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ARTICLE TYPE

Single-Walled Carbon Nanotubes as Anisotropic Relaxation Probes for Magnetic Resonance Imaging

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TXRF of metal contaminants before and after different oxidation times.

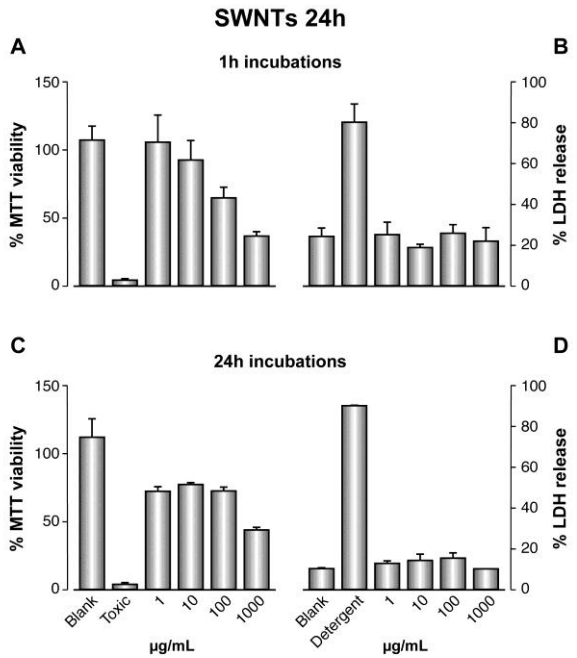
Supplementary Table I shows the results of TXRF analisis of the SNCNTs preparations before and after 24 or 48h oxidations, respectively. The oxidation protocol resulted in significant decreases in the paramagnetic metal content of the non oxidized starting materials.

Suplemmentary Table 1. TXRF analisis of the SNCNTs preparations before and after 24 or 48h oxidations.

	[Total] (ppm)	[Ni] (ppm)	Ni SCNWTs (%)	[Y] (ppm)	Y SCNWTs (%)
Untreated	802	141.14	17.6	34.75	4.3
24 hr oxidation	1132.5	35.939	3.2	7.296	0.64
48 hr oxidation	1133	66.741	5.9	13.302	1.17

Cellular Toxicity

We investigated cellular toxicity of SWCNTs oxidized for 24h using two different methods, either assesing mitochondrial function (MTT assay) or plasma membrane integrity (LDH assay), respectively. C6 cells were grown to confluence in Dulbecco's Modified Eagle Medium (DMEM) using 96 well plates. SWCNTs suspensions prepared on Fetal Bovine Serum (FBS) (Gibco, Life Technologies, Alcobendas, Madrid, ES) as described above, were added to each well in final concentrations of 1, 10, 100 and 1000 μg mL^{-1}, using three cell replicas for every SWCNTs concentration. The viability of the cellular preparations was determined spectrophotometrically (570nm, Spectramax, Molecular Devices, Downington, PA, USA) 1h and 24h after the addition of the different SWCNTs suspensions, using commercial kits to determine mitochondrial performance as the reduction of tetrazolium salts (MTT, Millipore, Billerica, MA, USA) (Suppl. Figure 1, left panels). As blank we used the medium from cells incubated in the absence nof SWCNTs while cell death was induced by adding the toxic hydrazine (5 mM) to the incubation medium. We observed approximatelly 100 % cell viability in the abscence of SWCNTs and roughly nulle viability in cells exposed to the toxic. A decrease in formazan production was detected at the highest SWCNTs concentartions, a finding that has been described previously to be due to a direct reaction of the nanotubes with the MTT molecule, rather to a citotoxic effect on the cells [Suppl. ref. 1]. To further investigate cytotoxicity, we performed additional tests using the same SWCNTs preparation but measuring the release of lactate dehydrogenase (LDH) from the cell supensions as an indicator of plasma membrane integrity and cell viability (Suppl. Figure 1, right panels). Briefly, SWCNTs were added to each well to final concentrations in the 1-1000 μg.mL^{-1} range. LDH activity was determined spectrophotometrically (340 nm, Spectramax, Molecular Devices, Downington, PA, USA) following the linear decrease in NADH absorbance in mixtures containing 50 mM HEPES pH: 7.0, 5 mM pyruvate and 0.6 mM NADH. We initiated the reaction with the addition of either aliquots from the incubation medium or from the cell lysate obtained after four cycles of freezing and thawing. We used as blanks, aliquots of the incubation medium containing no SWCNTs and as a maximum LDH release, that induced by adding the detergent sodium dodecylsulfate (2%) to the cell suspensions. We determined then the fractional LDH released (LDH medium/(LDH medium+LDH lysate) either after 1h or 24h incubations. While we observed no significant fractional LDH release in the blanks or close to 90 % after detergent treatment as expected , we detected no significant increase in fractional LDH release with increasinmg concentrations of SWCNTs either after 1h or 24h incubations. Taken together, our results revealed no significant cytotoxic effects of our SWCNTs preparations in the concentrations required for observable magnetic anisotropy effects.



Supplementary Figure 1. MTT assays (left column) and fractional LDH release assays (right column) of cellular viability for increasing concentrations of SWCNTs oxidized with nitric acid for 24h, either 1h (top row) or 24h (bottom row) after the addition to the cells of the SWCNTs suspensions (1-1000 µg.mL^{-1}) in Fetal Bovine Serum.

Supplementary Bibliography

Suppl Ref. 1. Wörle-Knirsch, J.M., Pulskamp, K., Krug, H.F., *Nanoletters* 2006, **6**,1261-68.

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