Supporting Information

Biodegradation of Carbon Nanohorns in Macrophage Cells

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1. Thermal gravimetric analyses of as-prepared, oxidized CNHs and S-CNHs

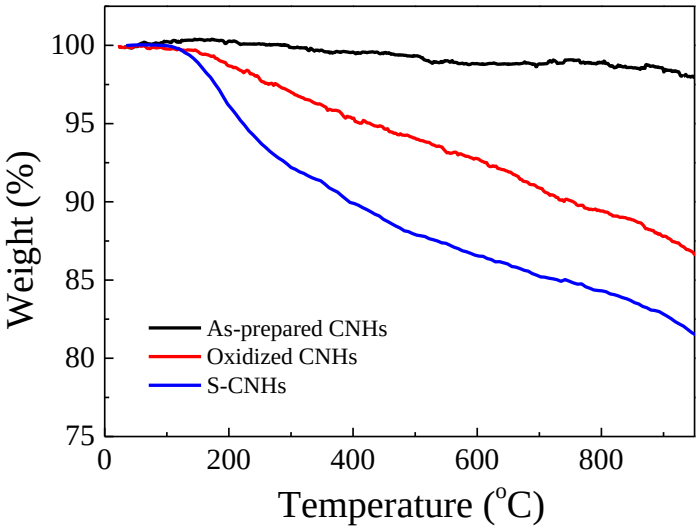


Figure S1. Thermal gravimetric analyses (TGA) of as-grown CNHs, oxidized CNHs prepared by light-assisted oxidation with H_2O_2, and S-CNHs prepared by strong acid treatment with H_2SO_4 and HNO_3. The TGA was performed in helium atmosphere from room temperature to 1000°C at a temperature increase rate of 5°C min^{-1}. The weight loss of S-CNHs was about 10 % from room temperature to 400 °C, which was higher than that of CNHs oxidized with H_2O_2 (about 4 %), indicating that S-CNHs had a larger amount of oxygen-containing functional groups than CNHs oxidized with H_2O_2.

2. Intracellular localization of CNHs after uptake by RAW 264.7 macrophages

RAW 264.7 cells (5×10^5 per dish) were seeded onto glass-bottomed dishes (Iwaki) and incubated for 24 h, after which CNHs were added (final concentration 0.01 mg ml^{-1}) for a further 24 h. The cells were then washed twice with PBS, placed into new medium containing 50 nM LysoTracker red DND-99 (Molecular Probes), and incubated for 20 min before being observed with a confocal microscope.

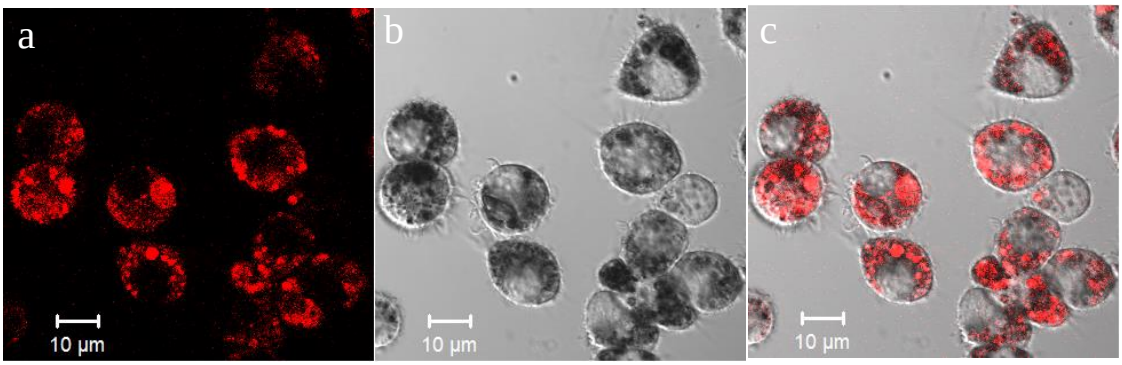


Figure S2. Intracellular localization of CNHs. (a) The lysosomes of RAW 264.7 cells were stained with LysoTracker dye (Molecular Probes). (b) DIC images in which CNHs are shown as black spots. (c) Merged image.

3. Differentiation of THP-1 cells by treatment with PMA

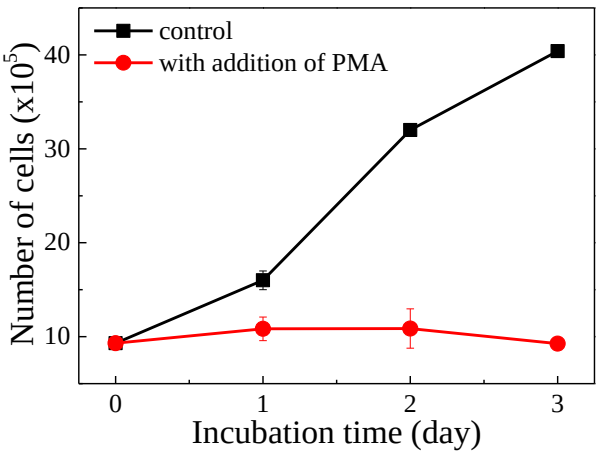


Figure S3. The numbers of THP-1 cells after treatment with or without PMA (50 ng ml^{-1}) to induce differentiation. Data are expressed as the mean ± SD of n = 3 independent replicates.

4. The effect of incubation with CNHs on the generation of ROS by THP-1 cells

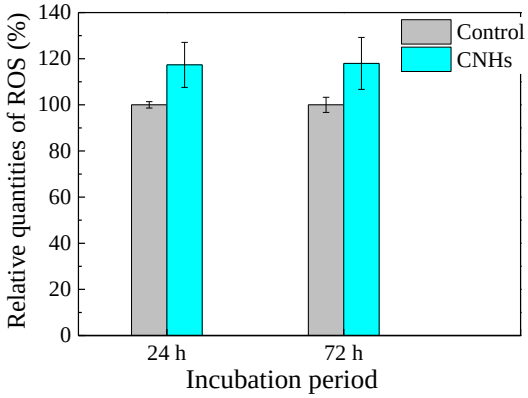


Figure S4. Generation of ROS in THP-1 cells incubated with CNHs (10 μg ml^{-1}) for 24 h or 72 h. Control cells were not treated with CNHs. Data represent the percentage of ROS relative to that in the control cells and are expressed as the mean ± SD of *n* = 3 independent replicates.

5. Raman spectra of as-prepared CNHs, oxidized CNHs and strong acid treated CNHs by H_2SO_4 and HNO_3.

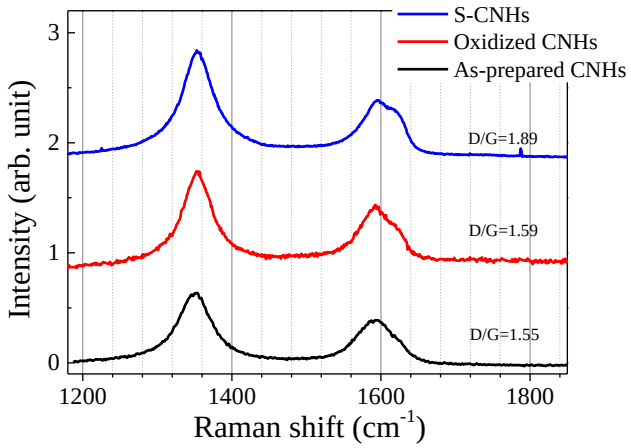


Figure S5. Raman spectra of as-prepared CNHs, oxidized CNHs generated by light-assisted oxidation with H_2O_2 and S-CNHs obtained by strong acid treatment with H_2SO_4 and HNO_3. All spectra showed two peaks at 1590 cm^{-1} (G band) and 1350 cm^{-1} (D band). The D/G bands peak height ratio for as-prepared CNHs, oxidized CNHs and S-CNHs were about 1.55, 1.59 and 1.89, respectively. In addition, the peak-shoulder at about 1620 cm^{-1} that indicates the defects on the wall of nanotubules [ref. 39] became obvious in the spectra of S-CNHs comparing with those of oxidized CNHs and

as-prepared CNHs. All these indicated that S-CNHs had more defects than CNHs oxidized with H_2O_2 and as-prepared CNHs.

6. TEM image of S-CNHs

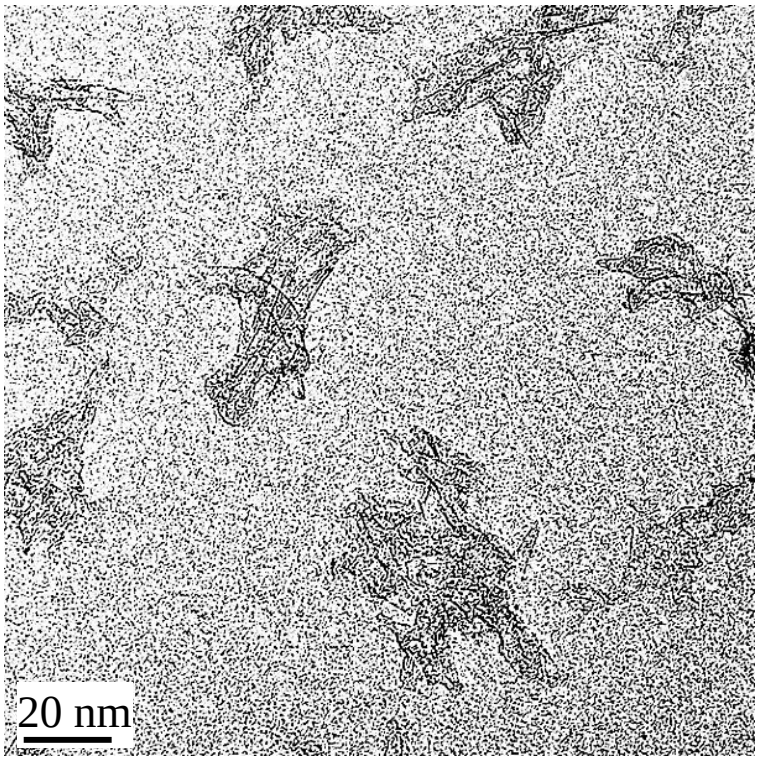


Figure S6. TEM image of S-CNHs. The strong acid treatment separated the original nanohorn-aggregates (about 100 nm) into smaller ones (20-50 nm). Although the defect-sites are difficult to be identified, the small-sized aggregates and unclear tubular structures of nanohorns suggested the defects existing in the S-CNHs.

7. The effect of strong acid treatment on the degradation of CNHs

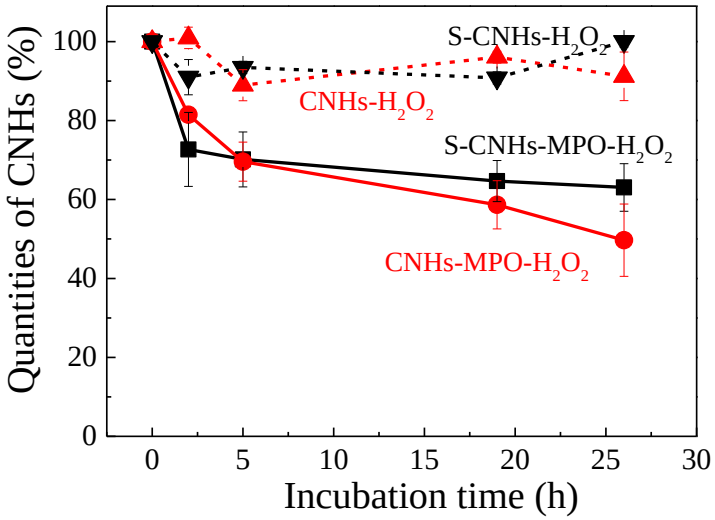
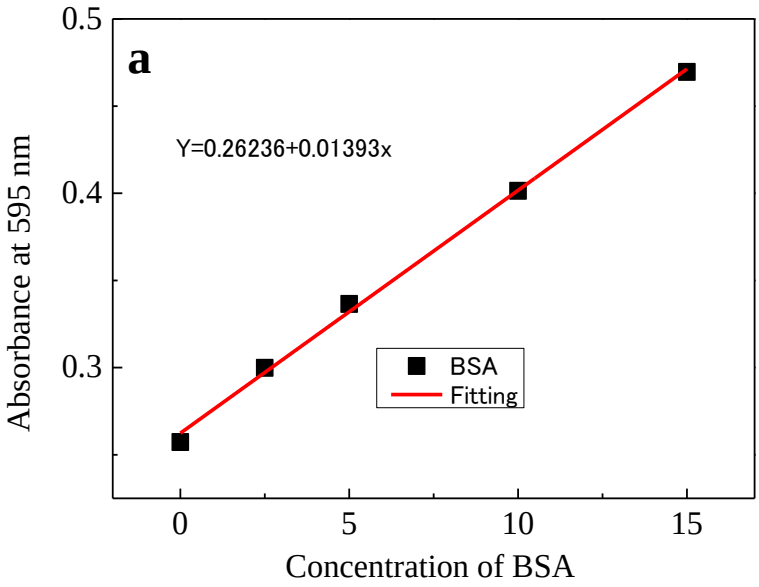


Figure S7. Degradation of CNHs following incubation with H_2O_2 alone or with a mixture of H_2O_2 with MPO, for CNHs prepared with strong acid (S-CNHs) or light assisted oxidation without strong acids (CNHs). The quantities of CNHs were estimated based on the optical absorbance at 700 nm. The data are expressed as the mean ± SD of n = 3 independent replicates.

8. The quantities of MPO attached to CNHs after 1 h, 2 h, and 5 h

The amount of MPO bound to CNHs after incubation for various periods, as determined using a Bradford assay (The Quick Start Bradford protein assay, BioRad). The measurement process was completely followed the protocol supplied by company (http://www.bio-rad.com/webroot/web/pdf/lsr/literature/4110065A.pdf). A CNH dispersion (0.3 ml of 1 mg ml^{-1}) was mixed with PBS (0.7 ml) containing MPO (50 µg) for 1–5 h and then centrifuged at 18000 g for 40 min. We took out the supernatant of centrifuge that containing free MPO (=not absorbed by CNHs), added Bradford assay solution in side, and incubated for 5 minutes, and then the optical absorbance at 595 nm was measured. The amount of MPO bounded to CNHs was calculated based on the standard curve obtained by bovine serum albumin (BSA).



b

MPO/CNHs	1 h	2 h	5 h
Absorbance at 595 nm	0.2829	0.28407	0.28855
Concentration (µg/ml)	1.16667	1.25926	1.61508
Attached on CNHs (µg/ml)	2.87103	2.77844	2.42262
Attached on CNHs (µg/mg)	57.4206	55.5688	48.4524

Figure S8. The amount of MPO bound to CNHs after incubation for various periods, as determined using a Bradford assay (The Quick Start Bradford protein assay, BioRad). (a) A standard curve obtained using bovine serum albumin; (b) Amounts of MPO attached to the CNHs after treatment for 1, 2, 5h with MPO (50µg/ml).

9. The effect of $FeCl_3$ on the oxidation of CNHs by H_2O_2

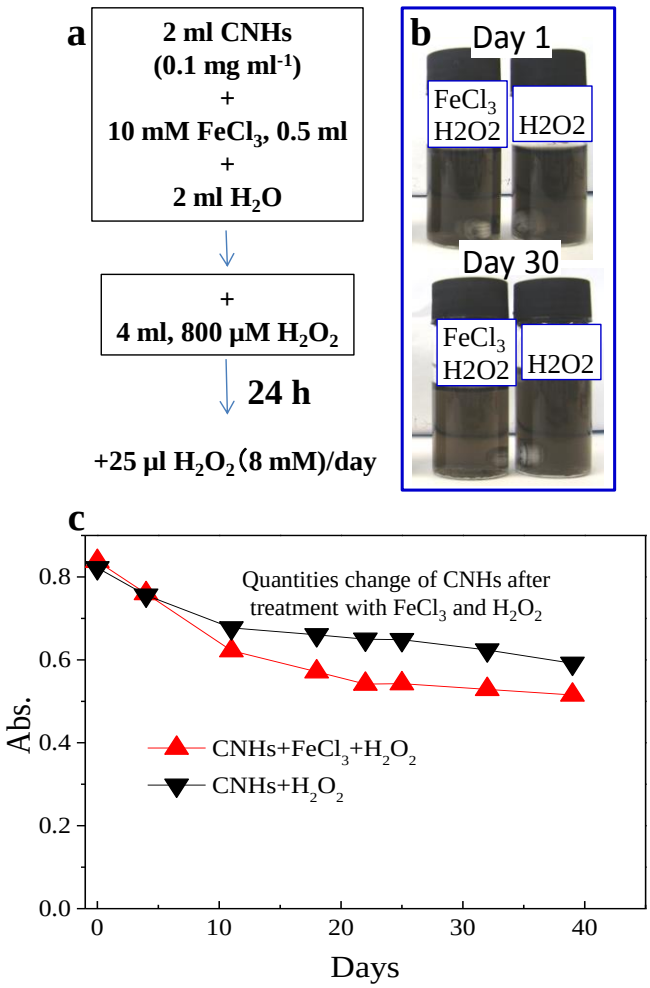


Figure S9. The effect of $FeCl_3$ on the degradation of CNHs. (a) An overview of the experimental process. CNHs (2 ml of 0.1 mg ml^{-1}) were mixed with an aqueous solution of $FeCl_3$ (0.5 ml of 10 mM) and water (2 ml). After sonication for 5 min, H_2O_2 (4 ml of 800 μM) was added and the suspension was stirred continually. After 24 h, H_2O_2 (25 μl of 8 mM) was added daily. (b) Photographs of the CNH suspensions treated with H_2O_2 and with or without $FeCl_3$. (c) The quantities of CNHs after treatment with H_2O_2 alone or H_2O_2 and $FeCl_3$, which were estimated by optical absorbance measurements of the CNH suspensions.