Note

The Preparation of Ag Nanoparticle-Modified Single-Walled Carbon Nanotubes and Their Antibacterial Activity

TOMOMI KOSAKA^{1*}, AKIE OHGAMI¹, TAKAKO NAKAMURA²,
TSUGUYORI OHANA² AND MASATOU ISHIHARA²

¹*Department of Chemistry, Tokyo Gakugei University, 4-1-1, Nukuikita-machi, Koganei, Tokyo 184-8501, Japan*

²*Research Center for Advanced Carbon Materials, National Institute of Advanced Industrial Science and Technology (AIST), 1-1-1, Higashi, Tsukuba, Ibaraki 305-8565, Japan*

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The sidewall modified single-walled carbon nanotubes (SWNTs) with amino-containing substituents were prepared using the radicals generated by the photolysis of acetonitrile. A subsequent treatment of modified SWNTs with the Ag colloid gave an attachment of Ag nanoparticles on the surface of SWNTs through the functionalized linkages. The Ag nanoparticle-modified SWNTs evaluated by antibacterial tests showed strong activities against *Escherichia coli* and *Staphylococcus aureus*. However, the use of Ag nanoparticle-modified SWNTs on simulated body fluid exhibited weaker antibacterial activity.

Key words : Single-walled carbon nanotubes/Chemical modification/Antibacterial activity/Ag nanoparticles.

Single-walled carbon nanotubes (SWNTs) have a wide range of applications due to their unique electronic, optical, mechanical (Dresselhaus et al., 1996; Saito et al., 1998), antimicrobial (Kang et al., 2007) and metallic or semiconducting properties depending on their diameter and helicity. The chemical functionalization of the sidewall of SWNTs has been expected to add novel functions to the original behavior.

Thiol-, thio- and amino-containing materials have attracted much attention because of their properties derived from the presence of sulfur- and nitrogen-containing functional groups. It is well-known that organic compounds containing thio- and thiol groups are important materials for biologically active substances and show good affinities to gold to give a self-assembled monolayer. Amino functionalities play an important role in constructing complex structures

with a combination of other functional groups. The sidewall modification of carbon nanotube surfaces with amino substituents has been investigated by the use of ammonia with the ball-milling method (Lin et al., 2003), a substitution of fluorinated SWNTs with diamines (Stevens et al., 2003), a conversion of nitro moieties to aniline (Guo and Li, 2005) and a further functionalization process of carboxylated SWNTs (Ramanathan et al., 2005; Gromov et al., 2005). However, these methods would create problems about the handling of the reactions due to the use of hazardous reactants and the necessity of multi-step reactions. Previously, we reported on the photochemical modification of SWNTs with fluorine functionalities by the use of radical reactions, which took place under mild conditions and gave no heavy modification (Nakamura et al., 2004) compared with conventional methods described above.

It is known that acetonitrile is photolysed to give CH_2CN radicals upon irradiation with VUV light (Lesiecki and Guillory, 1978 ; Halpern and Tang,

*Corresponding author. Tel : +81-42-329-7511, Fax : +81-42-329-7511.

1985 ; Cheng et al., 2000). Carbon nanotubes (CNTs) have poor solubility and tend to aggregate in the water. Then we considered that surface modification of CNTs has been effective to their antibacterial activity. Akasaka et al. (2005) reported that the apatite coating on multi-walled carbon nanotubes is achieved using simulated body fluid (SBF). SBF has been frequently used for the apatite coating to increase bioactivity and has been successfully applied to implant materials such as special dental and medical devices. Nonami et al. (2003) prepared apatite-coated TiO_2 composite and reported that the composite has better function of absorbing and decomposing acetaldehyde, nitrogen oxide and *Escherichia coli* than the uncoated TiO_2 . Accordingly, we considered that an apatite coating on Ag modified SWNTs might improve their antibacterial activity. In this paper, we prepared novel Ag modified SWNTs with amino substituents via the radical reactions produced by using the photolysis of acetonitrile and the attachment of Ag nanoparticles. We also investigated the antibacterial activity of the Ag modified SWNTs and those treated with SBF.

SWNTs (0.9–1.2 nm diameter, 10–50 μm length) and NaBH_4 were purchased from Aldrich Co. Inc. Acetonitrile and 10 mM aqueous solution of AgNO_3 were obtained from Kanto Chemical Co. Inc. A suspension of purified SWNTs (hereafter referred as p-SWNTs) in acetonitrile (4 ml) was irradiated with a Xe excimer lamp (USHIO UER20H-172C) for 3 h at room temperature under an argon atmosphere with stirring. After removing the acetonitrile solution from the reaction mixture, the residual powders were washed with acetonitrile. The attachment of silver nanoparticles on the surface of amino-functionalized SWNTs (hereafter referred as amino-SWNTs) was carried out by treating the amino-SWNTs (1.7 mg) with 10 mM aqueous solution of AgNO_3 (5 ml), then with 40 mM aqueous solution of NaBH_4 (10 ml) with stirring for 5 min. After finishing the reaction, the aqueous solution was removed from the reaction mixture, and then the samples were washed with water.

The Ag nanoparticle-modified SWNTs (hereafter referred as Ag-SWNTs) were dispersed in Dulbecco's phosphate-buffered saline (D-PBS) at a concentration of 10 mg/l by ultrasonication for 10 min. Then, the apatite crystallites were grown by immersing the samples at 36°C for 2 h. The D-PBS powder was purchased from Invitrogen Japan K.K. The composition of D-PBS was NaCl (8000 mg/l), CaCl_2 (100 mg/l), KH_2PO_4 (200 mg/l), Na_2HPO_4 (1150 mg/l), KCl (200 mg/l), MgCl_2 (47 mg/l). The apatite-coated Ag-SWNTs were referred as Ag-HA-SWNTs.

The obtained samples were analyzed by diffusion

reflectance infra-red Fourier transform spectroscopy (DRIFT), X-ray photoelectron spectroscopy (XPS), scanning electron microscopy (SEM), Raman spectroscopy, mass and UV-vis-NIR spectrometers.

DRIFT spectra were recorded with JASCO FT/IR-680 Plus spectrometers. XPS was measured by a PHI ESCA model 5800 using aluminum $\text{K}\alpha$ radiation. The chemically modified samples were analyzed using the mass spectrometer (JEOL JMS-AMSUN200) with the direct insertion method. Raman spectra were measured with JASCO Laser Raman Spectrometers NRS-2100 using the 514.5 nm line of an argon ion laser with a power of 100 mW. SEM was used to examine the surface morphology before and after the reactions. Samples were mounted on an Al stub using conducting carbon paste before examination in a Hitachi S-5000 model. UV-vis-NIR spectra were measured by Parkin-Elmer Lambda 900.

Escherichia coli NBRC 3972 and *Staphylococcus aureus* subsp. *aureus* NBRC 12732 were used for antibacterial tests. One loopful of the refrigerated bacteria was cultured in 1 ml nutrient broth at 36°C for 24 h. After being cultured, the bacteria were suspended in sterile deionized water to a concentration from 10^6 to 10^7 cells/ml. Suspensions of p-SWNTs (1.1 mg), Ag-SWNTs (1.1 mg) and Ag-HA-SWNTs (1.0 mg) with sterile deionized water (900 μl), a suspension of bacteria (100 μl) and nutrient broth (2 μl) were allowed to stand in the incubator at 36°C using 1 ml micro tubes. Then, the initial concentration of bacteria was from 10^5 to 10^6 cells/ml. The contaminated water was diluted to $10^2 \sim 10^4$ cells/ml by sterile deionized water using 1 ml micro tubes at each sampling. After 50 μl of the contaminated water in a micro tube had been dropped and spread on a growth medium-standard method agar in a petri dish, the bacteria formed small visible colonies after 24 h incubation at 36°C . The number of bacteria colonies was counted.

XPS spectra of SWNTs were measured before and after irradiation in the presence of acetonitrile (Fig. 1), showing a new peak at 400.5 eV of nitrogen 1s together with the sp^2 carbon peak at 284.6 eV after irradiation. In the carbon 1s region, the carbon peak was deconvoluted to the mixture of sp^2 carbon and carbon atoms bound to nitrogen atoms with a higher binding energy, compared with the spectra of p-SWNTs.

IR spectra of the samples before and after the treatment of irradiation with acetonitrile are shown in Fig. 2. New peaks at 2922 and 2856 cm^{-1} with a C-H stretching band and at 3434 cm^{-1} with a $-\text{NH}_2$ stretching band were observed, accompanied with a C=C bond peak (1631 cm^{-1}), as contrasted with no peaks in the case of p-SWNTs.

FIG. 1. XPS spectra of SWNTs were measured (a) before and (b) after irradiation in the presence of acetonitrile. C1s and N1s denote the carbon 1s and nitrogen 1s orbital photoelectron peaks, respectively. The inset graphs show the carbon 1s region.

FIG. 2. IR spectra of (a) p-SWNTs and (b) amino-SWNTs.

The mass spectrum of components generated by heating amino-SWNTs to 300°C is shown in Fig. 3a. A parent peak at m/z 43 ($C_2H_5N^+$) was ascribed to the CH_2CH_2NH group with fragmentation peaks m/z 29 ($C_2H_5^+$) consistent with the presence of the $-CH_2CH_2NH_2$ moiety. Moreover, the covalent functionalization of SWNTs was confirmed by UV-vis-NIR measurements in DMF as shown in Fig. 3b. It was known that a covalent sidewall modification on the surface of CNTs would result in changes in their original electronic behavior. In comparing the spectra of p-SWNTs and amino-SWNTs, it was found that the

FIG. 3. (a) Mass spectrum of amino-SWNTs after heating to 300°C. (b) UV-vis-NIR spectra of p-SWNTs and amino-SWNTs.

sidewall functionalization of SWNTs with amino substituents partly suppressed the original absorption of SWNTs (Park et al., 2003).

Figure 4 shows the Raman spectra of p-SWNTs and amino-SWNTs. Characteristic peaks were observed at 180, 165 and 149 cm^{-1} due to a breathing mode of p-SWNTs, accompanied with typical peaks at 1574 and 1337 cm^{-1} attributed to G and D band, respectively. The vibration mode at G band was attributed to the characteristic phonon dispersion of graphite and the one at D band arose from the disorder in the structure in the plane with graphite. In the case of amino-SWNTs, the breathing mode was suppressed by introduction of amino substituents. It was found that the peak intensity of the D band in amino-SWNTs increased in comparison to that of the p-SWNTs by covalent functionalization.

These analytical results suggest a plausible reaction mechanism as shown in Scheme 1. Hydrogen and CH_2CN radicals, generated by the photolysis of acetonitrile, reacted with the C=C bond on SWNTs

FIG. 4. Raman spectra of (a) p-SWNTs and (b) amino-SWNTs.

Scheme. 1. Chemical modification of SWNTs by photolysis of acetonitrile and subsequent treatment with Ag nanoparticles.

sidewall, then hydrogen radicals reacted with the CH_2CN moiety to give aminated SWNTs. This method is useful for the introduction of amino functionalities on the sidewall of SWNTs.

The attachment of Ag nanoparticles to amino-SWNTs formed by the photoreaction in acetonitrile was performed by the treatment with Ag colloids. As shown in Fig. 5a, the XPS spectrum shows a new peak at 369.0 eV of silver 3d together with the sp^2 carbon peak at 284.6 eV and nitrogen peak at 400.5 eV. The atomic ratio of C, N and Ag was estimated to be 93:2:5 in the Ag-SWNTs. As shown in Figure 5b and c, a SEM image of amino-SWNTs with Ag nanoparticles through the amino linkage indicates a

FIG. 5. (a) XPS spectrum of Ag-SWNTs. SEM images of (b) p-SWNTs and (c) Ag-SWNTs. Ag3p, Ag3d denote silver 3p and 3d orbital photoelectron peaks, respectively.

high dispersion of Ag nanoparticles on sidewall SWNTs and reveals no significant changes after the introduction of the substituents compared with that of p-SWNTs.

Antibacterial activity tests of p-SWNTs and Ag-SWNTs were performed against *E. coli* and *S. aureus* as shown in Table 1 and Table 2, respectively. In the case of the absence of SWNTs, the number of bacteria did not increase after 3 h. On the other hand, Ag-SWNTs showed a strong bactericidal activity, in which colonies of *E. coli* and *S. aureus* were not observed in 2 h and 3 h, respectively. These results indicate that Ag-SWNTs are useful as an antibacterial agent owing to the presence of Ag nanoparticles known to play an important role in strong bactericidal activities as reported previously (Matsumura et al., 2003 ; Sondi and Salopek-Sondi, 2004). In the case of Ag-HA-SWNTs, it was found that the SBF treatment resulted in a weaker antibacterial activity of Ag-SWNTs. According to XPS analysis as shown in Figure 6, it is confirmed that hydroxyapatite (HA) is formed on the surface of samples by soaking in SBF for 2 h at 36°C. However, we could not detect on improvement in the antibacterial activity contrary to our expectations. We also detected that colonies of *S. aureus* were not observed after 24h if p-SWNTs were in the suspension. Kang et al. (2007) reported that highly purified SWNTs have strong antimicrobial activity against *E. coli* K12. Our results against *S. aureus* were consistent with their conclusion. However, it is considered that further detailed experiments that include the interaction between p-SWNTs, Ag-SWNTs, Ag-HA-SWNTs and bacteria are needed.

The photolysis of acetonitrile in the presence of SWNTs led to a sidewall modification of the surface to introduce amino functional groups, respectively,

TABLE 1. The antibacterial activities of p-SWNTs, Ag-SWNTs and Ag-HA-SWNTs against *E.coli*. Values are shown as mean $\pm$ standard deviation obtained from three sets of data. (cfu/ml)

	0h	1h	2h	3h	24h
Control	2.0E+06 $\pm$ 2.0E+05	2.0E+06 $\pm$ 1.2E+05	2.0E+06 $\pm$ 1.7E+05	3.7E+06 $\pm$ 4.7E+05	1.1E+06 $\pm$ 1.4E+05
p-SWNTs	2.0E+06 $\pm$ 2.0E+05	1.8E+06 $\pm$ 2.8E+05	1.7E+06 $\pm$ 1.9E+05	2.6E+05 $\pm$ 0.0E+00	1.4E+06 $\pm$ 5.5E+05
Ag-SWNTs	7.9E+05 $\pm$ 1.4E+04	8.0E+02 $\pm$ 5.7E+02	<200	<200	<200
Ag-HA-SWNTs	9.8E+05 $\pm$ 5.7E+04	8.3E+04 $\pm$ 5.7E+03	2.4E+02 $\pm$ 0.0E+00	<200	<200

TABLE 2. The antibacterial activities of p-SWNTs, Ag-SWNTs and Ag-HA-SWNTs against *S.aureus*. Values are shown as mean $\pm$ standard deviation obtained from three sets of data. (cfu/ml)

	0h	1h	2h	3h	24h
Control	1.8E+06 $\pm$ 1.3E+05	1.9E+06 $\pm$ 3.2E+05	1.7E+06 $\pm$ 1.4E+05	1.7E+06 $\pm$ 9.0E+04	7.7E+06 $\pm$ 1.8E+06
p-SWNTs	1.8E+06 $\pm$ 1.3E+05	8.1E+05 $\pm$ 4.2E+04	2.1E+05 $\pm$ 7.6E+04	5.0E+04 $\pm$ 1.4E+04	<200
Ag-SWNTs	1.9E+06 $\pm$ 1.8E+05	8.7E+04 $\pm$ 9.9E+03	1.6E+03 $\pm$ 0.0E+00	<200	<200
Ag-HA-SWNTs	9.9E+05 $\pm$ 1.8E+05	7.4E+05 $\pm$ 1.9E+05	3.4E+05 $\pm$ 3.4E+04	1.1E+05 $\pm$ 2.0E+04	<200

FIG. 6. XPS spectra of Ag-SWNTs after SBF treatment. O1s, Ca2p, Cl2p and P2p denote the oxygen 1s, calcium 2p, chlorine 2p and phosphorus 2p orbital photoelectron peaks, respectively.

via a one-step reaction. These methods are useful for the modification of sidewall SWNTs with organic functional groups because byproducts are not formed and SWNTs electronic behavior is not destroyed unlike in the case of heavy modification. The modified

SWNTs with amino functionalities could be modified further by the attachment of Ag nanoparticles on sidewall SWNTs. The Ag nanoparticle-functionalised SWNTs evaluated by antibacterial tests with *E.coli* and *S.aureus* showed strong antibacterial activities. The apatite-coated Ag-SWNTs exhibited weaker antibacterial activity. However, it is considered that further detailed experiments to investigate the effect of the apatite coating on SWNTs on the absorbing function of the bacteria are needed.

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