Supporting information

Carbon Nanotube Degradation in Macrophages: Live Nanoscale Monitoring and Understanding of Biological Pathway

Dan Elgrabli[1‡], Walid Dachraoui[2‡], Cécilia Ménard-Moyon[3], Xiao Jie Liu[4], Dominique Bégin[4], Sylvie Bégin[5], Alberto Bianco[3], Florence Gazeau[1] and Damien Alloyeau[2*]*

(1) Laboratoire Matière et Systèmes Complexes, UMR7057 CNRS/Université Paris Diderot, Paris France.

(2) Laboratoire Matériaux et Phénomènes Quantiques, UMR7057 CNRS/Université Paris Diderot, Paris France.

(3) CNRS, Institut de Biologie Moléculaire et Cellulaire, Laboratoire d'Immunopathologie et Chimie Thérapeutique, 67000 Strasbourg, France

(4) Institut de Chimie et des Procédés pour L'Energie, l'Environnement et la Santé (ICPEES) UMR 7515, Université de Strasbourg, 25 rue Becquerel, 67087 Strasbourg cedex 2 France

(5) Institut de Physique et de Chimie de Strasbourg (IPCMS) UMR 7504 CNRS-Université de Strasbourg, 23 rue du Loess, BP 34 67034 Strasbourg cedex 2, France

* Damien Alloyeau: alloyeau.damien@gmail.com and Florence Gazeau : florence.gazeau@univ-paris-diderot.fr

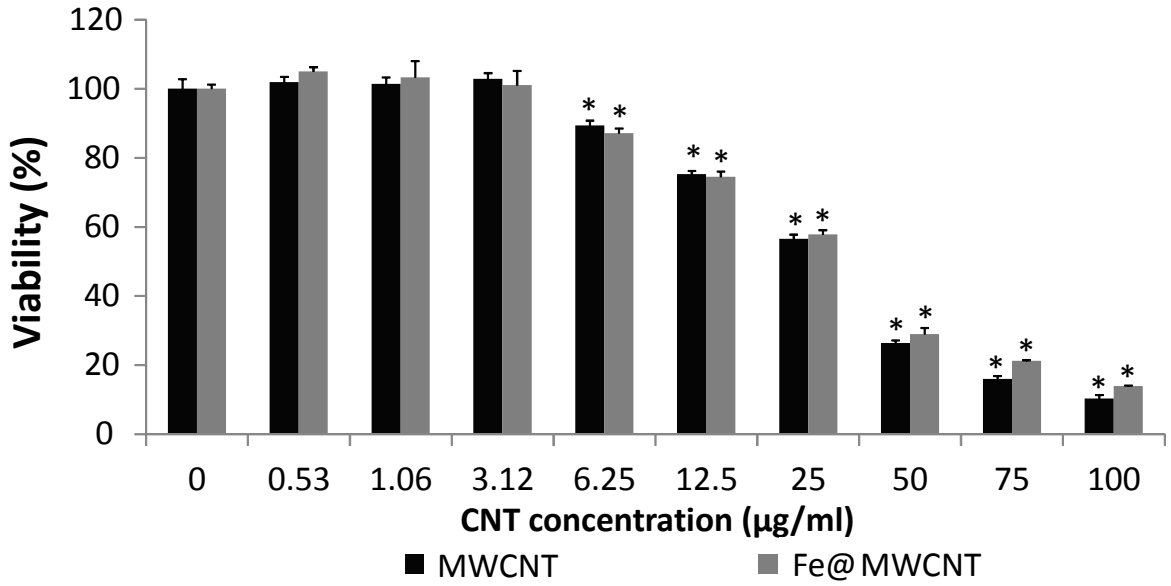


Figure S1:Cytotoxicity induced by MWCNTs and Fe@MWCNTs on THP-1 cells. Cell viability on THP-1 differentiated into macrophages treated for 24h with different concentrations of MWCNT or Fe@MWCNT was assessed by Alamar® Blue assay. Results are the mean ±SD of 3 separate experiments, each carried in triplicates. (*) Represents a statistically-significant difference from 0 µg/ml group ($p<0.05$).

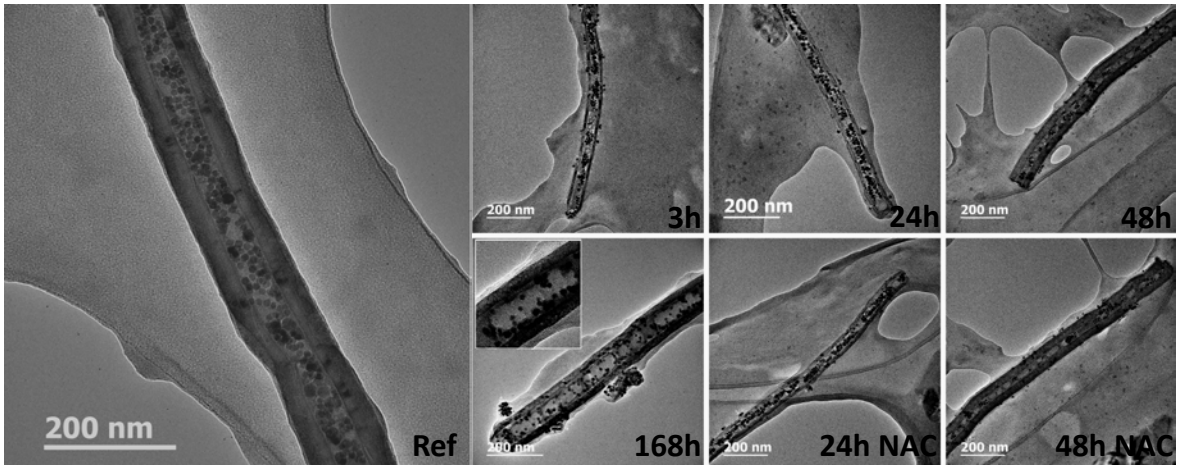


Figure S2: Fe@MWCNT degradation in THP-1 observed and quantified by TEM. TEM observations of Fe@MWCNTs before (Ref) and after exposure to cells. The incubation time and the presence of NAC in cells are indicated in the right down corner of each image.

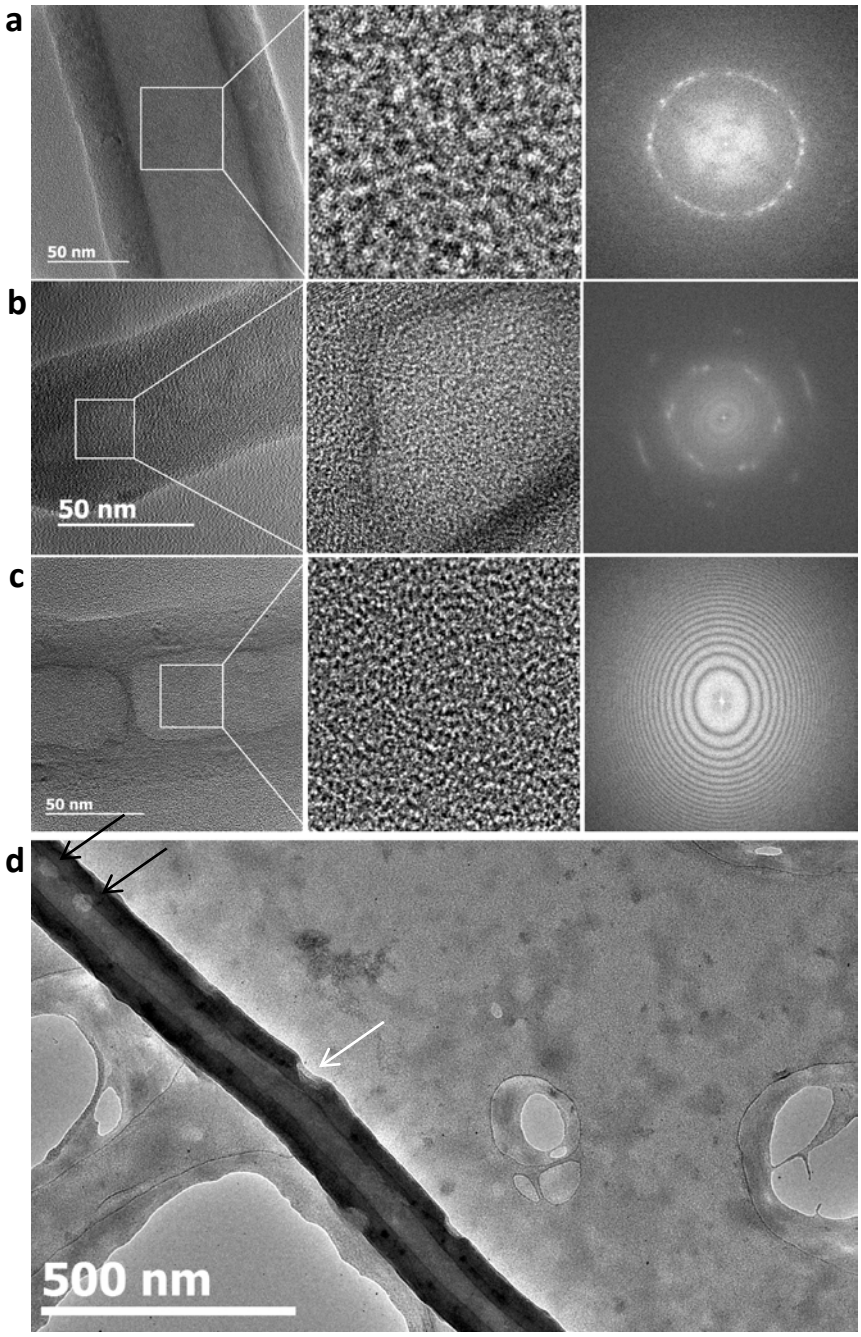


Figure S3: High-resolution analyses of MWCNTs degraded in macrophages. HRTEM image and corresponding fast Fourier transform (FFT) of **a**, an unperforated area, **b**, a perforated area with only one side of the tube attacked (FFT calculated over the hole shows the characteristic reflections for graphitic structure), **c**, an elongated perforated area through the whole nanotube (FFT calculated over the hole shows no characteristic reflections for graphitic structure). **d**, Lower-resolution image showing top views (black arrows) and side views (white arrows) of holes within the same nanotube. This indicates that MWCNTs are isotropically attacked.

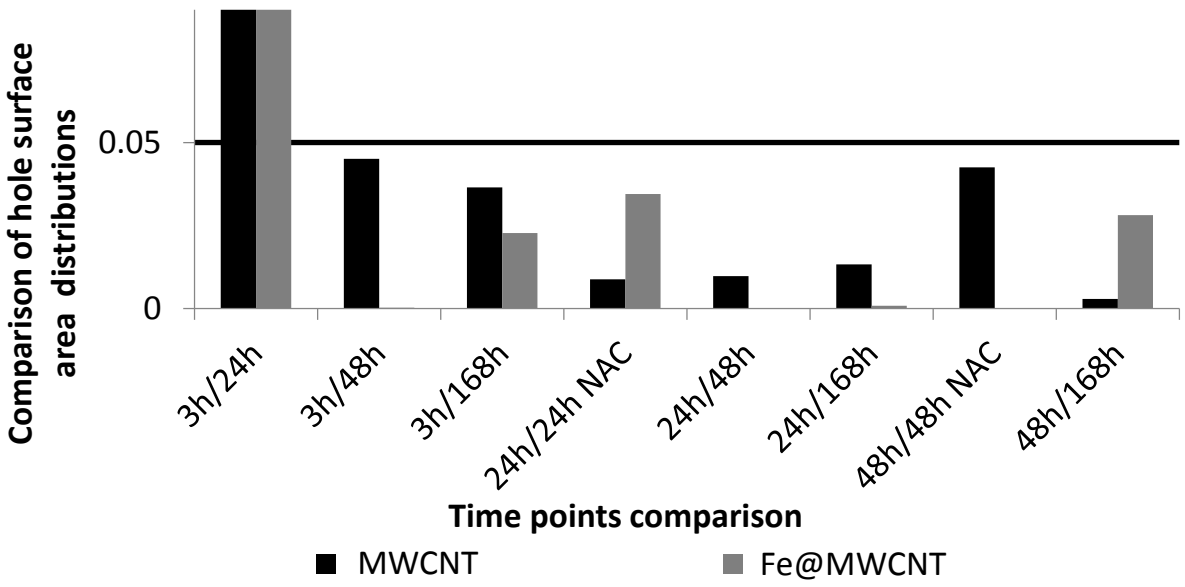


Figure S4: Comparaison of MWCNT and Fe@MWCNT holes induced by ROS. Comparison per pair of size distributions of the hole surface area in MWCNT and Fe@MWCNT for different incubation times. P-values obtained by Kolmogorov-Smirnov test were represented in y axis. P-values less than 0.05 show statistically-significant difference between the two distributions.

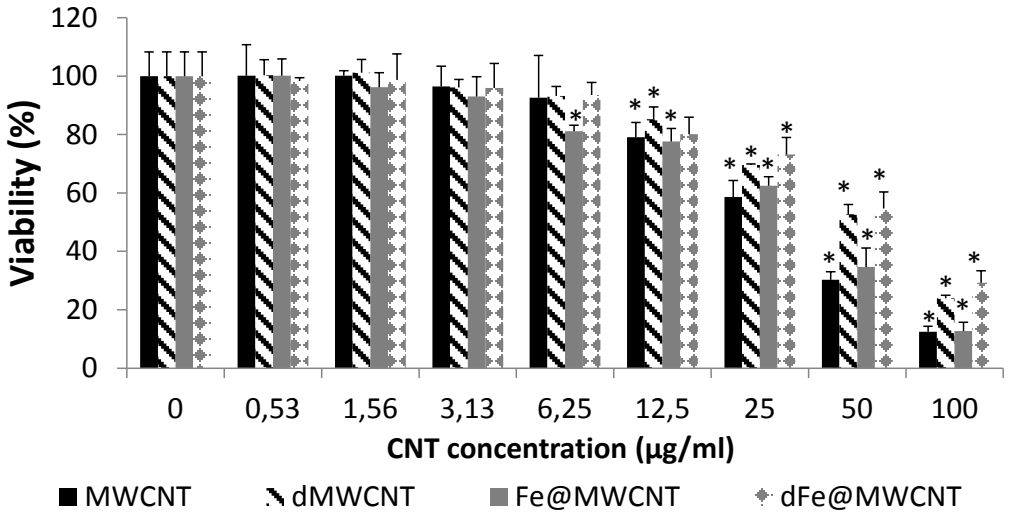


Figure S5 | Cytotoxicity induced by MWCNTs, degraded MWCNT (dMWCNT), Fe@MWCNT and degraded Fe@MWCNTs (dFe@MWCNTs) on THP-1 cells. Cell viability on THP-1 differentiated into macrophages treated for 24h with different concentrations of MWCNT, dMWCNT, Fe@MWCNTs or dFe@MWCNT was assessed by Alamar® Blue assay. Results are the mean ±SD of 3 separate experiments, each carried in triplicates. (*) represents a statistically-significant difference from 0 µg/ml group ($p<0.05$).

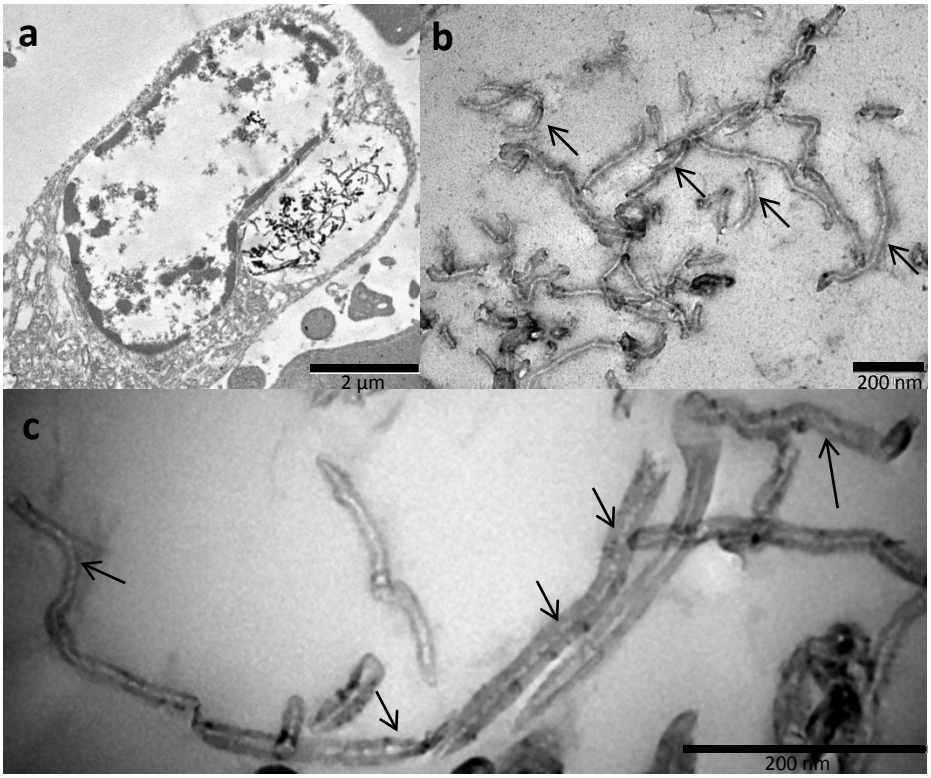


Figure S6 | MWCNT degradation in lymph nodes of mice observed 7 days after intra-tracheal instillation by TEM. a, in macrophages and **b and c**, in macrophage phagosome. As it was observed in vitro, many holes are observed in the graphitic structure of the CNTs (some are indicated by black arrows).

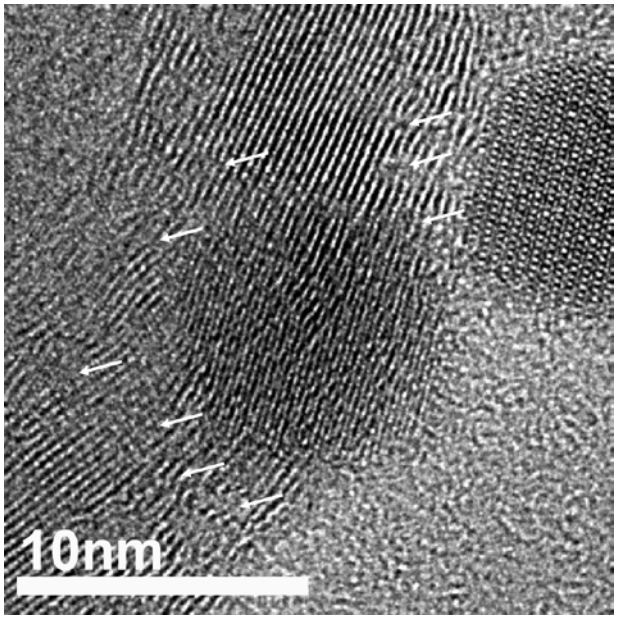
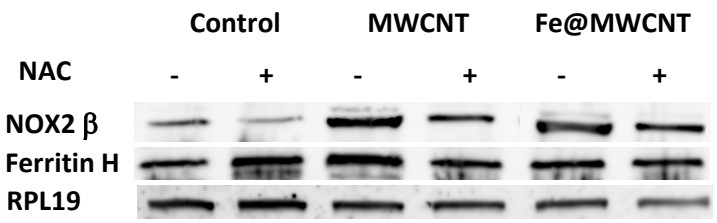


Figure S7: High-resolution analyses of Fe@MWCNTs. HRTEM image of an iron oxide nanoparticles inserted in-between the walls of a nanotubes. White arrows indicate the many structural defects of the graphitic layers close to the nanoparticle.



$Rd_{NOX2\beta}$ ±SD	1.00 ±0.03	0.84 ±0.09	2.56* ±0.47	1.78* ±0.36	2.43* ±0.23	1.45 ±0.65
$Rd_{Ferritin\ H}$ ±SD	1.00 ±0.05	1.15 ±0.25	2.12* ±0.19	1.25 ±0.12	1.40 ±0.38	1.26 ±0.17

Figure S8. Effect of MWCNTs and Fe@MWCNTs on $NOX_{2\beta}$ and Ferritin H protein level in presence or absence of NAC. THP-1 cells were treated with 5 µg/ml of MWCNT or Fe@MWCNT suspension. After 24h exposure, quantification of $NOX_{2\beta}$ and Ferritin H protein level were performed by western blot analysis. Results are the mean of densitometry ratio ±SD of 3 separate experiments. (*) Designates a statistically-significant difference from control group ($p<0.05$).

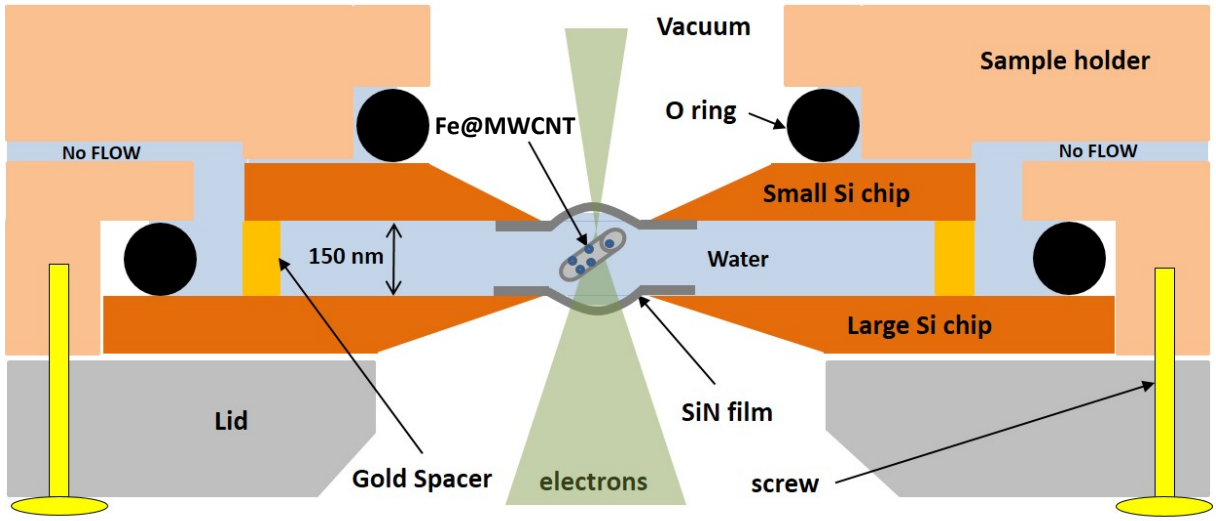


Figure S9: Schematic cross section of the sealed liquid cell. In the JEOL ARM microscope the small E-chip is on the top.