Supplementary Material (ESI) for Nanoscale

Biocompatible dispersions of Carbon nanotubes: a potential tool for intracellular transport of anticancer drugs

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Electronic Supplementary Information (ESI)

1. **Experimental section**

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   Figure S1 DLS analysis.
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Experimental Section

Materials. Poly(ethylene glycol) monomethyl ether (average molecular weight 2000 g mol^{-1}), propylene sulfide, thionyl bromide and thioacetic acid were obtained from Fluka (Buchs, Switzerland). Sodium methoxide, 2,2′-dithiopyridine and doxorubicin were obtained from Aldrich (Steinheim, Germany). All the solvents were purchased from Sigma-Aldrich and were not further purified. THF was used without stabilizers.
Pristine MWNTs (Nanocyl® 3150) were used in all the measurements. Ultra pure MilliQ water (Millipore Corp. model Direct-Q 3) with a resistivity of >18.2 MΩ-cm was used to prepare all solutions.

Dynamic Light Scattering analysis. Dynamic Light Scattering experiments were performed on a Zetasizer Nano ZS, Malvern Instrument Ltd., U.K. The data are reported in Figure S1. The initial concentration of PEG-PPS-FITC in the aqueous solution was 0.25 mg mL^{-1}. Albeit data obtained from DLS analysis evidence prevailingly strongly light scattering big aggregates, the almost complete disappearance of the smallest aggregates after gel filtration gives evidence of the removal of unbound copolymer.

Determination of DOX loading. Aqueous PBS buffered dispersion of $PEG_{44}PPS_{20}$ at concentration of 0.9 mg mL^{-1}, 2.3 mg mL^{-1} and 3.6 mg mL^{-1} were used to disperse MWNT in aqueous PBS buffered solution following previously published protocols.[1] Each dispersion was mixed with a solution of 50 μg mL^{-1} doxorubicin hydrochloride and DOX loading onto the nanotubes was performed by stirring the samples overnight. The obtained samples were spectrofluorimetrically characterized by using a Jasco FP-6500 spectrofluorimeter (See Figure S2).

Cell culture. Both Hela cells (human cervical cancer cells) and MDA-MB-435 cells (human breast cancer cells) were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin. All chemicals for cell culture were purchased from Gibco Invitrogen Corporation. Cells were maintained in culture at 37°C in a 5% CO_2 humidified incubator.

Confocal Microscopy imaging. The dispersion of PEG-PPS-FITC coated MWNTs were prepared as follows: 1 mg of pristine HiPCO MWNTs was added to 2 mL of 0.25 mg mL^{-1} PEG-PPS-FITC aqueous solution and sonicated with a ultrasonic bath sonicator (Bandelin Sonorex, 35 KHz) for 6 hr. Non-dispersed MWNTs and insoluble impurities such as graphite, amorphous carbon and metal catalysts were removed by centrifugation by using a Universal 32 (Hettich Zentrifugen) centrifuge for 15 min at 4000 RPM. Gel filtration was performed on the obtained dispersion, by using Sepharose 6B columns, in order to get rid of unbound block copolymer. Hela cells were plated in tissue culture dishes with cover glass bottom and maintained in culture up to 70% confluency. Cells were then incubated at 37 °C with 200 μL of the eluted dispersion of PEG-PPS-FITC coated MWNTs in 2 mL of Dulbecco's modified Eagle's medium (DMEM) for 48 hours. The final concentration of MWNTs was *ca*. 25 μg mL^{-1}. The cells were subsequently washed 3 times with phosphate buffered saline (PBS). Cellular nucleus was stained by incubating the cells with DAPI (250 μg/L) for 10 min at 37°C. Cells were then washed with PBS and observed by spinning disk confocal microscope (Perkin Elmer). The results are shown in Figure 2 of the Manuscript.

Viability test. The Alamar blue™ (AB) assay was used to determine the variation in cellular metabolic activity of MDA-MB-435 cells. Cells were washed and incubated with 10% (v/v) Alamar blue dye solution in DMEM for 1h at 37°C. Following the incubation, AB fluorescence, directly proportional to cell viability, was quantified at the respective excitation (550 nm) and emission (600

nm) wavelength using the plate reader (TECAN). Experiments were performed in quadruplicate. The cell viability of the untreated control was defined as 100%. Cytotoxicity data were expressed as mean percentage relative to the unexposed control ± standard deviation (SD).

Results

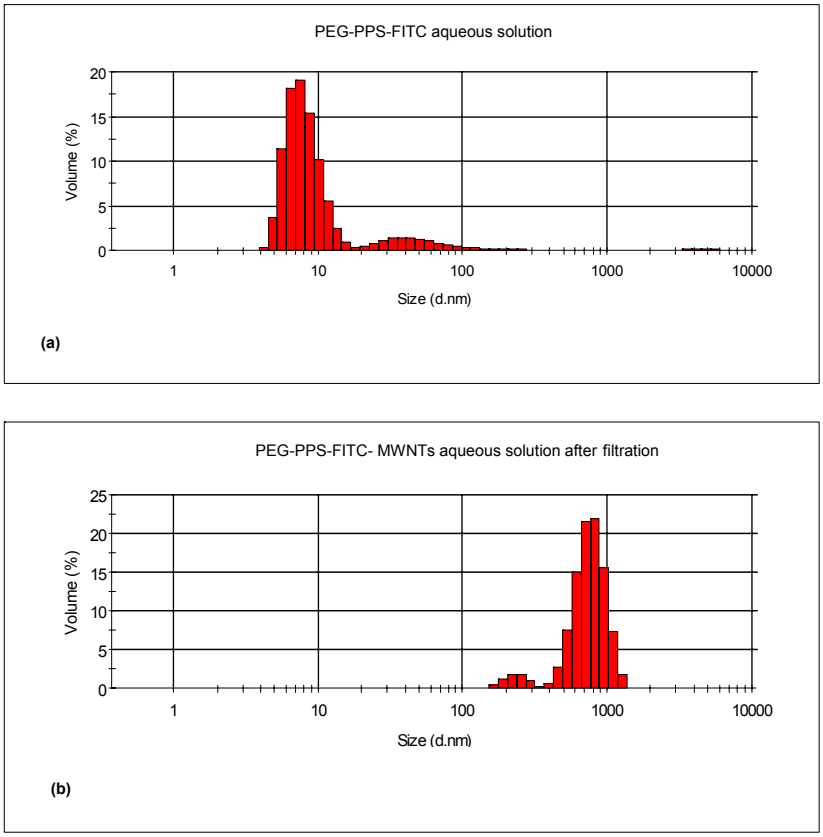

Figure S1 DLS analysis of (a) PEG-PPS-FITC aqueous solution and PEG-PPS-FITC coated MWNTs aqueous solution after (b) gel filtration (i.e. size exclusion chromatography on Sepharose 6B column).

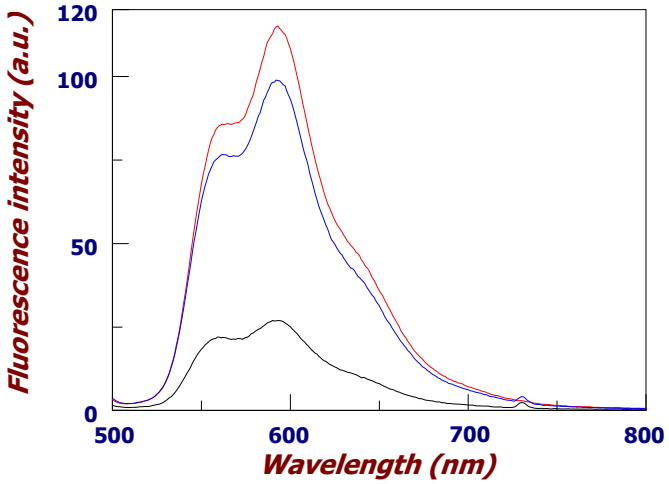

Figure S2 Fluorescence intensity of $PEG_{44}PPS_{20}$ coated MWNTs/DOX complexes prepared in the presence of different amounts of block copolymer: 0.45 mg mL^{-1} (black line), 1.15 mg mL^{-1} (blue line) and 1.8 mg mL^{-1} (red line). The concentration of DOX and MWNTs are kept constant at 25 µg mL^{-1}. The sample containing the lowest concentration of $PEG_{44}PPS_{20}$ (0.45 mg mL^{-1}) shows a considerable quenching in agreement with a high adsorption of DOX onto MWNT sidewalls.

Notes and references

1 E. M. Di Meo, A. Di Crescenzo, D. Velluto, C. O'Neil, D. Demurtas, J. A. Hubbell and A. Fontana, *Macromolecules,* 2010, **43**, 3429.