Supplementary Information (ESI)

Assessing DNA Damage from Enzyme-Oxidized Single-Walled Carbon Nanotubes

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Table S1. Degradation conditions studied in literature.

Degradation conditions	Carboxylated SWNT, Degradation time	Pristine SWNT
HRP + 0.04 mM H_2O_2 (4°C)	Yes, 16 weeks[1]	No[1]
HRP + 0.04 mM H_2O_2 (R.T.)	Yes, 10 days[2]	No[2]
H_2O_2 (0.04 mM)	No[2]	No[2]
Hemin + 0.8 mM H_2O_2	Yes[2]	Yes, 10 Days[2]
$FeCl_3$ + 0.8 mM H_2O_2	Yes[2]	Yes, 10 Days[2]
MPO + H_2O_2 + NaCl	Yes, 24 hours[3]	N/A[3]
MPO + H_2O_2	Yes[3]	N/A[3]
Sodium hypochlorite (NaClO)	Yes[3]	N/A[3]
H_2O_2 (1 mM)	Yes, 90 Days[4]	No[4]

N/A, not reported; No, no degradation were observed

Materials. Purified and low functionality pristine SWCNT (P2-SWNT) was purchased from CSI (Carbon Solutions Inc, Riverside, CA). Laboratory oxidized SWNTs were carboxylated as reported previously.[5] Briefly, approximately 13 mg of SWNTs was sonicated in 100 mL of piranha solution (1:3 volume ratio of 30% H_2O_2 and 98% H_2SO_4/H_2O_2, *Caution: piranha solution should be handled with extreme care*) for 24 hr at 0 °C. After 10 and 16 hr, 1.0 mL of H_2O_2 was supplemented into the reaction to compensate for H_2O_2 decomposition. The final dispersion was heated at 70 °C for 10 min, and subsequently diluted 10-fold, filtered on a 0.22 μm membrane filter, and washed with copious amounts of water to a neutral pH. Metallopolymer $[Ru(bpy)_2(PVP)_{10}](ClO_4)_2$ (RuPVP) was prepared and characterized following previous methods.[6] Male human liver microsomes were from BD Biosciences (liver microsomes contains major cytochrome P450 enzymes responsible for xenobiotic metabolism). Ethylenediaminetetraacetic acid disodium salt dehydrate (EDTA), calf thymus double stranded (ds)-DNA (Type I) and ABTS [2,2'-azinobis(ethylbenzthiazonline-6-sulfonate)] were purchased from Sigma. Water was purified with a Hydro Nanopure system to specific resistance >16 M. All other chemicals were reagent grade.

Raman Spectroscopy. 20 μl of cSWCNTs were applied and evaporated on a glass slide. Raman spectra were recorded using a Renishaw Ramanscope 2000 equipped with a Laser (514 nm) and a Leica microscope. The spectra shown in the figures are representative of the recorded spectra. The Raman spectrum of piranha solution oxidized SWCNT is shown in Figure S1. The peaks at around 1350 and 1580 cm^{-1} are the characteristic of SWCNTs, correspond with the graphite (G) and defect (D) bands of SWCNTs.[7] The G-band corresponds to the tangential vibration of the carbon atoms and the D band represents the defective graphitic structures.

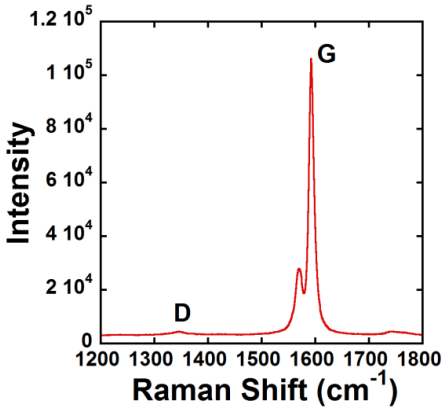

Figure S1. Raman spectra of oxidized, shortened SWNTs showing D band around 1350 cm^{-1} and G band at 1580 cm^{-1}. These cSWCNT was employed in reactions with HRP and H_2O_2.

HRP activity monitoring using ABTS assay. HRP was activated by hydrogen peroxide to oxidize ABTS to form a soluble end product with a characteristic absorbance at 405 nm.[8] 10 mg ABTS was dissolved in 100 mL of 0.05 M phosphate-citrate buffer at pH 5.0. 10 µL of diluted degradation solution containing SWCNT and HRP was added along with 25 µL of 30% hydrogen peroxide into 725 µL ABTS buffer. After mixing, the absorbance readings were taken every 15 seconds using a HP 8453 UV-Vis spectrophotometer. The HRP activity in solution can be calculated using the equation below.

0.76 is the total volume in milliliters of assay, 36.8 is the millimolar extinction coefficient of oxidized ABTS at 405nm, 0.01 is the volume in milliliter of enzyme used. Activity of initial HRP is obtained in the similar manner replacing the diluted degradation solution with HRP diluent. The activity of HRP incubated with PBS, cSWNT and (cSWNT+H_2O_2) at day 1, day 3 and day 5 was monitored and normalized using the initial HRP activity, as plotted in Figure S2.

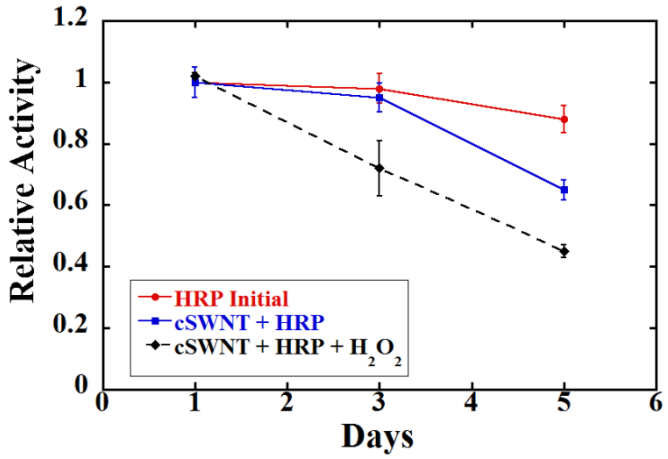

Figure S2. HRP activity under different conditions at room temperature. Error bar represents the standard deviation of HRP activity from three individual experiments.

Biodegradation of cSWNTs. cSWCNTs were biodegraded as reported previously.[2] Briefly, four vials (**S**, **C1**, **C2** and **C3**) were prepared by sonicating for 1 min approximately 0.5 mg of carboxylated SWCNTs into 4.0 mL of pH 7.4 phosphate buffered saline (PBS) per each vial. 4.0 mL HRP solutions (0.4 mg/mL in pH 7.4 PBS) was added to two vials of carboxylated SWNTs suspension (**S** and **C2**), creating a total volume of 8.0 mL. **C1** and **C3** vials were added 4.0 mL of pH 7.4 PBS buffer. Following one full day of incubation, an initial 8 mL of 0.8 mM H_2O_2 was added to **S** and **C1** start the degradation reaction, followed by daily additions of 100 µL of 0.4 mM H_2O_2. For vial **C2** and **C3**, pH 7.4 PBS buffer was added to replace H_2O_2. In the presence of cSWNT and H_2O_2, HRP gradually lost its activity and it retained about 50% of the initial activity after 5 days (Fig. S2). To keep a constant HRP activity in the degradation solution, 100 µL of 1.6 mg/ml fresh HRP was added to **S** and **C2** every five days.

Transmission electron microscopy for characterization of the degraded CNTs. Two desalting methods were used for preparation of TEM samples. In one method, SWCNTs were precipitated by centrifugation at 10000 g for 10 mins and the supernatant was discarded to remove the salts from the reaction buffers. The other method is to use molecular weight cut off filters (MWCO 3000) to separate low molecular weight salts from SWCNTs and residues with molecular weight above 3000 were retained above the filter after centrifugation. SWCNTs obtained in both ways were redispersed in methanol for characterization. TEM was performed on a Tecnai microscope with an accelerating voltage of 75 kV. 20 µL of a suspension of CNTs in methanol were deposited on a carbon TEM grid and dried.

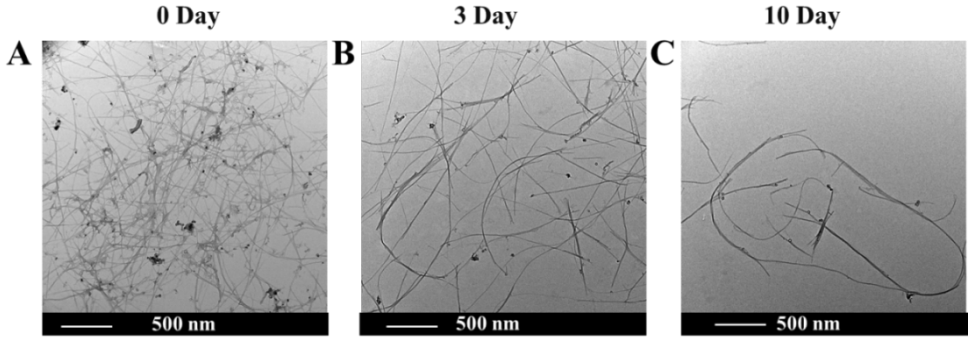

Figure S3. TEM micrographs of enzyme-catalyzed degradation of SWCNTs precipitated by centrifugation, redispersed in methanol, and applied to carbon TEM grid.

Electrochemiluminescent array for detection of potential DNA damage by extracts of degraded SWCNTs.
(1) Layer by Layer assembly of ECL array spots.

About 2 μL of DNA (2 mg mL^{-1}, 10 mM Tris pH 7.1 + 50 mM NaCl) were applied at demarcated locations on the PG block for 15 min to build up the base film. After the first DNA layer, two sets of RuPVP/DNA bilayers were applied successively with RuPVP (2.5 mg mL^{-1}, 88% H_2O, 12% ethanol) depositing for 5 min. The whole block was put in the ice bath for optimal absorption of cytosolic and microsomal enzymes from human liver. First, human liver cytosol (HLC) was applied for 30 mins absorption and another bilayer of RuPVP/DNA was followed. The last layer was human liver microsome for 30 min absorption. The final composition of thin films on array spots are $(DNA/RuPVP)_2$/DNA/RuPVP/DNA/HLC/RuPVP/DNA/HLM. For brevity, these assemblies are generally referred as DNA/RuPVP/enzyme films for the rest of the paper.

(2) Preparation of SWCNT extracts solution. Approximately 4 mL of dispersion samples of **S**, **C1**, **C2** and **C3** (every sample was duplicated), which have been incubated with designated for 1, 3, 5, 7 and 10 days, was acidified by the addition of 300 μL of 0.1 M HCl and extracted three times with ethyl acetate. After removal of ethyl acetate, products were then redispersed in 50 μL pure acetonitrile. The resulting solutions contain ethyl acetate extractable products from degraded SWCNT at different days and they were named as **S-xD** (**C1-xD**, **C2-xD or C3-xD**) for extracts from solution **S** (**C1**, **C2** or **C3**) after x day degradation period. As a result, these solutions were under the names of **S-1D**, **S-3D**, **S-5D**, **S-7D**, **S-10D**, **C1-1D**, **C1-3D**, **C1-5D**, **C1-7D**, **C1-10D**, **C2-1D**, **C2-3D**, **C2-5D**, **C2-7D**, **C2-10D**, **C3-1D**, **C3-3D**, **C3-5D**, **C3-7D**, **C3-10D.** Since two independent experiments was performed under each condition (**S**, **C1**, **C2** and **C3**), duplicate extracts were obtained.

(3) Incubation reactions. Incubations were done on arrays by spotting 50 μL incubating solutions onto four of the RuPVP/DNA/enzyme spots at 37 °C for up to 60 s. The incubating solution was 0.4 mL of 10 mM pH 6.0 MES buffer containing 1 mM EDTA, 5 mM $MgCl_2$, an NADPH regenerating system (10 mM G6P, 10 unit G6PDH, 0.8 mM NADP) donating electrons to the cytochrome P450 (cyt P450) enzymes and 2 μL of individual **c**SWCNT extracts solution. These cSWCNT extracts were from the same set of degradation experiment. The block was rinsed rapidly with water to stop the reaction. Control experiments were performed under the exact same conditions without NADPH system. This incubation experiment was repeated with the other set of extracts described above.

(4) ECL measurements. As previously,[9] the spotted array was placed in a 150 mL beaker filled to 60 mL with pH 7.0 buffer. The counter electrode was a platinum wire ring placed directly above the array electrode with an Ag/AgCl reference electrode placed to its right. The beaker was placed in the desired position of the gel documentation dark box. A potential of 1.25 V versus Ag/AgCl was applied to the array electrode for 30 s using a CH Instruments (Austin, TX) model 1232 electrochemical analyzer with ECL acquisition by the CCD camera on the "high sensitivity" setting. Data analysis and quantification was done using GeneSnap and GeneTools software provided by SynGene.

Comet assay for detection of potential DNA damage by extracts of degraded SWCNTs.

(1) Cell culture.

A549 adenocarcinomic human alveolar basal epithelial cells were a gift from Dr. Xiuling Lu from Pharmacy Department at the University of Connecticut. In brief, 1×10^6 A549 cells were seeded into 6 well plates and incubated 24hrs. Cells with 70% confluency were treated with cSWNT samples (S-3D, S-5D, S-7D) and control (C1-5D, C2-5D, C3-5D and 0.5% styrene oxide) in 5% CO_2 at 37 °C for 48h. cSWNT samples were vacuumed dried and resuspended in 1ml PBS. After incubation, the media was removed and Collagenase® was added to the cells. The cells were later kept at 37 °C for 10 minutes. Finally, the cells were washed, centrifuged and resuspended at $1x10^5$ cells/ml in 100 μL PBS.

(2) Comet assay

An OxiSelect™ Comet Assay kit (STA-350_ from Cell Biolab, San Diego, CA, was used. 10 μL of the cell suspension and 100 μL of low-melting agarose were mixed. 75ul of the mixture was immediately pipetted onto the pre-warmed OxiSelect™ Comet Slide.. The slides were maintained horizontally at 4°C in the dark for 15 minutes for solidifying the mixture. Slides were then immersed in pre-chilled lysis buffer at 4°C for 45 minutes in the dark. The lysis buffer was aspirated from the container and was replaced with pre-chilled alkaline solution (PH>13) for 30 minutes in the dark. Sildes were later transferred to electrophoresis chamber containing alkaline electrophoresis solution, and electrophoresis was performed at 20V 300mA for 20min. Slides were removed from the chamber, washed with water twice, and then was immediately placed in 70% ethanol for 5 min and air-dried overnight at room temperature. After completely dried, the slides were stained with Vista Green DNA Dye® and dried overnight in the dark.

The slides were read using an epifluorescent microscope with FITC filter and analyzed by Comet Assay IV from Perceptive Instruments Ltd (Bury St Edmunds, UK). Data were based on 50 randomly selected cells per sample. The tail moment has been considered to be an appropriated index of induced DNA damage, which can be measured using one of the following methods:

Eq. a: Olive Tail Moment= Tail DNA% × Tail Moment Length
Eq. b: Extent Tail Moment= Tail DNA% × Length of Tail

Tail moments were directly analyzed by the software.

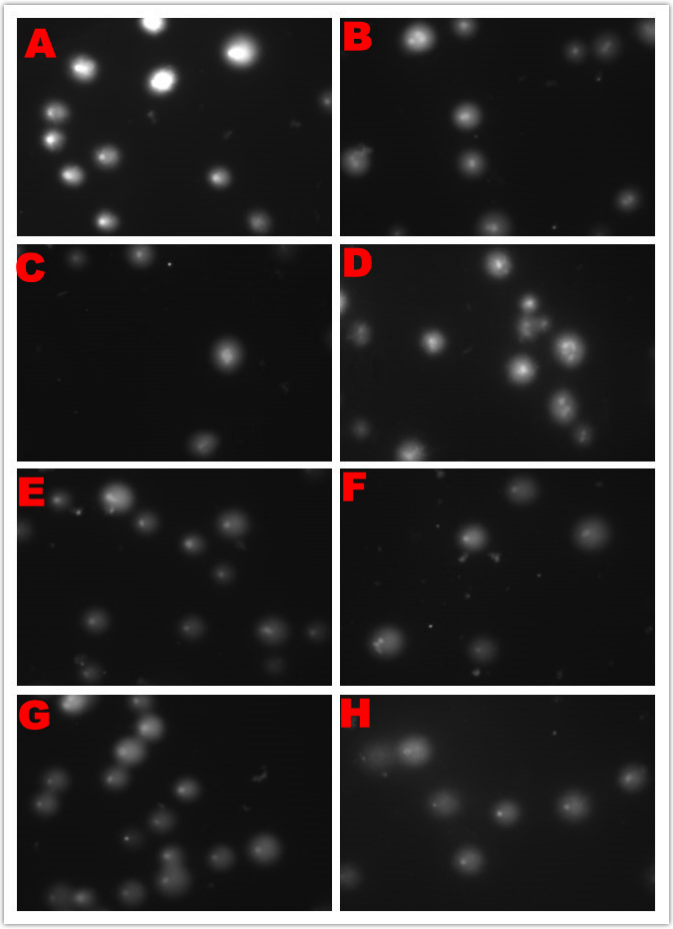

Figure S4. The comet assay response of cells incubated with different materials including A) PBS buffer; B) C1-5D extract; C) C2-5D extract; D) C3-5D extract; E) S-3D extract; F) S-5D extract; G) S-7D extract and H) styrene oxide (SO).

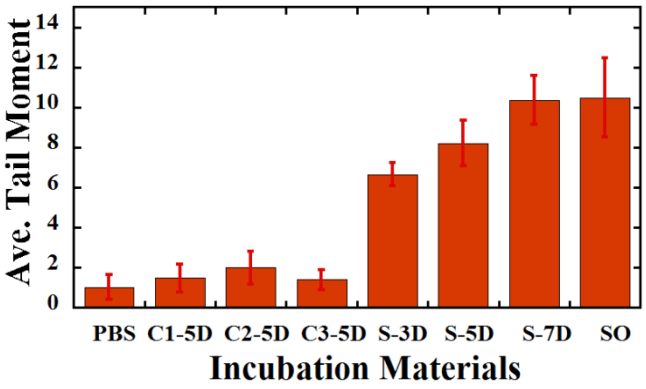

Figure S5. The average tail moment of cells incubated of materials as labeled. Error bar represents the standard deviation of the tail moment from 10 individual cells.

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