

Biodistribution of Aqueous Suspensions of Carbon Nanotubes in Mice and Their Biocompatibility

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In this study, we prepared two-types of water-dispersible carbon nanotubes (CNTs) and investigated their biodistribution in mice as well as bio-/cyto-compatibility. After administration, their organs were excised at various post-injection times, then observed using both optical and transmission electron microscopy (TEM). The color of the liver and lung markedly darkened, suggesting that administered CNTs reached these organs. By TEM observation, the CNTs were found in the liver and lung. They were observed even in the kidney and spleen, though their distributions in those organs were very low compared with that in liver and lung. Therefore, most of the administered CNTs would be accumulated in the liver or lung. However, the time profile of the body weight of CNT-administered mice was close to that of control mice. In addition, we estimated the cyocompatibility of the water-dispersible CNTs for hepatocytes. According to a TNF- α assay of the cells cultured with CNTs, the expression level was almost the same as that of the control. These results suggested that the water-dispersible CNTs have good bio-/cyto-compatibility under this condition.

Keywords: Biodistribution, Biocompatibility, Hydrophilic Carbon Nanotubes, Cytotoxicity.

1. INTRODUCTION

In recent years, carbon nanotubes (CNTs) have received much attention for their potential applications to electronic,^{1–3} mechanical,^{4–6} chemical^{7,8} and biotechnological fields.^{9,10} In particular, many researchers have paid attention to the interactions of CNTs with living organisms or the environment. The assessment of the effects on the human body is an important problem facing the potential use of CNTs for biomedical applications, such as drug or gene delivery systems, cancer tracking, and tissue engineering scaffolds.^{11–16} However, the effects of CNTs on animals, living organisms or cells have not been investigated enough.^{17–19} Some of the numerous materials considered biocompatible at the macro level have shown toxicity *in vitro* when the particle size reaches the micro-/nano-level.²⁰ In a previous study, we determined that even biocompatible materials such as Ti and TiO₂ cause inflammation with the decrease

of particle size.²¹ In a previous study, we reported that several micro- or nano-sized metal or metal oxide particles were accumulated in the liver.²² In the case of carbon nanotubes, some researchers reported that administered carbon nanotubes had reached the liver.²³ In general, the liver is one of the most vital organs present in animals and has a wide range of functions. Liver cells can metabolize, detoxify, and inactivate exogenous compounds such as drugs. The detection and investigation of CNTs in living organs is one of the problems in the elucidation of CNT biodistribution. For example, CNTs were administered to mice and the excised organs were homogenized. Columnchromatography was then carried out to detect CNTs. Many researchers have investigated the biodistribution of SWCNTs quantitatively based on radio-isotopic measurement after radioactive labeling of CNTs. Wang et al. prepared single-walled CNTs (SWCNTs) labeled ¹²⁵I and then determined the biodistribution in mice after administration.^{23a, 24a} Singh et al.^{24b} synthesized SWCNTs with a chelating molecule labeled the CNTs with radioactive metal, and then investigated the biodistribution. In addition, some researchers recently

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detected the biodistribution of SWCNTs based on Raman spectroscopy.^{23b,c} Though many researchers have reported the distribution of SWCNTs by comparison there has been insufficient investigation of multi-walled CNTs (MWCNTs). Guo^{25a} and Liu^{23d,e} et al. investigated the biodistribution of MWCNT-labeled radiotracers in mice. Jia et al.^{25b} reported the fluorescent imaging of the biodistribution of subcutaneously injected porphyrin-conjugated MWCNTs. Lacerda et al.^{25c} investigated the circulation and excretion behavior of chelating compounds tethered to MWCNTs in rats. We also directly observed specimens coming from mice injected with polycarboxylated MWCNTs using a transmission electron microscopy.^{23f} However, the organs or tissues, in which CNTs were mainly accumulated, varied from study to study. Therefore, the biodistribution of CNTs remains unclear.

In this study, we prepared two types of water-dispersible carbon nanotubes. The obtained compounds were characterized using Raman and Fourier-transfer infrared (FTIR) spectroscopy. To investigate the biodistribution and biocompatibility of CNTs, we injected the obtained CNT solution into mice intravenously and then monitored their body weight for 4 weeks post-injection. Specimens of excised organs from mice administered CNTs were also observed using a transmission electron microscope to visualize the biodistribution. To investigate the toxicity of CNTs *in vitro*, hepatocytes are representative of cells of human organisms, since the liver plays an important role in the defense system and in metabolism.

2. EXPERIMENTAL DETAILS

2.1. Materials and Syntheses

In this study, we prepared two types of water-dispersible carbon nanotubes. The MWCNTs used in this study were obtained from NanoLab (Brighton, MA, USA). They were heated at 500 °C and washed with HCl for purification. To prepare a water-dispersible carbon nanotube, a polycarboxylation reaction of MWCNTs was undertaken. The MWCNTs were dispersed in *o*-dichlorobenzene with sonication. A large excess of succinic acid peroxide was added to the solution, and the reaction mixtures were stirred at 80 °C. When the reaction was completed, the products were washed with tetrahydrofuran and then ethanol. To obtain other water-dispersible CNTs, the purified CNTs were dispersed in nitric acid and refluxed for 48 hrs. After each modification reaction, they were filtered and then dried in vacuo. The details are described in Refs. [23f and 26].

To identify the modification reaction processing, we measured the Raman and infrared spectra of the product using a Raman spectrometer system (Renishaw inVia Reflex) and an FTIR spectrometer (JASCO FT/IR-410). The morphologies of the obtained CNTs were observed by scanning electron microscopy (SEM: Hitachi S-4000)

and atomic force microscopy (AFM: Digital Instruments Nanoscope IIIa).

2.2. Biodistribution

Male mice (Jcl: ICR), 8–12 weeks old, were obtained from Nippon CLEA Japan, Inc. (Tokyo, Japan). The mice were randomly divided into groups (five mice per group). We injected 0.5 mL of the modified CNTs in aqueous dispersion (0.5 mg/mL) into the tail veins. After the water-dispersible CNTs were administered, the body weight of the mice was monitored for 4 weeks and compared with that of control mice injected with 0.5 mL of normal saline. CNT-administered mice were sacrificed at 1 day, 1 week, and 4 weeks post-injection. The lung, liver, spleen and kidney were excised and subjected to transmission electron microscopy (TEM: Hitachi H-800) at 75 kV. All operations on animals were in accord with the institutional animal use and care regulations of Hokkaido University.

2.3. Cytocompatibility

To estimate the cytotoxicity of water-dispersible CNTs, we exposed these particles to human hepatocytes, Hc cells (Cell Systems, Kirkland, WA, USA). Hc cells were seeded on 12-well plates (Hydro Cell, Wako, Osaka, Japan) at a density of approximately 1×10^4 per well and incubated at 37 °C in a humidified 5%-CO₂ atmosphere for 1 day ($n = 5$). After 1 day incubation, the culture medium was aspirated and another fresh medium including the CNTs was added. The concentrations of the particles were adjusted to 0, 0.01, 0.1, 1 and 10 ppm, respectively. We also used THP-1 as a representative of monocytes to compare with cell functions of Hc. During incubation, TNF- α in the supernatant of cell culture medium at 1 day after the incubation with CNTs was measured using ELISA kits (Bender MedSystems GmbH, Vienna, Austria). Details of the procedure were described in Ref. [26].

3. RESULTS AND DISCUSSION

3.1. Preparation of Water-Dispersible MWCNTs

We obtained a black precipitate (hydrophilic MWCNTs: MWCNT-(CH₂CH₂COOH)_n and MWCNT-(OH)_n), hereafter called CNT-COOH and CNT-OH, respectively (Fig. 1). The modification of the surfaces of the MWCNTs was characterized by Raman and FT-IR spectroscopy, respectively. Figure 2(a) is a typical Raman spectrum of pristine MWCNTs before modification. The relative intensity of the Raman peak of the *D*-band (~ 1340 cm⁻¹) increased after modification (Figs. 2(b and c)). The intensity reached almost the same value as that of the *G*-band (~ 1570 cm⁻¹). To characterize CNT-COOH, FT-IR spectroscopy was also carried out.²⁷ In

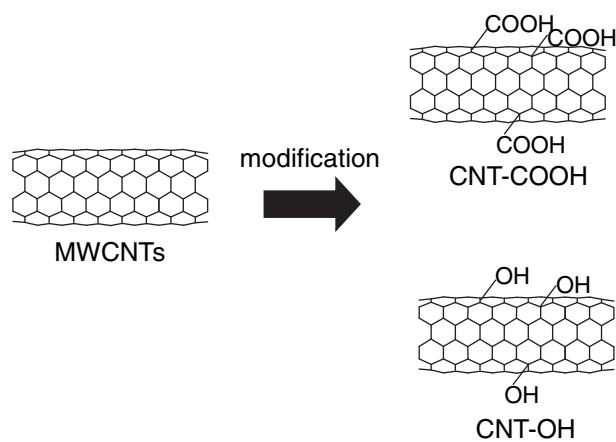


Fig. 1. Schematic structure of MWCNTs used.

a previous study,^{23f} we also determined that the introduction ratio of carboxyl groups to CNTs. The values varied from 3 to 10 wt%, depending on the reaction condition.

Figure 3 shows typical SEM images of pristine MWCNTs and poly-carboxylated MWCNTs (CNT-COOH), respectively. A large number of string-like carbon nanotubes were found to be mutually twisted. They formed a porous structure consisting of random, overlapped carbon nanotubes. The aggregation of CNTs was improved by the poly-carboxylation reaction, and disaggregated, single-string-like MWCNTs were observed (Fig. 3(b)). The CNT-OH also showed a disaggregated trend resulting from the poly-hydroxylation.

We also carried out atomic force microscopy to characterize the morphology of the CNTs obtained. A typical

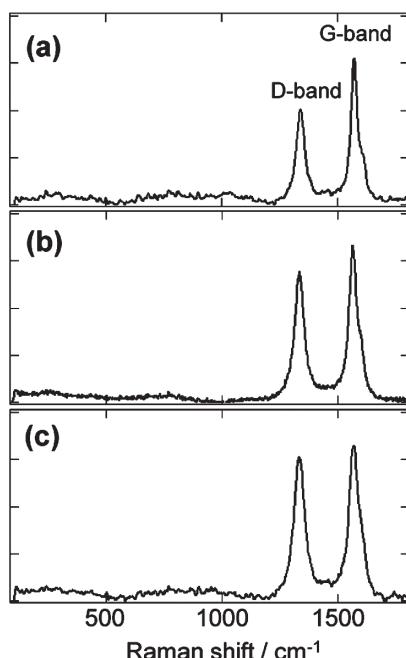


Fig. 2. Raman spectra of MWCNT materials: (a) pristine MWCNTs; (b) CNT-COOH; (c) CNT-OH.

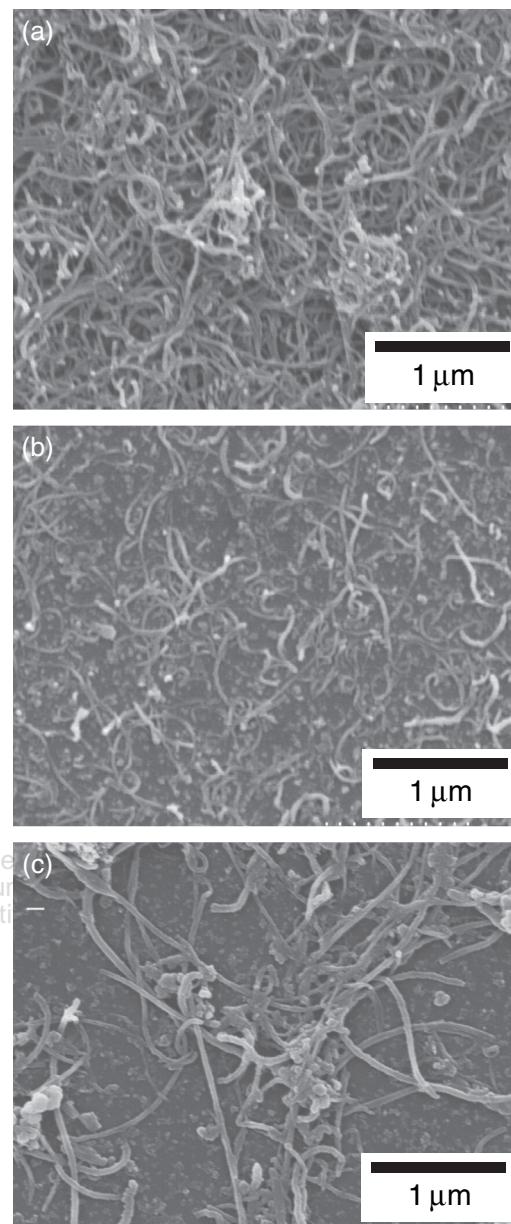


Fig. 3. Scanning electron microscopic images of: (a) pristine MWCNTs; (b) CNT-COOH; (c) CNT-OH.

AFM image of the CNT-COOH is shown in Figure 4. Curled, single-string-like nanotubes were observed. The CNTs ranged in length from 1.0 to 5.0 μ m, and their diameter was about 30 nm. The CNTs got twisted in three dimensions then formed a network structure of about 80 nm in height. These results were in good agreement with the SEM observation described above.

3.2. Biodistribution of Water-Dispersible CNTs in Mice

To elucidate biodistribution and acute toxicity, we administered 0.5 mL of water-dispersible CNT solution through the tail veins of mice. After administration of the CNTs

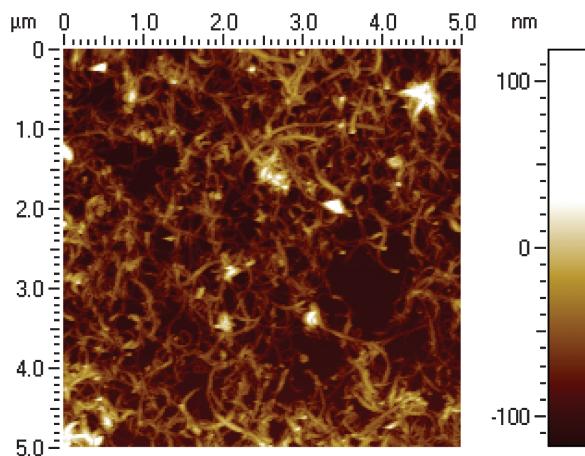


Fig. 4. Atomic force microscopic images of CNT-COOH.

(CNT-COOH or CNT-OH), the body weight of the mice was observed with post-injection time. The weights were normalized by the initial value of the two types of CNT-administered groups (31.7 ± 0.5 , 33.1 ± 0.2 g) and controls (34.6 ± 0.7 g), respectively. The amount of single-dose CNTs was 0.25 mg (0.5 mL of 0.5 mg/mL solution). The change in body weight over time between the water-dispersible CNTs-administered mice and the controls was almost the same (Fig. 5). When the dosage reached 0.5 or 1 mg, the mice showed a similar growth tendency.^{23f} These results suggested that the CNTs did not exhibit serious acute toxicity. Figure 6 shows optical images of the livers and lungs of mice administered CNTs. As shown in Figures 6(b and c), the lungs at 1 week post-injection showed drastically different color compared with that of the control group (administered saline, Fig. 6(a)). The same tendency was observed in the lungs, as shown in Figure 6(e). The color change can be attributable to the accumulation of CNT in the organs. However, the color of the organs tended to be restored at 4 weeks post-injection time, as shown in Figure 6(d). In this study, a distinct color change in the organs was seen only on the liver and lungs and not on the kidney and spleen. These results suggested that the administered CNTs temporarily accumulated in these organs after the

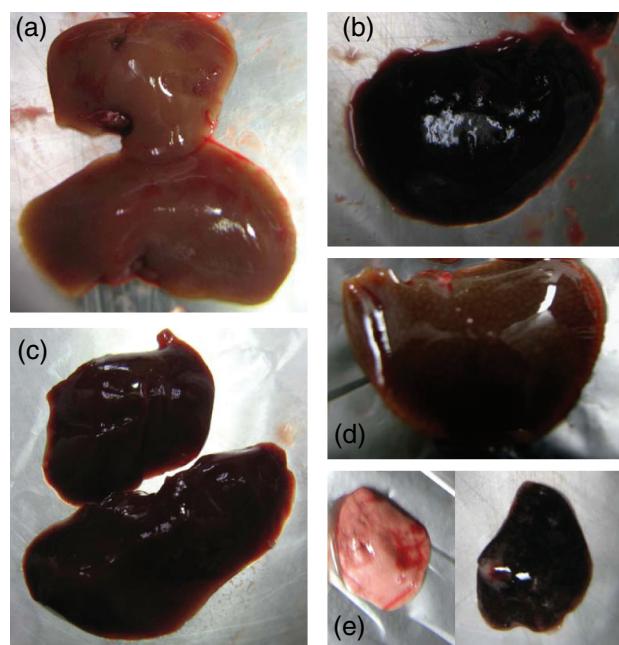


Fig. 6. Optical images of the liver and lung of mice. (a): control (saline injection), (b): CNT-COOH administered at 1 week post-injection, (c): CNT-OH administered at 1 week post-injection (d): 4 week post-injection, (e): lung at 1 week post-injection (left: control, right: CNT administration).

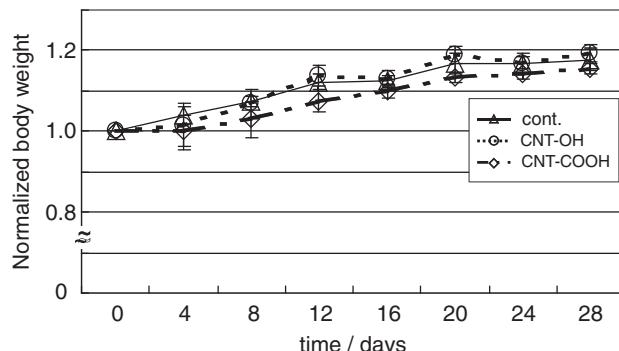


Fig. 5. Time-profile of the body weight of mice administered CNTs.

the amount in the liver remained. Guo et al.^{25a} reported that they had functionalized MWCNTs by glucosamine and labeled them by ^{99m}Tc . They injected the MWCNTs into mice intraperitoneally then investigated the biodistribution based on radioactive measurement. During the 24 hr study, the MWCNT derivatives were temporarily retained in the liver, lung, kidney and, especially, in the stomach. However, more than 70% of the injected MWCNTs were excreted as urine and feces in that period. One of the reasons why the CNTs accumulated particularly in the stomach would be the point of injection. Jia et al.^{25b}

investigated the biodistribution of porphyrin-conjugated MWCNTs based on fluorescent imaging. After subcutaneous injection, the MWCNTs were detected mainly in the subcutaneous connective mucosa even 130 days post-injection. The MWCNTs were also observed in the liver, kidney, spleen, and lung in small numbers compared with that in mucosa. On the other hand, Lacerda et al.^{25c} investigated the circulation and excretion behavior of chelating compounds tethered to MWCNTs in rats. At 24 hr post-injection, the intravenously administered MWCNTs were detected in urine. MWCNTs remaining in the body were

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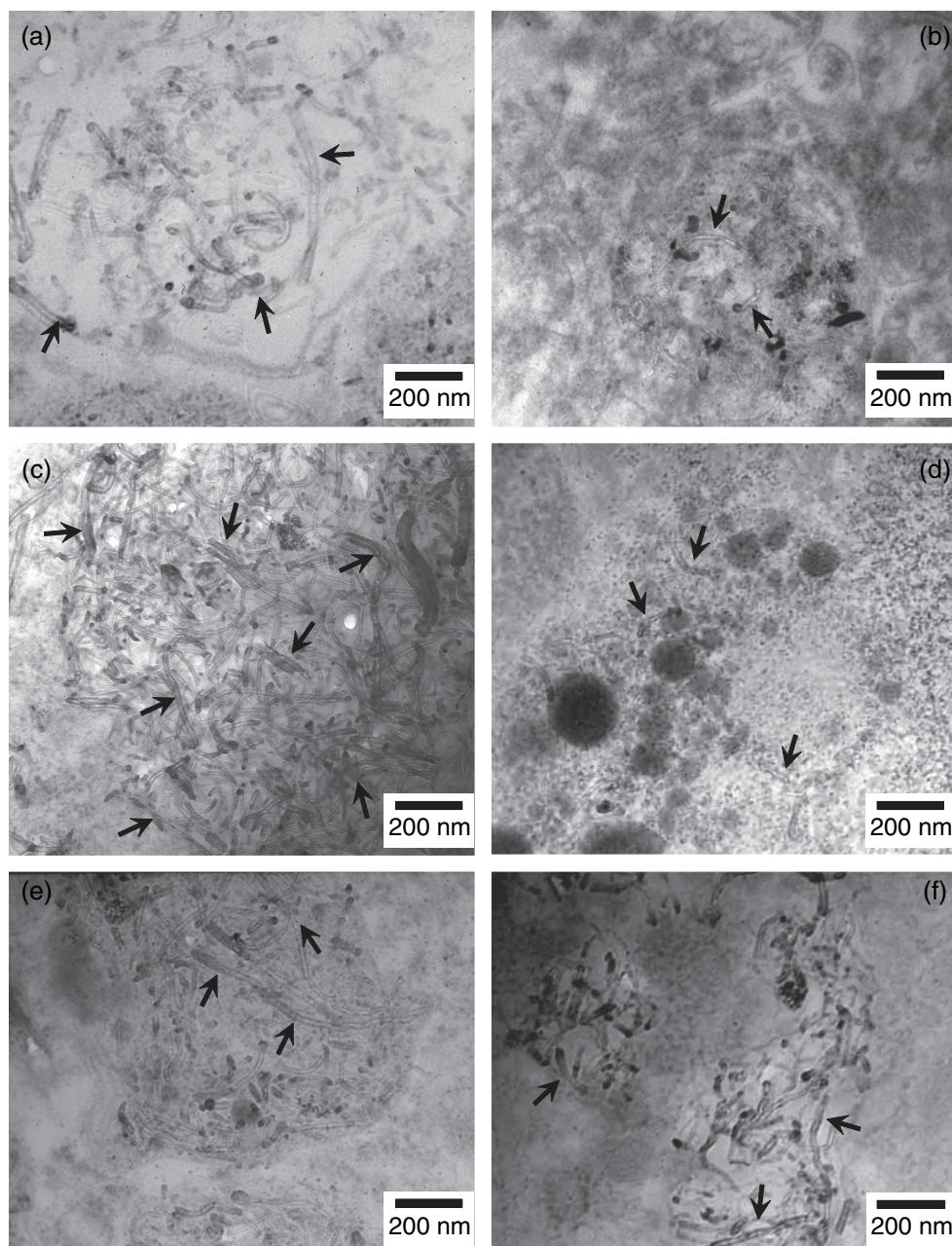


Fig. 7. Transmission electron microscopic images of excited organs of mice administered CNTs. (a): liver at 1 week post-injection, CNT-COOH administered, (b): liver at 1 week post-injection, CNT-OH administered, (c): lung at 1 week post-injection, (d): spleen at 1 week post-injection, (e): liver at 4 weeks post-injection, (f): lung at 4 weeks post-injection. (some CNTs indicated by black arrows)

observed mainly in the kidney. Though some groups investigated the accumulation/excretion behavior of MWCNTs in animals, the distribution ratio and the circulation-time profile were varied. One of the reasons for this different biodistribution is that the MWCNT surface was modified by individual water-soluble materials such as taurine, porphyrin, and diethylenetriaminopentaacetic compounds. It is assumed that the biocompatibility and the biodistribution behaviors of CNTs coated with water-soluble materials are quite different from those of pristine CNTs. These behaviors would be strongly affected by the water-soluble materials. To obtain high solubility, CNTs were functionalized by surfactants, saccharide, polyethyleneglycol, polyvinylpyrrolidone, alginate, protein, and DNA through covalent or non-covalent interactions.^{19, 22a, b, 28} Therefore, there are some problems in the elucidation of CNTs' biodistribution though elucidation of the biodistribution is essential for bioapplications of CNTs. According to those speculations, we prepared two types of water-dispersible MWCNTs presenting simple functional groups ($-\text{COOH}$ or $-\text{OH}$) on the surface and then investigated their biodistribution and biocompatibility. Though the surface nature of polycarboxylated CNTs was considered to be different from that of the pristine one, the difference may be smaller than that between the pristine one and the CNTs functionalized by water-soluble materials, because CNTs can be carboxylated under relatively mild conditions.

According to the results, the CNT-administered mice showed similar growing-time profiles in body weight to those of the controls after a dosage of 0.25 mg. Some researchers described above injected 0.1 mg or less of SWCNTs or MWCNTs into mice intravenously. Thus, the CNT dose in our study is higher than in those investigations. This suggests that the CNTs used in this study did not exhibit serious acute toxicity during the 4 week experiment, though they did accumulate in some organs.

3.3. Cytocompatibility of Water-Dispersible CNTs

According to the biodistribution of water-dispersible CNTs, the administered CNTs accumulated in the liver after administration and remained even at 4 weeks' post-injection. We carried out a cell function test of hepatocytes that come from human liver. Figure 8 shows the amounts of TNF- α released from monocytes (THP-1) and hepatocytes (Hc) at 24 hr after exposure to CNTs. THP-1 produced TNF- α in a dose-dependent response to the CNT content. Hc also produced a certain amount of TNF- α . However, the expression of TNF- α of hepatocytes was low and was very slightly different from that of the control. These results showed that CNTs induce phagocytosis and TNF- α emission to monocytes, but very little emission to hepatocytes. At the initial period (1 hour), THP-1 released TNF- α when the concentration of CNTs was high and Hc released a negligible level. In a previous study,²⁶

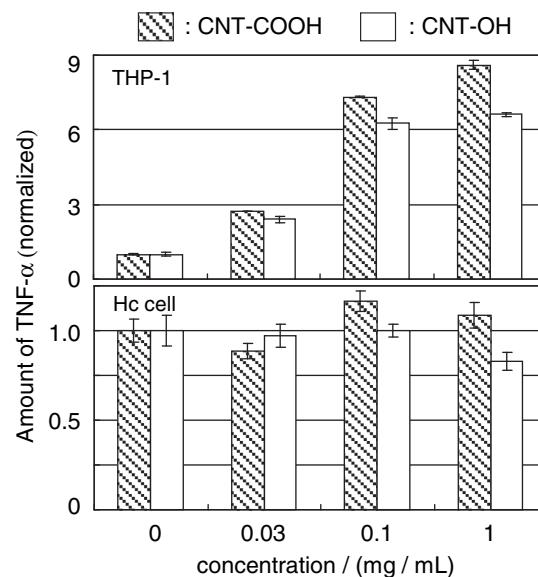


Fig. 8. Amount of TNF- α released from monocytes (THP-1) and human hepatocytes (Hc) at 1 day after exposure to hydrophilic CNTs

cytokine, TNF- α , and superoxide dismutase (SOD) activity were measured to evaluate the cytotoxicity of CNT-OH for cells. The CNTs have the capacity to stimulate both monocytes and hepatocytes to produce TNF- α , one of the most representative cytokines for inflammation, but the stimulated amount of TNF- α was much lower than that stimulated by LPS.²⁹ The amount of TNF- α released by hepatocytes after incubation with CNTs showed no significant difference compared to the control. Therefore, these results suggested that the water-dispersible CNTs would be a slight stimulus to hepatocytes but had very little cytotoxicity, which could lead to cell malfunction or cell death.

4. CONCLUSION

In this study, we prepared and characterized two types of water-dispersible carbon nanotubes. The obtained water-dispersible carbon nanotubes were administered to mice to investigate their biodistribution and bio-/cytocompatibility. The CNT exposure was at least twice that used by other researchers. Though the injected CNTs reached some organs, they did not cause significant acute toxicity in this study. We also estimated their cytocompatibility for hepatocytes because the CNTs accumulated in the liver through blood circulation. Both the CNTs indicated only slight stimulus responses the same level as the control. These results suggest that CNTs did not have significant acute toxicity *in vivo* and *in vitro* at least in this condition. In this study, we investigated two types of water-dispersible carbon nanotubes consisting of simple functional groups ($-\text{COOH}$ or $-\text{OH}$) instead of multifunctional compounds. Though the surface nature of the functionalized CNTs was considered to be different from that of the pristine one, the difference may be smaller than that

between the pristine one and the CNTs functionalized by water-soluble materials. Therefore, our results can help to understand the biodistribution of pristine CNTs. In addition, carboxyl groups can be involved in reactive sites. It has the potential for use as a platform for bioapplications with tethering, such as drugs, genes or DNA.

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References and Notes

1. M. Majumder, X. Zhan, R. Andrews, and B. J. Hinds, *Langmuir* 23, 8624 (2007).
2. W. I. Milne, K. B. K. Teo, G. A. J. Amaralunga, P. Legagneux, L. Gangloff, J. P. Schnell, V. Semet, V. Thien Binh, and G. J. Groening, *Mater. Chem.* 14, 933 (2004).
3. A. Javey, J. Guo, Q. Wang, M. Lundstrom, and H. Dai, *Nature* 424, 654 (2003).
4. B. S. Shim, Z. Y. Tang, M. P. Morabio, A. Agarwal, H. P. Hong, and N. A. Kotov, *Chem. Mater.* 19, 5467 (2007).
5. W. Wang, F. Watari, M. Ohmori, S. Liao, Y. Zhu, A. Yokoyama, M. Uo, H. Kimura, and A. J. Ohkubo, *Biomed. Mater. Res. B. Appl. Biomat.* 82, 223 (2007).
6. C. Velasco-Santos, A. L. Martinez-Hernandez, F. T. Fisher, R. Ruoff, and V. M. Castano, *Chem. Mater.* 15, 4470 (2003).
7. M. Prato, K. Kostarelos, and A. Bianco, *Accounts Chem. Res.* 41, 60 (2008).
8. B. S. Harrison and A. Atala, *Biomaterials* 28, 344 (2007).
9. A. Bianco, K. Kostarelos, C. D. Partidos, and M. Prato, *Chem. Commun.* 571 (2005).
10. X. Li, Y. B. Fan, and F. Watari, *Biomed. Mater.* 5, 22001 (2010).
11. Y. Lin, S. Taylor, H. P. Li, K. A. S. Fernando, L. W. Qu, W. Wang, L. R. Gu, B. Zhou, and Y. P. Sun, *J. Mater. Chem.* 14, 527 (2004).
12. W. H. De Jong and P. J. Born, *Int. J. Nanomedicine* 3, 133 (2008).
13. D. Pantarotto, R. Singh, D. McCarthy, M. Erhardt, J. P. Briand, M. Prato, K. Kostarelos, and A. Bianco, *Angew. Chem. Int. Ed. Engl.* 43, 5242 (2004).
14. B. S. Harrison and A. Atala, *Biomaterials* 28, 344 (2007).
15. N. Aoki, T. Akasaka, F. Watari, and A. Yokoyama, *Dent. Mater. J.* 26, 178 (2007).
16. M. Terada, S. Abe, T. Akasaka, M. Uo, Y. Kitagawa, and F. Watari, *Dent. Mater. J.* 26, 178 (2009).
17. L. P. Zanello, B. Zhao, H. Hu, and R. C. Haddon, *Nano Lett.* 6, 562 (2006).
18. E. Mooney, P. Dockery, U. Greiser, M. Murphy, and V. Barron, *Nano Lett.* 8, 2137 (2008).
19. (a) N. W. Shi Kam, T. C. Jessop, P. A. Wender, and H. Dai, *J. Am. Chem. Soc.* 126, 6850 (2004); (b) A. Bhirde, V. Patel, J. Gavard, G. Zhang, A. Sousa, A. Masedunskas, R. Leapman, R. Weigert, J. Silvio Gutkind, and J. Rusling, *ACS Nano* 3, 307 (2009).
20. F. Watari, N. Takashi, A. Yokoyama, M. Uo, T. Akasaka, Y. Sato, S. Abe, Y. Totsuka, and K. Tohji, *J. R. Soc. Interface* 6, S371 (2009).
21. F. Watari, S. Abe, C. Koyama, A. Yokoyama, T. Akasaka, M. Uo, M. Matsuoka, Y. Totsuka, M. Esaki, M. Morita, and T. Yonezawa, *J. Ceram. Soc. Jpn.* 116, 1 (2008).
22. (a) S. Abe, C. Koyama, M. Uo, T. Akasaka, Y. Kuboki, and F. Watari, *J. Nanosci. Nanotechnol.* 9, 4988 (2009); (b) S. Abe, I. Kida, M. Esaki, T. Akasaka, M. Uo, T. Hosono, Y. Sato, B. Jeyadevan, Y. Kuboki, M. Morita, K. Tohji, and F. Watari, *Bio-Med. Mater. Eng.* 19, 213 (2009); (c) S. Abe, I. Kida, M. Esaki, N. Iwadera, M. Mutoh, C. Koyama, T. Akasaka, M. Uo, Y. Kuboki, M. Morita, Y. Sato, K. Haneda, T. Yonezawa, B. Jeyadevan, K. Tohji, and F. Watari, *J. Ceram. Soc. Jpn.* 118, 525 (2010).
23. (a) S. Yang, W. Guo, Y. Lin, X. Deng, H. Wang, H. Sun, X. Wang, W. Wang, M. Chen, Y. Huang, and Y. Sun, *J. Phys. Chem. C* 111, 17761 (2007); (b) Z. Liu, C. Davis, W. Cai, L. He, X. Chen, and H. Dai, *Proc. Natl. Acad. Sci. USA* 105, 1410 (2008); (c) B. King, D. Yu, Y. Dai, S. Chang, D. Chen, and Y. Ding, *Carbon* 47, 1189 (2009); (d) X. Deng, G. Jia, H. Wang, H. Sun, X. Wang, S. Yang, T. Wang, and Y. Liu, *Carbon* 45, 1419 (2007); (e) X. Deng, S. Yang, H. Nie, H. Wang, and Y. Liu, *Nanotechnol.* 19, 75101 (2008); (f) S. Abe, D. Hayashi, T. Akasaka, M. Uo, Y. Kuboki, F. Watari, and T. Takada, *Nano Biomedicine* 1, 143 (2009).
24. (a) H. Wang, J. Wang, X. Deng, H. Sun, Z. Shi, Z. Gu, Y. Liu, and Y. Zhao, *J. Nanosci. Nanotechnol.* 4, 1019 (2004); (b) R. Singh, D. Pantarotto, L. Lacerda, G. Pastorin, C. Klumpp, M. Prato, A. Bianco, and K. Kostarelos, *Proc. Natl. Acad. Sci. USA* 103, 3357 (2006).
25. (a) J. Guo, X. Zhang, Q. Li, and W. Li, *Nucl. Med. Biol.* 34, 579 (2007); (b) F. Jia, L. Wu, J. Meng, M. Yang, H. Kong, T. Liu, and H. Xu, *J. Mater. Chem.* 19, 8950 (2009); (c) L. Lacerda, A. Soundararajan, R. Singh, G. Pastorin, K. Al-Jamal, J. Turton, P. Frederik, M. Herrero, S. Li, A. Bao, D. Emfietzoglou, S. Mather, W. Phillips, M. Prato, A. Bianco, B. Goins, and K. Kostarelos, *Adv. Mater.* 20, 225 (2008).
26. S. Itoh, T. Taira, Y. Yawaka, and F. Watari, *Nano Biomedicine* 1, 95 (2009).
27. The IR peak in the 2800–3000 cm^{-1} region is characteristic of C–H stretches: The broad band in the 3100–3600 cm^{-1} region is characteristic of acid O–H stretches: The dominant peak at 1719 cm^{-1} is assigned to the acid carbonyl stretching mode: The broad peak at 1169 cm^{-1} is identified as the C–O stretching mode.
28. (a) T. Akasaka and F. Watari, *Fullerene Nanotubes Carbon Nanostruct.* 16, 114 (2008); (b) R. Nap and I. Szleifer, *Langmuir* 21, 12072 (2005); (c) B. Fugetsu, S. Satoh, T. Shiba, T. Mizutani, Y. Nodasaka, K. Yamazaki, K. Shimizu, M. Shindoh, K. Shibata, N. Nishi, Y. Sato, K. Tohji, and F. Watari, *Bull. Chem. Soc. Jpn.* 77, 1945 (2004); (d) W. Huang, S. Taylor, K. Fu, Y. Lin, D. Zhang, T. Hanks, A. Rao, and Y. Sun, *Nano Lett.* 2, 311 (2002); (e) Y. Ma, P. Chiu, A. Sarrano, S. Ali, A. Chen, and H. He, *J. Am. Chem. Soc.* 130, 7921 (2008).
29. (a) Y. Sato, A. Yokoyama, K. Shibata, Y. Akimoto, S. Ogino, Y. Nodasaka, T. Kohgo, K. Tamura, T. Akasaka, M. Uo, K. Motomiya, B. Jeyadevan, M. Ishiguro, R. Hatakeyama, F. Watari, and K. Tohji, *Mol. Biosystems* 1, 176 (2005); (b) K. Kiura, Y. Sato, M. Yasuda, B. Fugetsu, F. Watari, K. Tohji, and K. Shibata, *J. Biomed. Nanotechnol.* 1, 359 (2005).

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