molecular pharmaceutics

articles

Carbon Nanohorns as Anticancer Drug Carriers

Kumiko Ajima,*,† Masako Yudasaka,*,†,‡ Tatsuya Murakami,§,|| Alan Maigné,†,||,⊥ Kiyotaka Shiba,||,# and Sumio Iijima†,‡,||,▽

JST/SORST, c/o NEC, 34 Miyukigaoka, Tsukuba, Ibaraki 305-8501, Japan, NEC, 34 Miyukigaoka, Tsukuba, Ibaraki 305-8501, Japan, JST/SORST, c/o Department of Protein Engineering, Cancer Institute, Tokyo 170-8455, Japan, Laboratoire de Physique des Solides, CNRS UMR 8502, 91405 Orsay, France, Department of Protein Engineering, Cancer Institute, Tokyo 170-8455, Japan, and Meijo University, 1-501 Shiogamaguchi, Tenpaku-ku, Nagoya 468-8502, Japan

Received July 19, 2005

Abstract: We demonstrate that oxidized single-wall carbon nanohorns (SWNHs), a type of single-wall nanotube, entrap cisplatin, an anticancer agent. We found that the cisplatin structure was maintained inside the SWNHs and that the cisplatin was slowly released from the SWNHs in aqueous environments. The released cisplatin was effective in terminating the growth of human lung-cancer cells, while the SWNHs themselves had no such effect. Cisplatin-incorporated oxidized SWNHs are thus a potential drug delivery system.

Keywords: Carbon nanotube; carbon nanohorn; anticancer drug; drug carrier; cisplatin

Introduction

Shell structures can be used for delivering anticancer drugs to tumors in various parts of the human body.[1] The drug is placed inside the shell, where it often conjugates with the shell-construction materials. The shells are usually made from organic materials. For example, liposomes,[2−4] microspheres,[5] polymeric shelles,[6] and polymeric micelles[7,8] are well investigated. In constructing a drug delivery system from organic materials, the combinations of shell materials, targeting molecules, and drugs are restricted to ensure stability, targeting efficiency, and drug effect. The restrictions

* Corresponding authors. K.A.: JST/SORST, NEC Fundamental Laboratories, 34 Miyukigaoka, Tsukuba, Ibaraki 305-8501, Japan; phone, +81-(0)29-856-1940; fax, +81-(0)29-850-1366; e-mail, kumikoajima@frl.cl.nec.co.jp. M.Y.: NEC, 34 Miyukigaoka, Tsukuba, Ibaraki 305-8501, Japan; phone, +81-(0)29-850-1190; fax, +81-(0)29-850-1366; e-mail, yudasaka@frl.cl.nec.co.jp.

† JST/SORST, c/o NEC.

‡ NEC.

§ JST/SORST, c/o Department of Protein Engineering, Cancer Institute.

|| E-mail: murakami@fujita-hu.ac.jp (T.M.); maigne@frl.cl.nec.co.jp (A.M.); kshiba@jfcr.or.jp (K.S.); iijimas@ccmfs.meijo-u.ac.jp (S.I.).

⊥ Laboratoire de Physique des Solides, CNRS UMR 8502.

Department of Protein Engineering, Cancer Institute.

▽ Meijo University.

(1) Allen, T. M.; Cullis, P. R. Drug delivery systems: entering the mainstream. *Science* **2004**, *303*, 1818−1822.

(2) Yatvin, M. B.; Weinstein, J. N.; Dennis, W. H.; Blumenthal, R. Design of liposomes for enhanced local release of drugs by hyperthermia. *Science* **1978**, *202*, 1290−1293.

(3) Allen, T. M. Liposomal drug formulations. *Drugs* **1998**, *56*, 747−756.

(4) Burger, K. N. J.; Staffhorst, R. W. H. M.; de Vijlder, H. C.; Velinova, M. J.; Bomans, P. H.; Frederik, P. M.; de Kruijff, B. Nanocapsules: lipid-coated aggregates of cisplatin with high cytotoxicity. *Nat. Med.* **2002**, *8*, 81−84.

(5) Matsumoto, A.; Matsukawa, Y.; Suzuki, T.; Yoshino, T.; Kobayashi, M. The polymer alloys method as a new preparation method of biodegradable microspheres: principle and application to cisplatin loaded microspheres. *J. Controlled Release* **1997**, *48*, 19−27.

(6) Maeda, H.; Sawa, T.; Kouno, T. Mechanism of tumor-targeted delivery of macromolecular drugs, including the EPR effect in solid tumor and clinical overview of the prototype polymeric drug SMANCS. *J. Controlled Release* **2001**, *74*, 47−61.

(7) Yokoyama, M.; Miyauchi, M.; Yamada, N.; Okano, T.; Sakurai, Y.; Kataoka, K.; Inoue, S. Characterization and anticancer activity of the micelle-forming polymeric anticancer drug adriamycin-conjugated poly(ethylene glycol)-poly(aspartic acid) block copolymer. *Cancer Res.* **1990**, *50*, 1693−1700.

10.1021/mp0500566 CCC: $30.25
Published on Web 10/05/2005

on these combinations could be reduced greatly if carbon nanotubes (CNTs)[9] were used as the shells because CNTs are stable chemically and mechanically, material incorporation into their inner hollow spaces is easy, and their outside walls can be chemically modified to achieve the desired targeting effect.

Of the various types of CNTs, single-wall carbon nanohorns (SWNHs)[10] are particularly suitable as a drug carrier to tumor tissue. SWNHs do not exist individually, and several hundred of them assemble to form a robust spherical aggregate with a diameter of 80−100 nm. This aggregate size matches the condition for achieving the enhanced permeability and retention (EPR) effect.[6,7,11−13] That is, they can permeate through the damaged vessels in tumor tissue and remain there because there is little lymphatic drainage.

Moreover, SWNHs have diameters of 2−5 nm, making them well suited for both incorporation[14−17] and slow release of materials[18] and drugs.[19] Slow release is critical in drug delivery to minimize the amount of drug lost before reaching the target. In addition, SWNHs do not exhibit cytotoxicity,[19] making them potentially applicable to drug delivery systems.

In this report, we present the results of experiments that show that cisplatin (CDDP, *cis*-diamminedichloroplatinum-(II)),[20,21] an anticancer agent, can be incorporated into and released from SWNHs. The release rate was low, and the released cisplatin maintained its cancer killing effect in vitro.

Experimental Section

Preparation of SWNHs. The SWNHs were produced by CO_2 laser ablation of graphite in an Ar (760 Torr) atmosphere at room temperature.[10] They were then treated in flowing oxygen gas at 570−580 °C for 15 min to form holes on their walls. The holes were about 0.5−1.5 nm in diameter.[14] These oxidized SWNHs (SWNHox) were further heated at 1200 °C in hydrogen gas for 3 h to remove the oxygen-containing functional groups attached to the hole edges.[22]

Cisplatin Incorporation into SWNHox. Cisplatin (Wako Pure Chemical Industries, Ltd.) was incorporated in the SWNHox using nanoprecipitation. The cisplatin was first dissolved in *N*,*N*-dimethylformamide (DMF, Kanto Chemical Co.) with a concentration of 4.15 mg mL^{-1}. SWNHox were then dispersed (1 mg/0.2 mL) in the solution, followed by evaporation of the DMF from the solution by flowing dry air over it at room temperature for about 5 days. The result was black CDDP-incorporated SWNHox (CDDP@SWNHox) powder at the bottom of the container. The inferred incorporation mechanism was that when the cisplatin concentration in the DMF exceeded its solubility limit, cisplatin molecules or clusters precipitated at stable sites, i.e., inside the nanometer-sized hollow spaces of the SWNHox.[18] The incorporation of cisplatin inside SWNHox was confirmed by high-resolution transmission electron microscopy (HR-TEM) (002B, Topcon, 120 keV), *Z*-contrast imaging with a scanning TEM (STEM) (HD-2300, Hitachi, 120 keV), and thermogravimetric analysis (TGA) (TA Instruments, Hi-Res TGA 2950).

Elemental Analysis of CDDP@SWNHox. Elemental analyses of the cisplatin clusters inside the SWNHs were done using low-dose energy-dispersive X-ray spectroscopy (XEDS) and electron energy loss spectroscopy (EELS).

The XEDS measurements were performed with an HR-TEM (002B, Topcon) equipped with an X-ray detector (5538A-4SUS-SN, Thermo Electron Inc.). It was operated at 120 keV with a 100 nm probe, the size of which matches that of the SWNHs aggregates. The electron dose was reduced until we did not observe any damage to the

(8) Nishiyama, N.; Okazaki, S.; Cabral, H.; Miyamoto, M.; Kato, Y.; Sugiyama, Y.; Nishio, K.; Matsumura, Y.; Kataoka, K. Novel cisplatin-incorporated polymeric micelles can eradicate solid tumors in mice. *Cancer Res.* **2003**, *63*, 8977−8983.

(9) Iijima, S. Helical microtubules of graphitic carbon. *Nature* **1991**, *354*, 56−58.

(10) Iijima, S.; Yudasaka, M.; Yamada, R.; Bandow, S.; Suenaga, K.; Kokai, F.; Takahashi, K. Nano-aggregates of single-walled graphitic carbon nano-horns. *Chem. Phys. Lett.* **1999**, *309*, 165−170.

(11) Maeda, H.; Seymour, L. W.; Miyamoto, Y. Conjugates of anticancer agents and polymers: advantages of macromolecular therapeutics in vivo. *Bioconjugate Chem.* **1992**, *3*, 351−362.

(12) Maeda, H.; Wu, J.; Sawa, T.; Matsumura, Y.; Hori, K. Tumor vascular permeability and the EPR effect in macromolecular therapeutics: a review. *J. Controlled Release* **2000**, *65*, 271−284.

(13) Ferrari, M. Cancer nanotechnology: opportunities and challenges. *Nature* **2005**, *5*, 161−171.

(14) Ajima, K.; Yudasaka, M.; Suenaga, K.; Kasuya, D.; Azami, T.; Iijima, S. Material storage mechanisms in porous nanocarbon. *Adv. Mater.* **2004**, *16*, 397−401.

(15) Murata, K.; Hashimoto, A.; Yudasaka, M.; Kasuya, D.; Kaneko, K.; Iijima, S.The use of charge transfer to enhance the methane-storage capacity of single-walled, nanostructured carbon. *Adv. Mater.* **2004**, *16*, 1520−1522.

(16) Hashimoto, A.; Yorimitsu, H.; Ajima, K.; Suenaga, K.; Isobe, H.; Miyawaki, J.; Yudasaka, M.; Iijima, S.; Nakamura, E. Selective deposition of a gadolinium(III) cluster in a hole opening of single-wall carbon nanohorn. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 8527−8530.

(17) Yuge, R.; Ichihashi, T.; Shimakawa, Y.; Kubo, Y.; Yudasaka, M.; Iijima, S. Preferential deposition of Pt nanoparticles inside single-walled carbon nanohorns. *Adv. Mater.* **2004**, *16*, 1420−1423.

(18) Yuge, R.; Yudasaka, M.; Miyawaki, J.; Kubo, Y.; Ichihashi, T.; Imai, H.; Nakamura, E.; Isobe, H.; Yorimitsu, H.; Iijima, S. Controlling the incorporation and release of C_{60} in nanometer-scale hollow spaces inside single-wall carbon nanohorns. *J. Phys. Chem. B* **2005**, *109*, 17861−17867.

(19) Murakami, T.; Ajima, K.; Miyawaki, J.; Yudasaka, M.; Iijima, S.; Shiba, K. Drug-loaded carbon nanohorns: adsorption and release of dexamethasone in vitro. *Mol. Pharm.* **2004**, *1*, 399−405.

(20) Rosenberg, B.; VanCamp, L.; Trosko, J. E.; Mansour, V. H. Platinum compounds: a new class of potent antitumor agents. *Nature* **1969**, *222*, 385−386.

(21) Lippert, B. *Cisplatin: chemistry and biochemistry of a leading anticancer drug*; Wiley-VCH: New York, 1999; pp 3−27.

(22) Miyawaki, J.; Yudasaka, M.; Iijima, S. Solvents on hole-edge structure for single-wall carbon nanotubes and single-wall carbon nanohorns. *J. Phys. Chem. B* **2004**, *108*, 10732−10735.

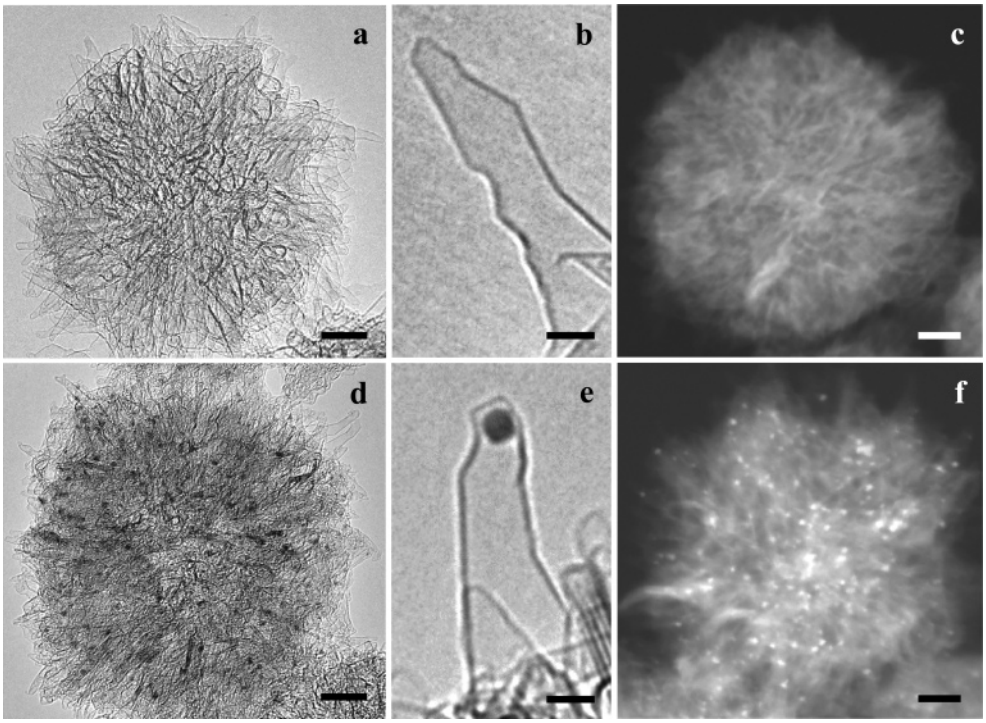

Figure 1. (a, b) HRTEM images of SWNHox (scale bars of 10 and 2 nm, respectively). (c) *Z*-Contrast image of SWNHox aggregate (10 nm). (d, e) HRTEM images of CDDP@SWNHox (10 and 2 nm) in which black spots are cisplatin clusters. (f) *Z*-Contrast image of CDDP@SWNHox in which bright spots are cisplatin clusters (10 nm).

CDDP@SWNHox. The peaks were identified using Bearden's tables.[23] The peaks were integrated using a digital top-hat filter to remove the background. The *k*-factor used for the quantification was calculated from a calibration with a cisplatin crystal (for the *k*-factor of Pt M_α1/Cl K_α) and with Pt inserted in the SWNHs (for the *k*-factors of Pt M_α1/C K and Pt L/C K). The EELS measurements were performed with an HRTEM (002BF, Topcon) with a Gatan imaging filter. It was operated at 100 keV with a 100 nm probe. The quantification of N and C was done using the integration method, and the partial cross sections were calculated using the Hartree−Slater method.[24]

Cisplatin Release from CDDP@SWNHox. The rates of cisplatin release from the CDDP@SWNHox were investigated by enclosing the CDDP@SWNHox inside a cellulose tube (Float-A-Lyzer, Biotech CE, Spectrum Laboratories Inc.; pore diameter of about 4 nm and cutoff molecular weight of about 50 kDa). The CDDP@SWNHox-cellulose tube was immersed in phosphate-buffered saline (PBS) or culture medium (RPMI 1640). The CDDP@SWNHox quantity in the PBS was 10 μg mL^{-1}. Cisplatin powder obtained by dissolving the purchased cisplatin in DMF and drying was used as a control. During the immersion in PBS or culture medium, the cisplatin dissolved out of the SWNHox and passed out of the cellulose tube. The cisplatin concentration outside the tube was measured as the concentration of Pt with an atomic adsorption spectrometer (Z-2700, Hitachi). After cisplatin release for 14 days, elemental analysis of the CDDP@SWNHox remaining in the tube was carried out using XEDS, performed in the same manner as for the CDDP@SWNHox.

Cell Viability Assays. The cytotoxicity of cisplatin released from the CDDP@SWNHox against human lung-cancer cells, NCI-H460, was evaluated with an assay using WST-1 (4-[3-(4-iodophenyl)-2-(4-nitrophenyl)-2*H*-5-tetrazolio]-1,3-benzene disulfonate) (Roche Diagnostics). This is a colorimetric assay for quantifying cell proliferation and cell viability and is based on the cleavage of the WST-1 into formazan by mitochondrial dehydrogenases in viable cells. The cells were seeded in RPMI-1640 culture medium with 5% fetal bovine serum and streptomycin (1 μL mL^{-1}), and the medium was placed in 96-well microplates. The initial concentration of cancer cells was 2.5×10^4 mL^{-1}. The cells were grown at 37 °C in humidified atmosphere with 5% CO_2 and 95% air for 4 h.

The cisplatin, CDDP@SWNHox, and SWNHox powders were dispersed in culture medium with various concentrations. These dispersions (500 μL) were added to the cancer cells cultured in the microplates. After incubation for 48 h, WST-1 diluted in the culture medium was added. The cell viabilities were monitored by measuring the decreased light absorbance at wavelengths from 450 to 595 nm using a microplate reader (680 XR microplate reader, Bio-Rad).

(23) Bearden, J. A. *NYO-10586*; U.S. Atomic Energy Commission: Oak Ridge, TN, 1964; p 533.

(24) Egerton, R. F. *Electron energy-loss spectroscopy in the electron microscope*, 2nd ed.; Plenum Press: New York, 1996; pp 279−284.

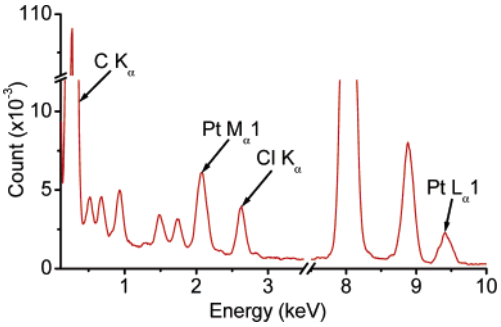

Figure 2. Low-dose energy-dispersive X-ray spectrum of individual aggregate of CDDP@SWNHox showing C K_α, Pt $M_\alpha1$, $L_\alpha1$, and Cl K_α peaks. The other peaks are assigned to O, Fe, Cu, Al, and Si, which originated from SWNHox, Cu grid disk (TEM sample holder), and microscope and XEDS detector bodies. The vertical axis represents the number of photons collected by the detector as a function of their energy, which is indicated by the horizontal axis.

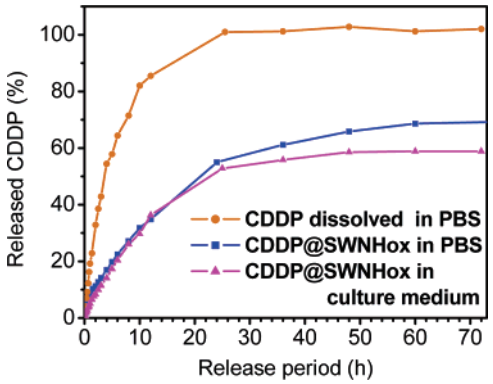

Figure 3. CDDP@SWNHox or cisplatin was confined within a dialyzing bag, which was immersed in PBS or culture medium. Quantities of cisplatin dialyzed were measured using atomic adsorption spectrometry. The vertical axis indicates (dialyzed cisplatin quantity)/(initial cisplatin quantity) in percent. The horizontal axis indicates release period.

Results and Discussion

HRTEM images of SWNHox (Figure 1a,b) indicated that they contained no cisplatin, while those of CDDP@SWNHox (Figure 1d,e) showed encapsulated cisplatin clusters (shown in the images as black spots). The clusters ranged in size from 1 to 2 nm. A typical cluster is shown in Figure 1e; the cluster size was about 2 nm. The distribution of cisplatin in SWNHox aggregates was observed more clearly in the *Z*-contrast images because the contrast in *Z*-contrast images is proportional to the square of the atomic number, so the Pt atoms appear with a brighter contrast than the C atoms. The Pt of the cisplatin clusters appeared as white spots in the *Z*-contrast images, and the clusters were homogeneously distributed over the entire SWNHox aggregate for CDDP@SWNHox (Figure 1f). No white spots were seen in the SWNHox images (Figure 1c).[25]

The quantity of incorporated cisplatin was estimated through TG measurements. The residue remaining after TG measurement at 1000 °C was Pt. From the amount of Pt residue and SWNHox, the mole ratio of Pt and C (Pt:C) was estimated to be about 1:100 (data not shown). This substantially agrees with the value estimated from XEDS measurements made on individual aggregates of CDDP@SWNHox (Figure 2), which was about 1:120 (from the C K peak and Pt $M_\alpha1$ peak). XEDS also revealed that the mole ratio of Pt:Cl was about 1:2 (from the Pt $M_\alpha1$ peak and Cl K_α peak). EELS measurements made on individual aggregates (data not shown) indicated that the mole ratio of N:C was 1:65 from quantification of the C K peak and the N K peak. These spectroscopic results suggest that the cisplatin (Pt:Cl:N = 1:2:2) structure was maintained during incorporation.

In applying CDDP@SWNHox to a drug delivery system, the cisplatin release needs to be slow to prevent drug dissipation before reaching the tumor. We found that the release rate of cisplatin from SWNHox in PBS and a culture medium was lower than the dissolution rate of free cisplatin powder, i.e., it took about 72 h for the cisplatin released from CDDP@SWNHox to saturate in PBS, while it took about 24 h for the free cisplatin (Figure 3). A similar curve for cisplatin release from CDDP@SWNHox in the culture medium was observed (Figure 3). After being released in PBS for 14 days, the CDDP@SWNHox was separated from the PBS using a centrifuge and washed with methanol to remove any remaining PBS. The CDDP@SWNHox structure was evaluated using HRTEM and XEDS measurements. Although the SWNHox appeared to be empty in the HRTEM images (Figure 4a,b), XEDS showed that Pt:C (from the Pt L peak and C K peak) was about 1:600, which meant that about 20% of the cisplatin remained in the SWNHox aggregates (Figure 4c).

We determined that the cisplatin released from the CDDP@SWNHox maintained its ability to reduce the viability of human lung-cancer cells, NCI-H460. The cancer cells multiplied and spread over the well bottoms after 48 h incubation at 37 °C (Figure 5a). However, the number of cells decreased when CDDP@SWNHox was added to the cell-culture medium (Figure 5b,c). Cisplatin is a proven DNA-damaging agent, arresting the cell cycle and preventing DNA replication.[26−28] Cells exposed to cisplatin eventually die due to apoptosis, in other words, programmed cell death.[29]

Since the number of cells (Figure 5d,e) treated with SWNHox was almost equal to the number of cells treated without CDDP@SWNHox (Figure 5a), and since no mor-

(25) Isaacson, M.; Ohtsuki, M.; Utlaut, M. In *Introduction to analytical electron microscopy*; Hren, J. J., Goldstein, J. I., Joy, D. C., Eds.; Plenum Press: New York, 1979; p 343.

(26) Cohen, S. M.; Lippard, S. J. *Progress in nucleic acid research and molecular biology*; Academic Press: New York, 2001; Vol. 67, pp 93−130.

(27) Eastman, A. Interstrand cross-links and sequence specificity in the reaction of *cis*-dichloro(ethylenediamine)platinum(II) with DNA. *Biochemistry* **1985**, *24*, 5027−5032.

(28) Eastman, A. Reevaluation of interaction of *cis*-dichloro(ethylenediamine)platinum(II) with DNA. *Biochemistry* **1986**, *25*, 3912−3915.

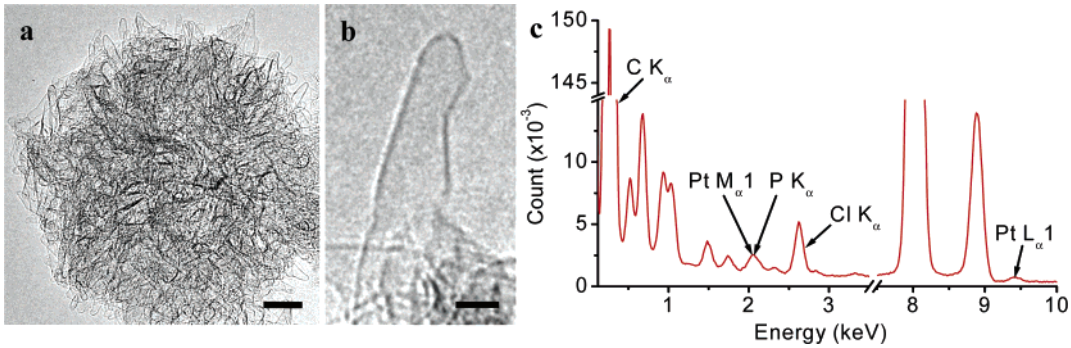

Figure 4. Structure of CDDP@SWNHox after cisplatin was released by immersion in PBS for 14 days. (a, b) HRTEM images exhibit no black spots, i.e., cisplatin clusters (scale bars of 10 and 2 nm, respectively). (c) Low-dose energy-dispersive X-ray spectrum of individual aggregate. Origins of O, Fe, Cu, Al, and Si were the same as described in the Figure 2 caption. Elements of P, Na, and most of Cl originated from PBS, so the Cl K_α peak was useless in estimating the cisplatin quantity. Peaks for Pt $M_\alpha 1$ originating from cisplatin and P K_α from PBS overlapped at around 2 keV, so we used Pt $L_\alpha 1$ to estimate the Pt:C ratio, which indicated that about 20% of cisplatin was not released. The vertical axis represents the number of photons collected by the detector as a function of their energy, which is indicated by the horizontal axis.

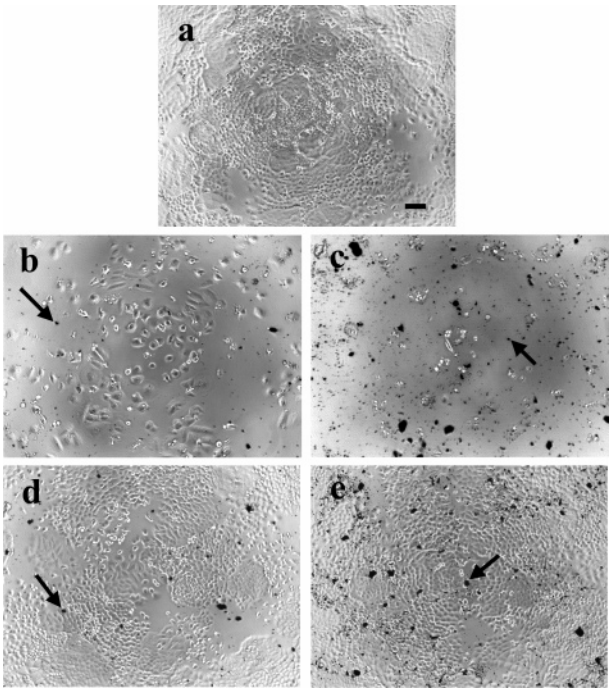

Figure 5. Optical microscopic images of cancer cells after incubation for 48 h (scale bar of 100 μm). Additions were (a) nothing, (b) CDDP@SWNHox (cisplatin: 3 μM), (c) CDDP@SWNHox (cisplatin: 12 μM), (d) SWNHox (5 μg mL^{-1}), and (e) SWNHox (20 μg mL^{-1}).

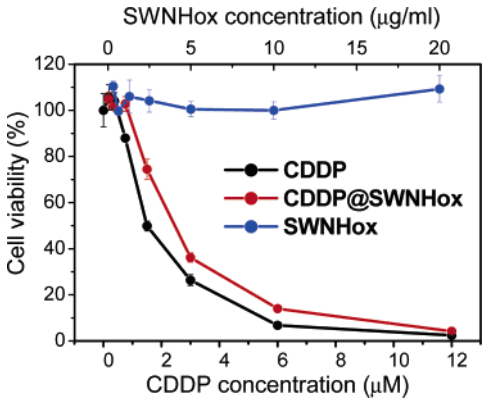

Figure 6. Inhibition of human lung-cancer cell growth due to adding cisplatin, CDDP@SWNHox, and SWNHox. Cytotoxicity of these additions against human lung-cancer cells, NCI-H460, was evaluated with an assay using WST-1 after 48 h incubation.

phological change was observed, SWNHox apparently does not promote or suppress the growth of cancer cells. The black spots in Figures 5b and 4e were CDDP@SWNHox or SWNHox, which often gathered together, forming agglomerates of micrometer-order size.

Cytotoxicity evaluated using WST-1 assay[30,31] showed that the anticancer activity of CDDP@SWNHox increased with the CDDP concentration (Figure 6). The effect of cisplatin released from CDDP@SWNHox on the viability of cancer cells was a little less than that of the free cisplatin (Figure 6), apparently due to the slow release of cisplatin from CDDP@SWNHox. This was also seen in the our finding that 40% of the cisplatin was not released from the SWNHox even after 48 h (Figure 3) or that 20% was not released even after 14 days (Figure 4c).

In addition to the optical microscopic observation (Figure 5d,e), WST-1 assay confirmed that SWNHox itself had no discernible effect on cancer cell growth (Figure 6). WST-1 assay has also demonstrated that SWNHox are not toxic

(29) Iwaki, T.; Iwaki, A.; Fukumaki, Y.; Schlehofer, J. Alpha B-crystallin in C6 glioma cells supports their survival in elevated extracellular K^+: the implication of a protective role of alpha B-crystallin accumulation in reactive glia. *Brain Res.* **1995**, *673*, 47−52.

(30) Barry, M. A.; Behnke, C. A.; Eastman, A. Activation of programmed cell death (apoptosis) by cisplatin, other anticancer drugs, toxins and hyperthermia. *Biochem. Pharmacol.* **1990**, *40*, 2353−2362.

(31) Takenouchi, T.; Munekata, E. Trophic effects of substance P and beta-amyloid peptide on dibutyryl cyclic AMP-differentiated human leukemic (HL-60) cells. *Life Sci.* **1995**, *56*, 479−484.

against noncancerous cells, such as mouse bone marrow stromal and osteoblastic cells.[19] While these results suggest that SWNHs are suitable drug delivery systems, further investigation is needed on the effects of SWNHs both in vitro and in vivo.

Conclusion

Experiments demonstrated that cisplatin is incorporated inside the nanometer-sized hollow spaces of single-wall carbon nanohorns treated in flowing oxygen gas at 570−580 °C for 15 min. The slow release of incorporated cisplatin was observed in phosphate-buffered saline and cell-culture media, and the released cisplatin exhibited anticancer effects. These results suggest that oxidized SWNHs are potentially useful as a drug carrier.

Acknowledgment. We are grateful to Dr. Takeshi Azami and Dr. Daisuke Kasuya of NEC Corporation for preparing the single-wall carbon nanohorns and to Dr. Jin Miyawaki and Dr. Katsuyuki Murata for helping with the thermogravimetric and atomic absorption spectroscopy measurements. A.M. is supported by a JSPS predoctoral fellowship.

MP0500566