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Optimum Hole-Opening Condition for Cisplatin Incorporation in Single-Wall Carbon Nanohorns and Its Release

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We enclosed cisplatin (CDDP), an anticancer drug, inside single-wall carbon nanohorns (SWNH) with holes opened by being heated from room temperature to a target temperature (475−580 °C) in flowing dry air, with an increase rate of 1 °C/min. The optimum target temperature was found to be 500 °C, in terms of the least amount of CDDP deposited outside the SWNH, when the quantity of CDDP encapsulated inside the SWNH was 12 wt %. The incorporated CDDP was slowly released from the SWNH in phosphate buffer saline, and the released quantity was 80%, which was greatly improved from the previous value of 15%. This indicated that a CDDP-containing SWNH could become more potentially useful for biological applications.

Introduction

Single-wall carbon nanohorns (SWNH), a type of single-wall carbon nanotube, have diameters of 2−5 nm and lengths of 40−50 nm, and thousands of SWNHs assemble to form a spherical aggregate with a diameter of 80−100 nm.[1] Because the inner spaces of SWNHs are large, it is possible to incorporate various materials,[2−5] even drugs, inside SWNHs, and often to release them.[6,7] Further, in vivo visualization of SWNHs was demonstrated to be possible by attaching magnetite nanoparticles on the SWNHs.[8] On the other hand, the aggregate sizes fit to the "enhanced permeability and retention" effect, which would enable passive targeting to the tumors.[9] Thus, we consider that the SWNHs are potentially useful as drug carriers, and in this report, we show the optimum hole-opening of SWNHs for the drug incorporation and release.

We previously reported that cisplatin, *cis*-$(NH_3)_2PtCl_2$ (CDDP), an anticancer drug, can be incorporated inside SWNHs having holes with hydrogen-terminated edges (NHh) and that 75% of the incorporated CDDP was slowly released from inside the NHh when immersed in phosphate-buffered saline (PBS).[7,10] Unfortunately, the hydrophobic property of an NHh would not be adequate enough for the biological application of one as a CDDP carrier because it hinders the dispersion in PBS. On the other hand, SWNHs having holes with their edges terminated by oxygen-containing functional groups (NHox) could handle dispersion in PBS better than NHh because their functional groups are hydrophilic. Therefore, it is assumed that the NHox is more appropriate for use as CDDP carriers. However, the release of CDDP from inside NHox in PBS was considerably suppressed, which was caused by the sodium ions in PBS: the sodium ion replaced the hydrogen of the oxygen-containing functional groups at the hole edges, which resulted in plugging of the holes, hindering CDDP release.[10]

For CDDP-incorporating NHox (CDDP@NHox) to be used as a CDDP-releasing carrier in vivo, the hole-size enlargement and control of the number of functional groups at the hole edges of NHox might be effective at producing the necessary CDDP releasing quantities, at a slow release rate. We show in this report that such requirements were satisfied by applying the special hole-opening method.

Experimental Section

We prepared SWNHs by CO_2 laser ablation of graphite in an Ar atmosphere at room temperature.[1] The SWNHs were about 95% pure, and the 5% impurity was graphitic particles.[11] To change the diameters of the NHox holes, the holes were opened up by combusting the as-grown SWNHs in dry air by heating them up from room temperature to target oxidation temperatures (Tox) of 475, 500, 525, 550, 565, and 580 °C at a rise rate of 1 °C/min. Then the SWNHs were allowed to cool naturally to room temperature, and the method is described as slow combustion.[12] In our previous study, the NHox was prepared by heat treatment at 570−580 °C for 10 min in flowing oxygen gas,[7,10] a method described as quick combustion.[12] We refer to the NHox prepared by the quick combustion and the slow combustion as "NHox(quick)" and "NHox(slow)", respectively.

Electron energy loss spectroscopy (EELS) measurements were performed using a STEM HD2300 operated at 120 keV, with a parallel electron energy loss spectrometer to clarify the level of oxygen in the NHox(quick) and NHox(slow).

Incorporation of CDDP inside NHox was carried out as follows. NHox (50 mg) was mixed with a CDDP (15 mg) solution of DMF (5 mL), and the DMF was evaporated

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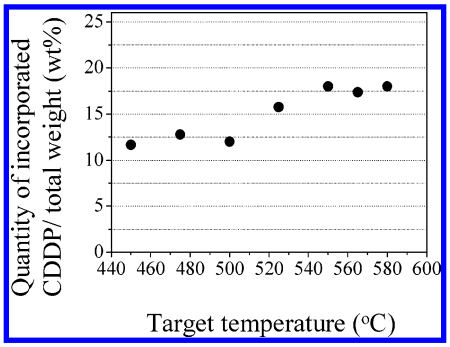

Figure 1. Quantities of CDDP incorporated into NHox with holes opened by the slow combustion method at various target temperatures. Each datum representing the amount of CDDP was averaged among the three specimens.

completely in a flowing N_2 gas at room temperature. At the bottom of the container, the CDDP@NHox(slow) was obtained.[12]

To estimate the quantities of CDDP in the CDDP@NHox-(slow), the CDDP@NHox powder was dispersed in an aqueous solution of 0.1% Tween 20 and homogenized with an ultrasonic processor (240 W for 15 min), and their platinum concentrations in the solutions were measured by atomic absorption spectrum. The CDDP quantities were estimated from platinum concentrations.

X-ray diffraction (XRD) measurements were used to check for CDDP outside the NHox(slow). The powder of NHox(slow) with target temperature or CDDP was prepared on the silicon substrate and measured by XRD from 5−35°.

To estimate the release rate of CDDP from the CDDP@NHox-(slow) in PBS, a dialysis method was performed. We dispersed CDDP@NHox(slow) (contained CDDP, 1.2 mg) in PBS (2 mL) inside a dialysis-membrane cylinder (molecular weight cut off is 10 000) and immersed the cylinder in PBS (598 mL). The CDDP diffused out of the cylinder membrane in PBS was sampled (1 mL) from time to time, and their atomic absorption spectra were measured. From the measured atomic absorption spectrum of their samples, we estimated the quantity of platinum in each sample, which we then used to estimate the quantity of CDDP release.

Results and Discussion

Our previous report indicated that, with the increase of Tox, the sizes and numbers of holes of the NHox(quick, slow) increased, and the adsorption quantities of N_2 or *m*-xylene reached maximum at 500 °C of Tox.[12] Therefore, we expected that the CDDP quantities encapsulated in NHox(slow) would also maximize at these Tox's. However, our result indicated that the quantity of incorporated CDDP in CDDP@NHox(slow) did not really depend on the Tox, only slightly increasing with the increase in Tox from 475 to 580 °C (Figure 1).

Before considering the CDDP quantities in CDDP@NHox-(slow) presented in Figure 1, we show that not all of the CDDP was enclosed inside NHox(slow), but a little was located on the outside. This was exhibited using X-ray diffraction (XRD): The peaks at 13.5−14.5° and at about 28.5° (Figure 2) correspond to CDDP crystals exhibiting sharp peaks or amorphous of their crystals exhibiting broad peaks. In Figure 2, the peak heights of the CDDP crystals were normalized at a peak height at 26°, which was a diffraction peak from graphitic particles contained in NHox(slow).[11] By comparing the shapes and heights of the CDDP-crystal peaks in Figure 2, it becomes apparent that the quantity of CDDP that was located outside

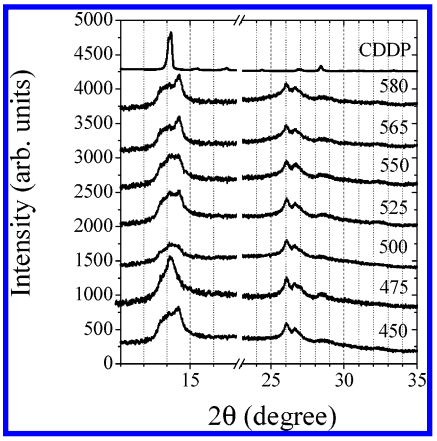

Figure 2. XRD patterns of CDDP and CDDP@NHox with various target temperatures. The peaks at 13.5−14.5° and about 28.5° corresponded to diffractions by the CDDP crystals existing outside of NHox. The peak heights of the CDDP crystals were normalized at the peak height at 26°, which was a diffraction peak from graphitic particles, which are impurities.

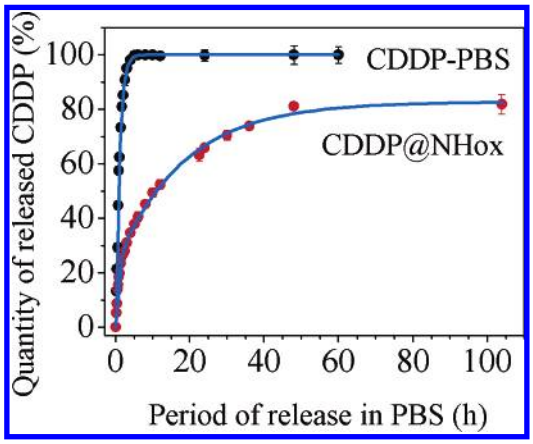

Figure 3. Release of CDDP from CDDP@NHox in PBS evaluated by the dialysis method. The CDDP quantities in PBS solutions outside the membrane were measured with an atomic absorption spectrometer. The release quantity was plotted as a percentage of the initial quantity of CDDP Table 1. Parameters obtained from numerical analysis of release profiles in Figure 3. The equation used was: released quantity (%) $= A - A_1 \exp(-t/t_1) - A_2 \exp(-t/t_2)$, where $A = A_1 + A_2$ is the final released quantity.

NHox(slow) was at its least when the Tox was 500 °C. The XRD pattern of CDDP@NHox(slow, Tox, 500 °C) showed the smallest peak height of a CDDP crystal and the absence of sharp peaks at 13.5−14.5° and about 28.5°. Thus, in terms of the least amount of CDDP found outside of NHox(slow), the optimum Tox was 500 °C. The CDDP was not well incorporated inside NHox(slow) when the Tox was low or high because the pore volumes of those NHox(slow)'s were low.[12]

We confirmed that CDDP@NHox(slow, Tox, 500 °C) abundantly and slowly released CDDP in PBS. Figure 3 indicates that the release saturated at about 80% after 50 h immersion. This means that the CDDP release from NHox(slow, Tox, 500 °C) did not suffer from the plug effect, which was caused by the attachment of sodium in the PBS to the hole edges of NHox(slow).[10] For NHox(quick) in flowing oxygen gas at 570−580 °C for 10 min, CDDP@NHox(quick) could release only 15% of CDDP in PBS.[10] When comparing the NHox(slow) with the NHox(quick), the hole sizes might be larger, making the sodium plugs ineffective at hindering the release of CDDP from NHox(slow). The other possible reason may be that the number of oxygen-containing functional groups on the hole

TABLE 1: Parameters Obtained as a Result of Numerical Analysis of the Release Profiles in Figure 3[a]

	A_1 (%)	A_1 (%)	A_1 (%)	A_1 (%)
CDDP@NHox	60	23	18.3	0.77
CDDP/PBS solution	N/A	107	N/A	0.96

[a] Equation used was: released quantity (%) $= A - A_1 \exp(-t/t_1) - A_2 \exp(-t/t_2)$, where $A = A_1 + A_2$ is the final released quantity.

edges of NHox(slow) would be less than NHox(quick), which was confirmed as follows.

To examine the quantities of oxygen contained in the two types of NHox(quick, slow), EELS measurements were performed. By measuring the EELS spectrum of the individual aggregate of NHox(quick, slow), we found that the quantity of oxygen in NHox obtained by slow combustion was extremely small, less than 1% in mole, which was much smaller than that of about 3% of NHox(quick). The mechanism that brought about this difference is not clear. We inferred from these EELS results that the NHox(slow) had less oxygen-containing functional groups on the hole edges, and therefore, the plug effect by sodium in PBS did not stop the release of CDDP from the NHox(slow).

To estimate how much CDDP was located outside/inside NHox(slow), we numerically analyzed the release profiles in Figure 3 by assuming that the CDDP deposited outside NHox(slow) was released more quickly than that enclosed inside. Therefore, the profile was fitted using two exponential functions.

$$\text{Released quantity (\%)} = A - A_1 \exp(-t/t_1) - A_2 \exp(-t/t_2), \text{ where } A = A_1 + A_2 \text{ is the final released quantity}$$

Here, 1 and 2 denote the slow and quick release processes, respectively. The fitted curve is indicated with a blue line in Figure 3, which well simulated the experimental values. The fitted parameters are listed in Table 1, and it shows that the parameters of the quick-releasing components, A_2 and t_2, are close to those obtained by fitting the release profiles measured using a CDDP−PBS solution (Figure 3) instead of CDDP@NHox(slow). These coincidences supported the validity of the simulation. From Table 1, the quantity ratio of CDDP inside and outside is estimated to be 62:23 ($= A_1{:}A_2$), that is, about 72% of CDDP was enclosed inside the NHox(slow).

Conclusion

We optimized the hole-opening condition for the CDDP incorporation in terms of the least CDDP quantity deposited outside of NHox(slow). The obtained CDDP@NHox(slow) abundantly released the CDDP, which was about 80%, and slowly for 50 h, which is favorable for being using as CDDP carriers in vivo. We concluded that, compared to the CDDP quantity released in PBS from CDDP@NHox(quick) with CDDP@NHox(slow) used in our previous studies, the latter is much higher, which might be due to the larger-sized holes and less oxygen-containing functional groups at the hole edges of the NHox(slow) used in this study and that the release of CDDP from CDDP@NHox might be controllable by the SWNHs' oxidation methods.

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