT film on polystyrene petri dishes. It has been found that the evaporation of water led to formation of large individual A-CNT bundles on the substrate surface. In addition, these bundles could easily be peeled off from the substrate surface, showing that the A-CNTs had poor bonding strength. M-CNTs, in comparison, formed a uniform deposition layer on the substrate surface and bonded robustly to the substrate. This is due to the strong electrostatic repulsion effect of negatively charged oxygen functional groups on the surface of M-CNTs and this appears to prevent the formation of large CNT bundles. The uniform and tightly bonded M-CNT film on a substrate has the benefits of improving contact surfaces and the binding capability that is not the case for silver nanoparticles alone and thus enhances their anti-bacterial activity [29].

Transmission electron microscopy (TEM) was carried out to understand the structural changes to M-CNTs. As shown in Fig.4a-b, the pristine CNTs used in this work have an average diameter of 100 nm and a small aspect ratio about 100 (Fig.4a). Such a small aspect ratio of CNTs has the benefit of reducing the twisting among CNTs as well as improving their dispersion. Furthermore, the pristine CNTs, as shown in Fig.4b, have a highly oriented structure comprising of numerous parallel graphitic layers, demonstrating the high crystallinity of the pristine CNTs. In comparison to that, some obvious morphology changes were noticed on the structure of M-CNTs. As seen from Fig.4c, the cross-sectional view of M-CNTs shows a sharp contrast between the porous outer layer and the tight inner layer, and the porous outer layer has an average thickness of 10 nm. This structural change to the M-CNTs was caused by the combined effect of both deep oxidation and low-power ultrasonication of the multi-oxidation process. The lattice structure close to the surface area of the M-CNTs was partly disrupted and formed small nanoscale defects due to the effect of deep oxidation. The inner graphitic layers were not affected by the modification process and still kept their original highly oriented structure, as shown in Fig.4d, which helped to maintain the mechanical strength of the M-CNTs. TEM images of M-CNTs are consistent with the previous material characterization results, showing a superior efficiency of the multi-oxidation process in creating nanoscale defects on CNT surfaces.

Fig.4. (a-b) TEM images of pristine CNTs with different magnifications. (c) TEM cross-sectional view of M-CNTs. (d) TEM image of the graphitic lattice change of M-CNTs. (e-f) TEM images of M-CNTs carrying monodispersed silver nanoparticles with different magnifications. (g-h) TEM images of the out layer of M-CNTs carrying monodispersed silver nanoparticles. (i-j) TEM images of A-CNTs carrying large silver nanoparticles with different magnifications.

3.2 Silver nanoparticle growth on M-CNTs

The growth of silver nanoparticles was carried out in a silver complex solution where a stable reaction speed was maintained to refine nanoparticle's size and geometry. Fig.4e-f show the morphology of M-CNTs carrying the silver nanoparticles. As seen, large amounts of monodispersed nanoparticles with a mean diameter of 3 nm were uniformly deposited around the whole M-CNT surface and formed a densely packed nanoparticle layer, and interestingly, these nanoparticles penetrated into the porous out layer of M-CNTs. After extracting the tight inner part of M-CNT, the out layer with the tremendous silver nanoparticles can still remain the original shell structure, instead of forming individual particles (Fig.4g-h), indicating the strong binding capability of M-CNTs to the attached nanoparticles. Furthermore, there were no detected large-sized particles or obvious agglomeration on the surface of the M-CNTs, indicating the highly uniform diameter and distribution of nanoparticles. The reason for such a good coating effect is that the large number of nanoscale defects created on the surface of M-CNTs restricted the growth of individual particles and also prevented their agglomerations. Fig.4i-j show the morphology of A-CNTs carrying silver nanoparticles. It was found that the surface of A-CNTs was lightly coated by a very limited amount of silver nanoparticles. In addition, the silver nanoparticles had irregular shapes and different sizes ranging from 10 nm to 200 nm. Such a poor silver coating probably resulted from insufficient nucleation sites for

the growth and attachment of silver nanoparticles. These results show the importance of our developed CNT surface modification process to the uniformity of nanoparticles.

An X-ray diffraction (XRD) study was employed to identify the elementary composition of the nanoparticle layer. As shown in Fig.5a, the spectrum shows three new intensive peaks appearing at angles of 37.9° , 44.1° , and 64.8° . They are indexed as the (111), (200) and (220) reflections of silver's face-centered cubic (FCC) structure, implying the presence of silver with high crystallinity [30]. Due to the use of chelating agents, the developed silver nanoparticle growth method has advantages of stable reaction speed, a steady process, and good controllability of the metallic nanoparticle size and uniformity, which is superior to many reported approaches. For example, glucose, the most common silver reducing agent for mirror fabrication, can only grow very limited amount of silver nanoparticles on the surface of CNTs. Furthermore, the generated silver nanoparticles showed irregular shapes and variable sizes ranging from 10 nm to 200 nm [31]. Such non-uniform silver growth is likely a result of the fast reaction between the silver cations and glucose. Besides using conventional glucose for reduction, previously reported silver growth methods employing, for example, sodium borohydride or dimethylamine borane species as reducing agents unavoidably lead to the generation of hydrogen gas during the reactions [32,33]. Hydrogen bubbles can take over the nucleation sites on the surface of the CNTs and interfere in the morphology and coverage ratio of silver nanoparticles.

Fig.5. (a) X-ray diffraction (XRD) patterns of M-CNTs carrying silver nanoparticles. (b) The change of silver weight ratios deposited on the surface of CNTs as a function of the reaction time as measured by TGA. (c) UV-visible spectroscopy of the fabricated M-CNT carrying

silver nanoparticle aqueous dispersion freshly made and after one week. (d) The time-resolved UV-visible absorbance intensities of the M-CNT carrying silver nanoparticle dispersion at the wavelength of 417 nm over one week.

To precisely control the size and amount of silver nanoparticles, a systematic study of silver deposition was performed by TGA analysis. Different M-CNT samples with varied silver nanoparticle growth time were collected and burnt completely in a dry air flow to record the change of silver mass ratios expressed as W_{Ag}/W_C . The expressions W_{Ag} and W_C represent the weights of silver and M-CNTs respectively. Fig.5b shows the change of silver mass ratio as a function of growth time. According to the curve, there was a short initial stage lasting for 10 min at the beginning of silver growth. During this stage, the reaction speed was relatively slow, possible reasons could be that most of silver and cobalt ions were encapsulated by the chelating agent (EDTA) and formed stable metal ion chelates, Ag^+ -EDTA and Co^{2+} -EDTA, respectively. The reaction speed at this stage was mainly dominated by the slow spreading and decomposition processes of metal ion chelates. After this initial stage, reactions between silver cations and cobalt cations became much more active due to the self-catalytic effect of silver. As a result, large amount of silver nanoparticles were generated and deposited onto the surface of the M-CNTs. This growth stage continued for about 40 min and the W_{Ag}/W_C ratio increased from 0.5 to 5. Importantly, a stable reaction speed was exhibited during this stage due to the slow decomposition of the chelated compounds. Therefore the rapid growth of silver nanoparticles is avoided and the benefit was obtaining a monodispersed nanoparticle layer. The reaction then reached a plateau after 60 minutes with a final W_{Ag}/W_C ratio of approximately 6 and diminished completely after prolonged reaction time due to the depletion of reactants in the solution bath. According to the calculation of silver input-output ratio, up to 70% of the silver in the solution reacted while the reduction of the remaining 30% was prevented by the chelating agents. Further prolongation of reaction time would result in no further increase in the silver mass ratio, indicating that the growth of silver nanoparticles had been completed.

Due to the strong SPR absorption of silver nanoparticles in the visible region, the dispersability and stability of the fabricated M-CNT/Ag in an aqueous dispersion was characterized by UV-visible spectroscopy. As shown in Fig.5c, the absorption spectra of the freshly-prepared dispersion and the same dispersion after one week exhibited similar surface plasmon absorption bands with a maximum of 417 nm. It has been reported that the exact position and shape of the SPR absorption band are strongly dependent on the particle shape and size [34]. According to the literature, the number of SPR peaks increases as the symmetry

of the nanoparticle decreases, meaning that agglomeration of silver nanoparticles would lead to the increase of SPR peaks in the UV-visible spectrum [35]. Therefore, the single SPR peak in both two spectra indicates the presence of spherical silver nanoparticles with no agglomeration. Additionally, the position of SPR peak is also closely related the particle size. When silver nanoparticles aggregate, the SPR peak tends to shift towards longer wavelengths due to the increased particle size [36]. However, the SPR peak of the dispersion still showed a maximum absorption band at the wavelength of 417 nm after one week, confirming the good uniformity of the dispersion. Fig.5d shows the time-resolved UV-visible absorbance intensities of the M-CNT/Ag dispersion at the wavelength of 417 nm. It can be seen that the dispersion showed relatively stable SPR absorbance intensities over a week period, indicating the good stability of the dispersion.

3.3 Anti-bacterial activity of M-CNT/Ag

Due to the intrinsic anti-bacterial property of Ag NPs and large surface area of M-CNT, the fabricated M-CNT/Ag was used for killing bacteria. The anti-bacterial activity of M-CNT/Ag was compared to commercial Ag NPs with an average size of 5 nm by monitoring the respective growth and time killing assay of *E.coli* bacteria. Fig.6a shows the optical density (OD) changes of Ag NPs and M-CNT/Ag as a function of growth time respectively. Blank samples without any anti-bacterial agents served as the control group. It was found that the control group was more opaque compared to the other two samples, indicating normal growth of the bacteria. Compared to that, the transparency of the culture medium was consistent for Ag NPs at high concentrations ($\geq 25 \mu\text{g/ml}$), showing the strong bacteriostatic effect of silver nanoparticles. However, the bacterial growth at a low concentration of $12.5 \mu\text{g/ml}$ exhibited a relatively similar growth pattern to the control group, demonstrating a very weak bacteriostatic effect (top image of Fig.6a). A possible reason is that Ag NPs tend to form agglomeration which decreased the contact areas between the silver nanoparticles and bacteria and made the bacteriostatic effect of Ag NPs less obvious at low concentrations. On the other hand, M-CNT/Ag at high concentrations ($\geq 25 \mu\text{g/ml}$) also exhibited very strong bacteriostatic effect to the growth of bacteria, as shown in the bottom image of Fig.6a. Interestingly, M-CNT/Ag at a lower concentration of $12.5 \mu\text{g/ml}$ showed a much more restricted growth of bacteria within the first 8 h than the Ag NPs. The bacteria growth speed of within the first 8 h was very slow, indicating a higher bacteriostatic effect of M-CNT/Ag at low concentrations.

Fig.6. (a) The optical density (OD) changes of Ag NPs and M-CNT/Ag as a function of growth time respectively. Ag NPs and M-CNT/Ag with different concentrations (12.5 µg/ml, 25 µg/ml and 50 µg/ml) were measured respectively. (b) The time killing assay of *E.coli* in the presence of Ag NPs and M-CNT/Ag with different concentrations (12.5 µg/ml, 25 µg/ml and 50 µg/ml). (c) Anti-bacterial activities of Ag NPs and M-CNT/Ag with different concentrations (12.5 µg/ml, 25 µg/ml and 50 µg/ml) against *E.coli* biofilm. Blank samples without any anti-bacterial agents acted as the control group in all of the measurements.

Fig.6b depicts the time killing assay of *E.coli* in the presence of Ag NPs and M-CNT/Ag. Herein, both Ag NPs and M-CNT/Ag did not show obvious bactericidal effects against *E.coli* at a low concentration of 12.5 µg/ml. Nevertheless, the viability of the bacteria still decreased compared to the control sample due to silver's inhibitory effect on bacterial growth. It has also been found that M-CNT/Ag at the concentration of 12.5 µg/ml was more effective than Ag NPs in the initial 2-6 hours. This is due to the fact of large contact areas between the bacteria and the M-CNT/Ag that caused immediate damage to the bacteria and thus slow down the initial growth speed. With continued growth of bacteria, the inhibitory effect of M-CNT/Ag became less obvious because the bacteria outnumbered the available M-CNT/Ag. The inhibitory effect at low concentrations was not detected from Ag NPs. In the case of high concentrations (25 µg/ml and 50 µg/ml), the bactericidal effect of M-CNT/Ag can be clearly observed with the increase of interaction time between the bacteria and M-CNT/Ag, where the bacteria were neutralized in a more linear fashion. For Ag NPs, it also showed bactericidal activity at high concentrations but the overall bacterial killing rate and final bacterial viability were much less than M-CNT/Ag. Moreover, it has been noticed that the bactericidal effect of Ag NPs reached a threshold after 8 hours, meaning that the bacterial killing speed of Ag NPs was almost in a state of equilibrium with the bacterial growing speed. These results show that the M-CNT/Ag hybrids have stronger anti-bacterial activity and better reliability than Ag NPs at all concentrations.

Anti-bacterial activities of Ag NPs and M-CNT/Ag against *E.coli* bacteria on biofilm were also evaluated, and the results are shown in Fig.6c. It was found that both Ag NPs and M-

CNT/Ag at a low concentration of 12.5 $\mu\text{g/ml}$ did not show obvious bactericidal effects against bacteria on biofilm due to the strong resistance of biofilm bacteria to anti-bacterial agents. For higher concentrations (25 $\mu\text{g/ml}$ and 50 $\mu\text{g/ml}$), the viability of the bacteria treated by M-CNT/Ag was only half that for Ag NPs, showing the superior anti-bacterial performance of M-CNT/Ag hybrids compared to Ag NPs. Scanning electron microscope (SEM) images (Fig.7) show the structural damage of *E.coli* bacteria treated with Ag NPs and M-CNT/Ag respectively. As seen in Fig.7a-b, *E.coli* bacteria from the control group showed intact morphology and the cells maintained a normal physicality. In contrast, the treatment of Ag NPs (50 $\mu\text{g/ml}$) caused obvious shrinkage of the cell walls (Fig.7c-d) due to the lysing effect of silver. For M-CNT/Ag (50 $\mu\text{g/ml}$), it can be clearly observed that most of the cell walls were completely destroyed and the ratio of damaged/dead *E.coli* was much higher than that of Ag NPs (Fig.7e-f). This result shows that the very strong anti-bacterial activity of M-CNT/Ag is actually attributed to the combined effect of deposited silver nanoparticles and M-CNTs. For example, the interaction of the sharp edges of M-CNTs and rough surfaces with the bacteria cell wall resulted in obvious physical damage to cell membranes and led to the loss of bacterial membrane integrity. The silver nanoparticles on the surface of M-CNTs then caused irreversible damage to the proteins, DNA and lipids after exposure to the bacterial cytoplasmic matrix. Moreover, the significant amount of oxygen functional groups on the M-CNTs was able to generate large amount of reactive oxygen species (ROS); which induced oxidative stress onto the bacteria thereby lysing them. As a whole, the membrane and oxidative stresses induced by M-CNTs, together with the lysing effect of silver nanoparticles, significantly enhanced the anti-bacterial activity of the M-CNT/Ag hybrids.

Fig.7. SEM images showing the structural damages of *E.coli* biofilm cells treated by Ag NPs and M-CNT/Ag respectively. (a-b) The morphology of *E.coli* biofilm cells from the control group. (c-d) The morphology of *E.coli* biofilm cells treated by Ag NPs (50 µg/ml). (e-f) The morphology of *E.coli* biofilm cells treated by M-CNT/Ag (50 µg/ml).

4. Conclusion

In this paper, a new surface modification process was developed to modify the chemically inert graphitic surface of CNTs for close bonding with silver nanoparticles. The multi-oxidation process is capable of creating a porous out layer with abundant nanoscale defects and bond large amounts of oxygen-containing groups onto the surface of the CNTs. The M-CNTs show excellent dispersability and stability in water without using any surfactants, Monodispersed silver nanoparticles with an average size of 3 nm were closely bonded on the surface of M-CNTs without any obvious agglomerations. Due to the combined anti-bacterial effects of M-CNTs and silver nanoparticles and good reliability of the hybrid nanostructures, the fabricated M-CNT/Ag exhibited greatly enhanced anti-bacterial activity compared to commercial silver nanoparticles. With these superior properties, the developed surface modification process could be widely used for improving the performances of many CNT based hybrid nanomaterials in diverse applications, such as catalysts, optical spectroscopies, conductive inks, water sterilization, and biosensors.

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