

A study on bactericidal properties of Ag coated carbon nanotubes

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Abstract

A silver layer is coated on carbon nanotubes (CNTs) by ion beam assisted deposition (IBAD). Standard agar dilution method is used to evaluate the bactericidal rate against Gram positive *S. aureus* and negative *E. coli*. The structure and the chemical states are investigated by scanning electron microscope (SEM) and X-ray photoelectron spectroscopy (XPS). The results show that Ag coated CNTs possess very high bactericidal rate. In comparison with the Ag coated pyrolytic carbon sample, the Ag coated CNTs show stronger bactericidal property.

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1. Introduction

With the development of biomedical materials, more and more artificial organs and parts are used in clinic to ease patient's agony and elongate their lifetime. Biomedical materials are those materials which can be used to assist or substitute human being's tissue and organs. Because of contacting tissue, blood, cells and protein, their biocompatibility is a very important factor. Biomedical materials can be divided into metal and nonmetal materials. Metal biomaterials are used as frame plates and screws in orthopedics [1]. The nonmetal biomaterials are used as heart valves and artificial tubes [2]. Recently, pyrolytic carbon is used to make artificial heart valves due to its fantastic mechanical property and biocompatibility [3,4]. As an artificial heart valve, not only is it important to have good mechanical properties such as wear resistance, fatigue resistance but also to possess bactericidal property. In the past decade, for the sake of safety, an inorganic antibacterial method,

such as doping silver or copper to the alloy has replaced the organic agents [5]. Metallic ions from the alloy can penetrate the wall and membrane of bacterial cells and inhibit their breeding. In order to enhance antibacterial property of artificial heart valve, a silver implanted pyrolytic carbon valve was reported in our previous work [6]. Due to carbon nanotubes possess fantastic properties such as high adsorption, huge surface ratio etc., it must be widely used in biomedical area. Combining the merits both from silver and carbon nanotubes, it is possible to grow carbon nanotubes on the surface of pyrolytic carbon to enhance its surface area and then coated Ag on it to protect bacteria.

In this paper, Ag coated carbon nanotubes are put forward firstly in bactericidal application for biomedical materials. The chemical vapor deposition (CVD) is used to grow carbon nanotubes on pyrolytic carbon substrate and then the IBAD is used to coat Ag on the surface of carbon nanotubes. Then the bactericidal property of Ag coated carbon nanotubes is evaluated by standard agar dilution method with bacteria of *E. coli* and *S. aureus*. In addition, SEM and XPS analyses are performed to investigate the Ag distribution and chemical states.

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2. Experimental details

The pyrolytic carbon plate was cut into circle pieces having 13 mm in diameter and 4 mm thick. The samples were ground and polished to a mirror finish prior to growing carbon nanotubes by CVD.

The carbon nanotubes were grown on pyrolytic carbon by decomposing ethylene on Ni/Co catalyst nanoparticles at 850 °C during CVD process at University of Western Ontario, Canada. The synthesis was performed in a specifically designed chemical vapor deposition (CVD) reactor, as described previously [7]. This reactor takes advantage of the resistivity of the pyrolytic carbon in the electrical circuit, which heats the Ni–Co catalytic nanoparticles pre-deposited on the pyrolytic carbon by Joule effect, to decompose ethylene for the growth of carbon nanotubes. The catalyst preparation of Co–Ni nanoparticles was briefly described by the following steps. The first step involves the hydrolysis of 2(4-chlorosulfonylphenyl) ethyl trichlorosilane (United Chemical Technologies) and exchange proton in sulfonic acid silicate with Co, Ni ions. This will give a combined transition metal ion sulfonate solution. Then the pyrolytic carbon, which is used to support the nanotubes, was immersed in the solution for 10 s and dried with a filter paper. The Co–Ni ions absorbed on the pyrolytic carbon is reduced under hydrogen at 500 °C to obtain Co–Ni catalyst nanoparticles for the growth of multi-walled carbon nanotubes (MWCNTs) by decomposing ethylene at about 850 °C. The further experimental details were described in the previous report [7]. The carbon nanotubes were directly used without further treatments.

After the growing carbon nanotubes, the samples were coated with Ag by IBAD in our group using a multifunctional thin film deposition facility. The IBAD facility has two Kaufmann ion sources which can provide low and high

energy ion beam. An argon beam is used to sputter Ag target providing Ag atoms, simultaneously. Another argon ion beam is used to assist the Ag deposition so as to enhance the adhesion. The experimental condition of IBAD is listed in Table 1. The description of all samples is shown in Table 2.

The bactericidal properties of Ag coated carbon nanotubes on pyrolytic carbon substrate and Ag coated pyrolytic carbon samples were measured using Gram positive *S. aureus* and negative *E. coli* as the test bacteria, because these two kinds of bacteria are very common and typical infection to human body. The experimental procedures were described in our previous work [6]. The bactericidal rate K can be calculated by following formula:

$$K = \frac{(A - B)}{A} \times 100\%,$$

where A and B are the number of bacteria colonies corresponding to the reference sample and Ag coated samples, respectively.

XPS analysis was performed using a X-ray photoelectron spectrometer in model of PHI5300 made in US. It operated at 250 W and 1256.6 eV with Mg K α radiation. The working pressure in the XPS chamber was approximately 4×10^{-7} Pa. The charging effect of the samples during analyses was corrected using a value of 285.0 eV for the binding energy of the main peak C 1s. SEM characterization was conducted using a SEM facility in model of LEO 1530 VP made in GER.

3. Results and discussion

3.1. Bactericidal effects

The *E. coli* and *S. aureus* were used as the testing bacteria in this test. The specimens with *E. coli* and *S. aureus* were incubated at 25 °C for 24 h. The number of the live bacteria colonies in a fixed area were counted and the antibacterial rate were measured by the standard agar dilution method.

Fig. 1 presents the breeding status of *E. coli* corresponding to Ag coated carbon nanotubes sample (A1), Ag coated pyrolytic carbon sample (B1), and CNTs sample without Ag coating (A0) and pyrolytic carbon substrate sample (B0). After the *E. coli* had been cultivated for 24 h, a few bacterial colonies presented on the glass utensil corresponding to Ag coated pyrolytic carbon sample (B1) than that of pyrolytic carbon substrate (B0). There was nearly

Table 1
Technical parameters of Ag coated CNTs samples by IBAD

Parameters of IBAD	Value
Base vacuum	3×10^{-4} Pa
High energy Ar ions bombarding energy	1.4 keV
High energy Ar ions beam current	20 mA
Low energy Ar bombarding energy	100 eV
Low energy Ar ions beam current	5 mA
Deposition vacuum	2.0×10^{-2} Pa
Deposition temperature	RT

Table 2
Describing and numbering of the samples

Sample	Substrate	Sample	Substrate	Ag deposition time (min)
A0	CNTs	B0	Pyrolytic carbon	0
A1	CNTs	B1	Pyrolytic carbon	5
A2	CNTs	B2	Pyrolytic carbon	10
A3	CNTs	B3	Pyrolytic carbon	30
A4	CNTs	B4	Pyrolytic carbon	60

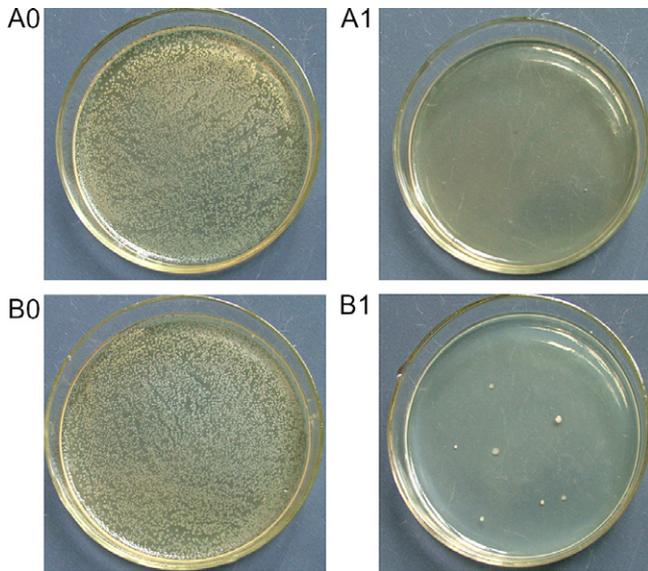


Fig. 1. The colonies of *E. coli* corresponding different samples: CNTs sample without Ag coating (A0), pyrolytic carbon substrate (B0), Ag coated CNTs sample (A1) and Ag coated pyrolytic carbon (B1).

no bacterial colony on the glass utensil corresponding to Ag coated CNTs (A1) than that of CNTs sample without Ag coating (A0). This result reveals that coating Ag provides pyrolytic carbon and CNTs with strong antibacterial property. Furthermore, the bacteria colony corresponding to Ag coated CNTs (A1) is much less than that of Ag coated on pyrolytic carbon (B1) under the same condition. This result suggests that Ag coated CNTs can improve the antibacterial property in great deal.

The bactericidal rate K depends on the thickness of Ag film which is associated with deposition time. This thickness dependence is shown in Fig. 2, where two curves are

used to show the different effect on Ag coated CNTs samples and Ag coated pyrolytic carbon samples. It can be observed that the bactericidal rate K increases with the thickness of Ag film on both samples. However, for the same thickness, the bactericidal rate K of Ag coated CNTs is much higher than that of Ag coated pyrolytic carbon sample. Taking the samples A1 and B1, for example, the sample A1 has stronger antibacterial ratio than the sample B1. The reason should be attributed to the huge surface area caused by carbon nanotubes which are coated with Ag. Therefore, the Ag coated CNTs samples can provide more opportunities to make Ag atoms contact with the bacteria resulting in the death of the bacteria [8]. These silver ions can kill bacteria by penetrating the cell wall and membrane and inhibiting their breeding. At same time, the silver ions can extract the electrons from the bacteria, causing their cytoplasm to run off and oxidizing their cell nucleus [9]. From the Fig. 2 it can also be observed that the antibacterial rate against *S. aureus* is lower than that against *E. coli*. It may be caused by the different thickness of cell wall of different bacteria. The cell wall of *E. coli* is about 10 nm, but the cell wall of *S. aureus* is about 100 nm. So, it is easy for the silver atoms to penetrate *E. coli*'s wall than that of *S. aureus* showing the high bactericidal ratio.

3.2. SEM and XPS analysis

It is very important to control the deposition rate in Ag coating. If the deposition rate is too high, the Ag may cover the CNTs bunch which results in a reduced the surface area. In our experiment, the deposition rate was controlled within 3.33 nm/min. In 10 min, the thickness of coated Ag is about 33 nm. This guarantees that the Ag is only coated

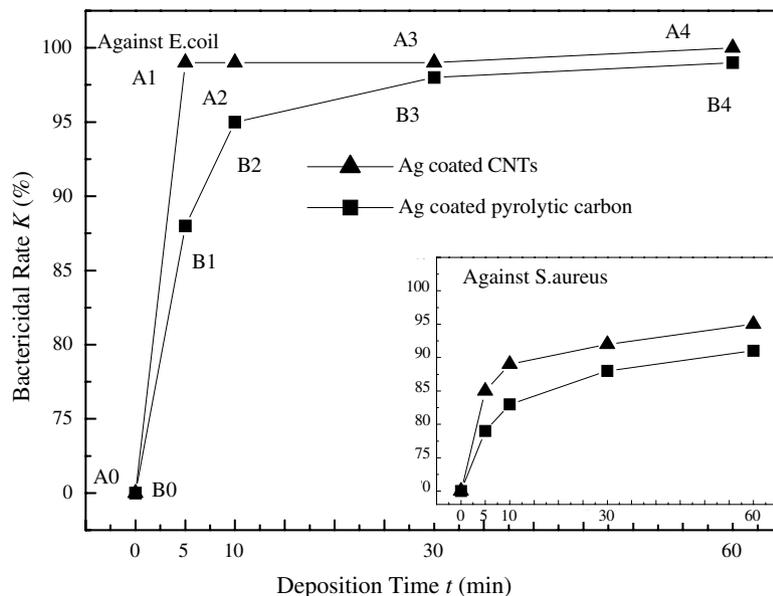


Fig. 2. The bactericidal rate of Ag coated CNTs samples and Ag coated pyrolytic carbon samples.

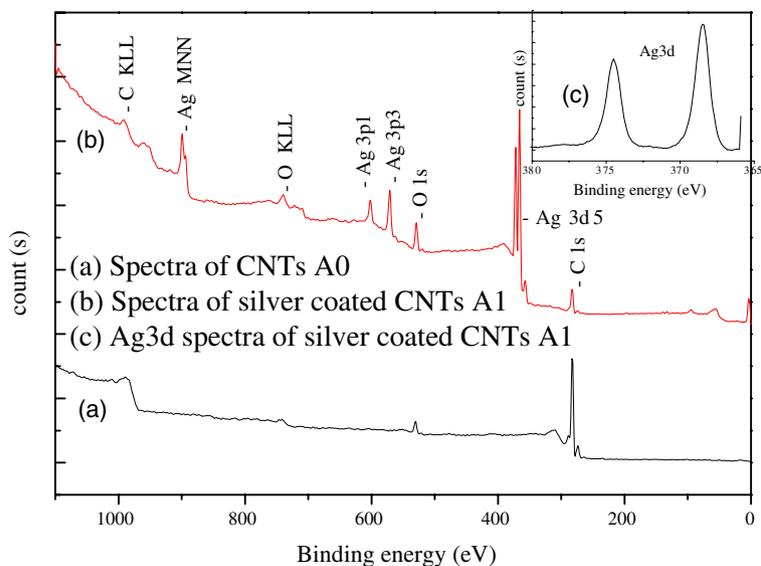


Fig. 3. (a) XPS spectra of CNTs sample A0, (b) Ag coated CNTs sample A1 and (c) Expanded XPS spectrum of Ag3d for Ag coated CNTs sample A1

on a single CNT. Fig. 4(a) shows SEM image of Ag coated CNTs at deposition rate of 3.33 nm/min. It is clear that the Ag is covered on single CNT walls not on CNT bunch. In comparison, Fig. 4(b) shows the TEM photos of original CNTs without Ag coating. It shows that the single CNT

is not covered by silver as a bunch. This SEM characterization is consistent with the result of bactericidal analysis. In order to further examine if the Ag covered on a single CNT or covered on a CNT bunch, XPS analysis is performed.

Fig. 3 shows the XPS spectra of CNTs samples before and after Ag coating. The spectrum (a) corresponds to the sample (A0) before Ag coating. It can be observed that a strong carbon peak of C1s at 275 eV showing CNTs on substrate. The spectrum (b) corresponds to the sample (A1) after Ag coating and shows that there are strong Ag3d5 peak at 364.52 eV and Ag3p1, Ag3p3 at 602.17 eV, 570.45 eV, respectively and O1s at 528.16 eV. The silver deposition on CNTs substrate results in a silver rich thin film with some silver oxides making carbon peak of C1s weak. The extend XPS spectra (c) of Ag3d on the right corner proves that the Ag on the CNTs is in the state of pure Ag and its oxide. Ag and its oxide coated on CNTs and pyrolytic carbon play a key role in killing bacteria.

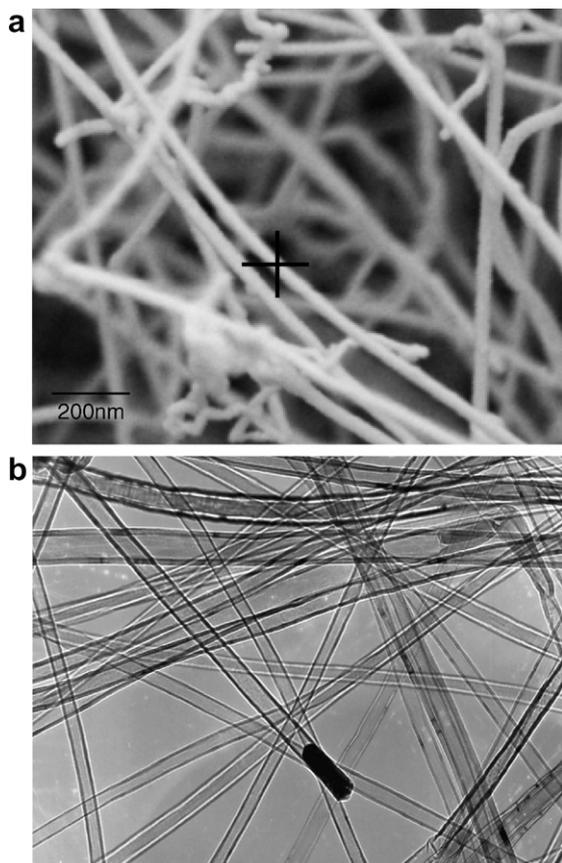


Fig. 4. (a) SEM photo of Ag coated CNTs sample (A1) and (b) TEM photo of uncoated CNTs sample A0.

4. Conclusion

We have presented the bactericidal properties of Ag coated carbon nanotubes on pyrolytic carbon substrate by IBAD. The results of our experiments show that the Ag coated CNTs by IBAD possess strong bactericidal property. The bactericidal rate of Ag coated CNTs is larger than that of Ag coated pyrolytic carbon under the same condition. XPS shows that Ag coated on CNTs is in the state of pure Ag element and Ag oxides. SEM characterization indicates that the Ag is coated on single CNT rather than on CNT bunch. The carbon nanotubes enlarge the surface area of Ag coating, which provides high probability for Ag atoms to contact the bacteria leading the death of the bacteria. Ag coated CNTs may be a new candidate in

manufacturing artificial heart valves or some other biomedical devices.

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