

The Effect of Dispersed MWCNTs Using SDBS Surfactant on Bacterial Growth

J.E. Park, G.R. Kim, D.J. Yoon, C.H. Sin, I.S. Park, T.S. Bea, and M.H. Lee

Abstract—Carbon nanotubes (CNTs) are attractive because of their excellent chemical durability mechanical strength and electrical properties. Therefore there is interest in CNTs for not only electrical and mechanical application, but also biological and medical application.

In this study, the dispersion power of surfactant-treated multiwalled carbon nanotubes (MWCNTs) and their effect on the antibacterial activity were examined. Surfactant was used sodium dodecyl-benzenesulfonate (SDBS). UV-vis absorbance and transmission electron microscopy(TEM) were used to characterize the dispersion of MWCNTs in the aqueous phase, showing that the surfactant molecules had been adsorbed onto the MWCNTs surface.

The surfactant-treated MWCNTs exhibited antimicrobial activities to streptococcus mutans. The optical density growth curves and viable cell number determined by the plating method suggested that the antimicrobial activity of surfactant-treated MWCNTs was both concentration and treatment time-dependent.

Keywords—MWCNT, SDBS, surfactant, antibacterial.

I. INTRODUCTION

CNTs were discovered in 1991 as a small byproduct of fullerene synthesis.[1] Multiwalled carbon nanotubes(MWCNTs) show some excellent properties, including high moduli of elasticity, high aspect ratios, excellent thermal and electrical conductivities, and optical and magnetic properties. Due to their excellent optical, electrical, and mechanical properties, carbon nanotubes enjoy a preeminent status in the panoply of nanomaterials, finding wide range of applications in biosensors [2], composites [3], field emission devices [4], electronic components [5], probe tips [6], etc. Delocalization of π -electrons renders them conducting and alleviates adsorption of various chemical moieties via π - π stacking interaction [7]. A high aspect ratio makes them prone to entanglement and bundling. Particularly, carbon nanotubes are bundled with strong van der Waals interaction energy of ca. 500 eV/ μ m of tube-tube contact [8]. Such high interaction energy renders CNT dispersion a challenging task. So, they

need to be functionalized or modified to improve their dispersion ability via covalent or non-covalent attachment of functional groups. The non-covalent method of functionalization is preferred over covalent method because the pore textural properties of CNTs are not affected. This is because the π system of graphene sheets is undisturbed, meaning to say that the external surface area of the tubes will not be affected [9]. Indeed, non-covalent surfactant modification has become a frequently used approach for increasing CNT dispersions due to its simple modification procedures, involving only ultrasonication and centrifugation or filtration, and a capacity for preserving the nanotube structures and properties [10-15].

A wide variety of surfactants have been investigated to date for dispersion of carbon nanotubes, such as sodium dodecyl-benzenesulphonate (SDBS) [16], octyl phenol ethoxylate (TritonX-100) [17], hexadecyltrimethylammonium bromide (CTAB) [18], and sodium dodecyl sulfate (SDS) [19]. However, one usually needs high concentration of surfactants to obtain stable CNT dispersions, which is inconvenient for further CNT processing into composite materials. The introduction of suspensions containing high concentration of surfactants inside the resin/polymer matrices increases the viscosity drastically. This results in a higher viscosity profile, so for further applications like composite processing, the whole process needs to be optimized.

In this study, a surfactant SDBS was used to modify MWCNTs and increase their dispersity in the aqueous phase. Besides, the antibacterial activity of SDBS-treated MWCNTs was also reported. Streptococcus mutans (S. mutans) was used as the testing microorganism. Their antibacterial activity was investigated by determining the optical density (OD) growth curves. The mechanism for the bacterial inactivation of SDBS-modified MWCNTs was discussed.

II. MATERIALS AND METHODS

A. Purification of MWCNTs

MWCNTs were purchased from CNT CO. Ltd. (Korea) with average of 15nm; they synthesized using the chemical vapor deposition technique. Purification method was as follows. 100mg of as-pure MWCNTs were heated in air at 450°C for 90min. The heating MWCNTs were then introduced into a flask containing 6M HCl to remove the metal catalyst (Fe, Mo and Cr). Afterwards, the acid solution was filtered through a membrane filter and the filtered cake was transferred to a flask with 3M NaOH and heated under reflux at 100°C for at least 12h to remove the aluminium oxides. The resulting suspension was filtered and the filtered cake was washed with distilled

J.E. Park, I.S. Park, T.S. Bae, M.H. Lee is with the Institute of Oral Bioscience, School of Dentistry, Chonbuk National University, Chonbuk, 561-756, South Korea (phone& fax: 82632704040; e-mail: pje312@naver.com, ilsong@jbun.ac.kr, bts@jbun.ac.kr, lmh@jbun.ac.kr).

D.J. Yoon is Center for Practical Use of Rare Materials, Sunchon National University, Sunchon, 540-742, South Korea (e-mail: djyoon@snu.ac.kr).

C.H. Sin is Seohae Environment Science Institute, Jeonju, 561-211, South Korea (e-mail: 1005shin@hanmail.net).

M.H.Lee, I.S.Park Corresponding Author is with the Institute of Oral Bioscience, School of Dentistry, Chonbuk National University, Chonbuk, 561-756, South Korea (lmh, ilsong@jbun.ac.kr). T. C. Author is with the Electrical Engineering Department, University of Colorado, Boulder, CO 80309 USA, on leave from the National Research Institute for Metals, Tsukuba, Japan (e-mail: author@nrim.go.jp).

water (DW) until became pH7. Finally, the samples were dried in a vacuum at 55°C for 24h.

B. Preparation of SDBS-treated MWCNTs

In a procedure, 10 mg purified MWCNTs (p-MWCNTs) were added to 50mL SDBS (Sigma–Aldrich, its chemical structure is shown in Fig. 1) solution with concentration of 1wt.%. The dispersion was ultrasonicated for 4h to obtain the SDBS-adsorbed MWCNTs. The resulting suspension was centrifuged at 20,000g for 1h. With UV–vis characterization, the upper supernatant fluid was collected. In other cases, the solution was filtered through a membrane filter (0.20µm) and the filtered cake was washed three times with DW and dried in a vacuum at 55°C for 24h prior to use.

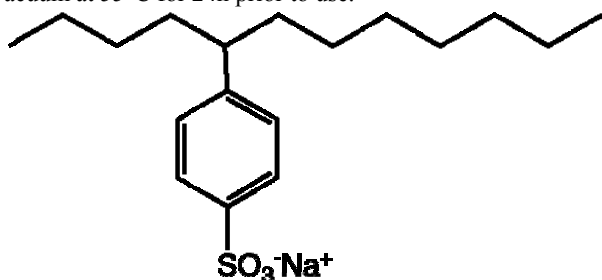


Fig. 1 The chemical structure of SDBS surfactant

C. Characterization of SDBS-treated MWCNTs

The dispersion of SDBS-treated MWCNTs was confirmed using a UV–vis spectrophotometer (Hitachi U-2800) operating between the ranges of 250–1000nm. In the first set of experiments, a baseline correction was carried out using a pure 1wt.% SDBS solution so that their absorbance could be subtracted from the MWCNT dispersions. In the second set of experiments, the baseline was equilibrated and the SDBS-treated MWCNTs were analyzed with the corresponding SDBS concentration.

A transmission electron microscopy (TEM, JEM-2010, JEOL, Japan) image was used to examine the SDBS-adsorbed MWCNTs. The samples were redispersed in DW at a concentration of 0.5mg/mL. A 10µL drop of the above suspension was placed onto a carbon-coated TEM grid (200mesh, purchased from TED PELLA INC.) and viewed under TEM.

D. Preparation of Bacterial Cells

S. mutans JC2 were grown in brain heart infusion (BHI) broth at 37°C under aerobic conditions and harvested in the midexponential growth phase. The cells were washed twice and resuspended in a saline solution (0.9% NaCl) to remove the residual macromolecules and other growth medium constituents.

E. SDBS-treated MWCNT Treatment to Bacterial Cells

150 µL of the bacterial cell suspensions were introduced into 1.5 mL micro centrifuge tubes. 50 µL of the sample suspensions with a desired concentration was added into the tubes. The control samples contained 150 µL of the cell

suspension with 50 µL of DW. The tubes were kept rotating on a shaker at 170 rpm for the required treatment times at room temperature.

F. OD Growth Curve Measurements

After treatment, the mixtures in the tubes were removed and placed into 4 mL of BHI broth. The resulting samples were then incubated at 37 °C in an incubator. Bacterial cell growth was monitored by measuring the OD at 600 nm every 2 h on a spectrophotometer. The OD growth curves were obtained by plotting the OD values vs. the growth time.

G. SEM Imaging

The samples were filtered through a 0.20 µm PVDF membrane (Millipore), fixed with 2.5% glutaraldehyde and post-fixed in 1% osmium tetroxide. Subsequently, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 80%, 90%, 95% and 100%, v/v) and dried at room temperature. The dried samples were then sputter-coated with gold for the field emission scanning electron microscopy (FE-SEM, S-4800 Hitachi, Japan) examinations.

H. Statistical Analysis

All experiments were carried out in triplicate and are expressed as the mean ± standard deviation (SD). Single-factor analysis of variance (ANOVA) was used to assess the statistical significance of the results. A *p* value < 0.05 was considered significant.

III. RESULTS AND DISCUSSION

Bundled CNTs are not active in the UV-vis region. Only individual CNTs absorb in this region [19]. So, a dispersion of CNTs can be characterized by UV-vis absorption spectroscopy. Fig. 2 show photographs of D.W mixed with pure MWCNT(A) and SDBS treated MWCNT(B). also Fig. 3 show the dispersion ability of p-MWCNTs and surfactant-modified MWCNTs detected using a UV–vis spectrophotometer at 500 nm. Absorbance values were recorded at 500nm as reported in previous studies [20-24].

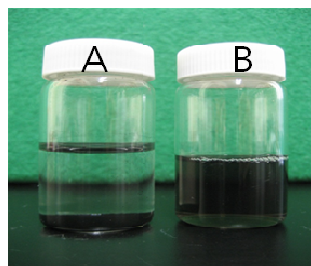


Fig. 2 Photograph of (A) pure MWCNTs, (B) SDBS treated MWCNTs

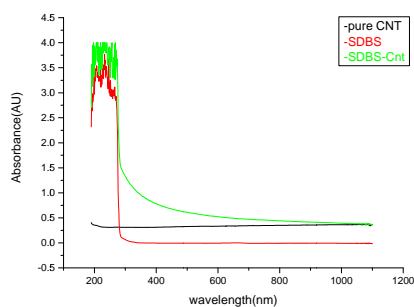


Fig. 3 UV-vis spectra

With the assistance of surfactants, the solubility of the MWCNTs was higher than that in the absence of surfactants. CNTs suspended in water are closely related to the adsorption capacity of the surfactants. This experimentally observed trend for dispersing power of surfactants can also be explained on the basis of their chemical structures. In order to disperse nanotubes in water, surfactant molecules orient themselves in such a fashion that hydrophobic tail groups face toward the nanotube surface while hydrophilic head groups face toward the aqueous phase, producing a lowering of the nanotube/water interfacial tension. Thus, the dispersing power of the surfactant depends on how firmly it adsorbs onto the nanotube surface and produces by this adsorption energy barriers of sufficient height to aggregation. Molecules having the benzene ring structure adsorb more strongly to the graphitic surface due to π - π stacking type interaction [11], [25]. Generally, hydrophobic tail groups tend to lie flat on the graphitic surface because graphitic unit cells match well with the methylene units of hydrocarbon chains[26]. Thus, efficiency of adsorption and consequently dispersing power of surfactants are greatly affected by the tail length of the surfactant. Longer tails means high spatial volume and more steric hindrance, thus providing greater repulsive forces between individual carbon nanotubes [27]. Besides this, surfactants with unsaturated bonds in their tail groups contribute more toward nanotube dispersion [27].

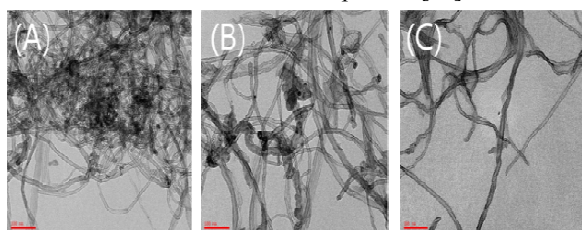


Fig. 4 TEM image (A) as-pure-MWCNTs, (B) Pure MWCNTs, (C) SDBS treated MWCNTs

Fig. 4 show the TEM images of as-p-MWCNTs, p-MWCNTs and surfactant-modified MWCNTs. Large agglomerates and closely packed CNTs are prevalent in the pristine state (A,B), which become significantly loosened and highly dispersed after the surface treatment with SDBS surfactant without the breakage of MWCNTs (C). This suggests that the use of an SDBS surfactant rise to a better dispersion power.

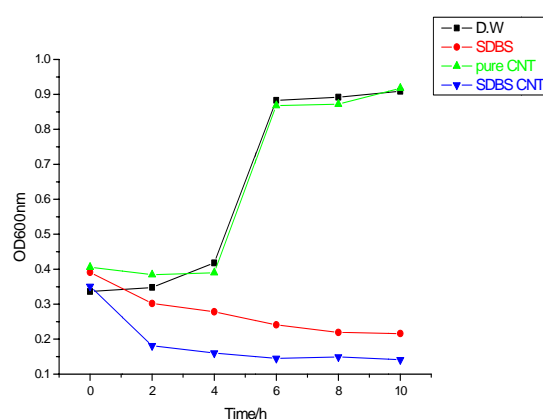


Fig. 5 OD growth curves

Fig. 5 shows the representative OD growth curves of *S. mutans* after being treated with the SDBS-MWCNTs for 60 min. The antibacterial activity of surfactant-modified MWCNTs increases.

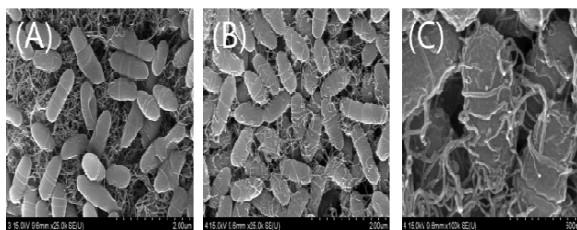
Fig. 6 SEM image of *s. mutans* treated (A) pure MWCNTs, (B) SDBS MWCNTs, (C) high magnification of image (B)

Fig. 6 shows FE-SEM images of the bacteria cells interacting with pure CNT and SDBS-MWCNTs. (A) looks like that the cell are just setting on top the MWCNTs clusters instead of tightly interacting with the MWCNTs. But (B, C) it can seen clearly at edge that individual SDBS-MWCNTs attach one end the cells and stick out the other end from the cells, which act like needles surrounding the cells. Several studies showed that highly dispersed CNTs, whether SWCNTs or MWCNTs, had a strong ability to adhere to bacterial cells due to van der Waals interactions between them [28]. With SWCNTs, they can not only capture cells, but also cause cell death due to the direct physical puncture, resulting in damages to the outer membrane of cells. However, in contrast to SWCNTs, although MWCNTs can also capture bacteria cells, they cannot effectively kill them, which is probably due to a large diameter of the MWCNTs compared to that of SWCNTs. This was confirmed by Akasaka and Watari who reported that MWCNTs (diameter \approx 30 nm) had a strong capacity to adhere to or capture bacteria, but the use of MWCNTs that could adhere to bacteria via physical sorption was not linked to antibacterial resistance [29]. Yang and Arias also found that MWCNTs with -OH and -COOH groups could form cell aggregates, but they did not exhibit antibacterial activity [30].

IV. CONCLUSION

The dispersion ability of surfactant-treated MWCNTs and its antibacterial activity to *S. mutans* were investigated. Both the UV-vis absorption and TEM images suggest that the surfactant treated have an ability to disperse MWCNTs in the aqueous phase. The OD growth curves confirmed that the surfactant-treated MWCNTs showed high antibacterial activity. Moreover, the SEM images also showed the well interactions between the bacterial cells and surfactant-treated MWCNTs. The binding of surfactant molecules to MWCNTs increases its dispersing ability in aqueous solution. Well dispersed CNT increased the antibacterial activity.

ACKNOWLEDGMENT

This work was financially supported by the 'National Research Foundation of Korea (NRF) grant funded by the Korea government (MEST) (No. 2011-0028709)' and **핵심**.

REFERENCES

- [1] S. Iijima, "Helical microtubules of graphitic carbon," *nature*, vol. 354, pp. 56-58, 1991.
- [2] X. Tang, S. Bansaruntip, N. Nakayama, E. Yenilmez, Y.-I. Chang, and Q. Wang, "Carbon Nanotube DNA Sensor and Sensing Mechanism," *Nano Letters*, vol. 6, pp. 1632-1636, 2006/08/01 2006.
- [3] L. M. Clayton, A. K. Sikder, A. Kumar, M. Cinke, M. Meyyappan, T. G. Gerasimov, and J. P. Harmon, "Transparent Poly(methyl methacrylate)/Single-Walled Carbon Nanotube (PMMA/SWNT) Composite Films with Increased Dielectric Constants," *Advanced Functional Materials*, vol. 15, pp. 101-106, 2005.
- [4] J. Koohsorkhi, Y. Abdi, S. Mohajerzadeh, H. Hosseinzadegan, Y. Komijani, and E. A. Soleimani, "Fabrication of self-defined gated field emission devices on silicon substrates using PECVD-grown carbon nano-tubes," *Carbon*, vol. 44, pp. 2797-2803, 2006.
- [5] E. Frackowiak and F. Béguin, "Electrochemical storage of energy in carbon nanotubes and nanostructured carbons," *Carbon*, vol. 40, pp. 1775-1787, 2002.
- [6] H. Dai, J. H. Hafner, A. G. Rinzler, D. T. Colbert, and R. E. Smalley, "Nanotubes as nanoprobe in scanning probe microscopy," *nature*, vol. 384, pp. 147-150, 1996.
- [7] M. Terrones, "Science and technology of the twenty-first century: synthesis, properties, and applications of carbon nanotubes," *Annual Review of Materials Research*, vol. 33, pp. 419-501, 2003.
- [8] L. Girifalco, M. Hodak, and R. S. Lee, "Carbon nanotubes, buckyballs, ropes, and a universal graphitic potential," *Physical Review B*, vol. 62, p. 13104, 2000.
- [9] B. Shi, X. Zhuang, X. Yan, J. Lu, and H. Tang, "Adsorption of atrazine by natural organic matter and surfactant dispersed carbon nanotubes," *Journal of Environmental Sciences*, vol. 22, pp. 1195-1202, 2010.
- [10] M. Bystrzejewski, A. Huczko, H. Lange, T. Gemming, B. Büchner, and M. Rummeli, "Dispersion and diameter separation of multi-wall carbon nanotubes in aqueous solutions," *Journal of Colloid and Interface Science*, vol. 345, pp. 138-142, 2010.
- [11] M. Islam, E. Rojas, D. Bergey, A. Johnson, and A. Yodh, "High weight fraction surfactant solubilization of single-wall carbon nanotubes in water," *Nano Letters*, vol. 3, pp. 269-273, 2003.
- [12] J. Rausch, R. C. Zhuang, and E. Mäder, "Surfactant assisted dispersion of functionalized multi-walled carbon nanotubes in aqueous media," *Composites Part A: Applied Science and Manufacturing*, vol. 41, pp. 1038-1046, 2010.
- [13] Y. Geng, M. Y. Liu, J. Li, X. M. Shi, and J. K. Kim, "Effects of surfactant treatment on mechanical and electrical properties of CNT/epoxy nanocomposites," *Composites Part A: Applied Science and Manufacturing*, vol. 39, pp. 1876-1883, 2008.
- [14] Y. Bai, D. Lin, F. Wu, Z. Wang, and B. Xing, "Adsorption of Triton X-series surfactants and its role in stabilizing multi-walled carbon nanotube suspensions," *Chemosphere*, vol. 79, pp. 362-367, 2010.
- [15] R. Rastogi, R. Kaushal, S. Tripathi, A. L. Sharma, I. Kaur, and L. M. Bharadwaj, "Comparative study of carbon nanotube dispersion using surfactants," *Journal of Colloid and Interface Science*, vol. 328, pp. 421-428, 2008.
- [16] J. Hilding, E. A. Grulke, Z. G. Zhang, and F. Lockwood, "Dispersion of carbon nanotubes in liquids," *Journal of Dispersion Science and Technology*, vol. 24, pp. 1-41, 2003.
- [17] H. Wang, W. Zhou, D. L. Ho, K. I. Winey, J. E. Fischer, C. J. Glinka, and E. K. Hobbie, "Dispersing single-walled carbon nanotubes with surfactants: a small angle neutron scattering study," *Nano Letters*, vol. 4, pp. 1789-1793, 2004.
- [18] A. Ryabenko, T. Dorofeeva, and G. Zvereva, "UV-VIS-NIR spectroscopy study of sensitivity of single-wall carbon nanotubes to chemical processing and Van-der-Waals SWNT/SWNT interaction. Verification of the SWNT content measurements by absorption spectroscopy," *Carbon*, vol. 42, pp. 1523-1535, 2004.
- [19] J. Yu, N. Grossiord, C. E. Koning, and J. Loos, "Controlling the dispersion of multi-wall carbon nanotubes in aqueous surfactant solution," *Carbon*, vol. 45, pp. 618-623, 2007.
- [20] W. H. Huang, "Quantum stabilization of compact space by extra fuzzy space," *Physics Letters B*, vol. 537, pp. 311-320, 2002.
- [21] K. D. Ausman, R. Piner, O. Lourie, R. S. Ruoff, and M. Korobov, "Organic solvent dispersions of single-walled carbon nanotubes: toward solutions of pristine nanotubes," *The Journal of Physical Chemistry B*, vol. 104, pp. 8911-8915, 2000.
- [22] O. K. Kim, J. Je, J. W. Baldwin, S. Kooi, P. E. Pehrsson, and L. J. Buckley, "Solubilization of single-wall carbon nanotubes by supramolecular encapsulation of helical amylose," *Journal of the American Chemical Society*, vol. 125, pp. 4426-4427, 2003.
- [23] V. A. Sinani, M. K. Gheith, A. A. Yaroslavov, A. A. Rakhnyanskaya, K. Sun, A. A. Mamedov, J. P. Wicksted, and N. A. Kotov, "Aqueous dispersions of single-wall and multiwall carbon nanotubes with designed amphiphilic polycations," *J. Am. Chem. Soc.*, vol. 127, pp. 3463-3472, 2005.
- [24] A. Ikeda, T. Hamano, K. Hayashi, and J. Kikuchi, "Water-solubilization of nucleotides-coated single-walled carbon nanotubes using a high-speed vibration milling technique," *Organic Letters*, vol. 8, pp. 1153-1156, 2006.
- [25] J. F. Liu and W. A. Ducker, "Self-assembled supramolecular structures of charged polymers at the graphite/liquid interface," *Langmuir*, vol. 16, pp. 3467-3473, 2000.
- [26] D. M. Cyr, B. Venkataraman, and G. W. Flynn, "STM investigations of organic molecules physisorbed at the liquid-solid interface," *Chemistry of materials*, vol. 8, pp. 1600-1615, 1996.
- [27] D. H. Napper, *Polymeric stabilization of colloidal dispersions* vol. 7: Academic Press London, 1983.
- [28] C. Yang, J. Mamouni, Y. Tang, and L. Yang, "Antimicrobial activity of single-walled carbon nanotubes: length effect," *Langmuir*, 2010.
- [29] T. Akasaka and F. Watari, "Capture of bacteria by flexible carbon nanotubes," *Acta Biomaterialia*, vol. 5, pp. 607-612, 2009.
- [30] L. R. Arias and L. Yang, "Inactivation of bacterial pathogens by carbon nanotubes in suspensions," *Langmuir*, vol. 25, pp. 3003-3012, 2009.