In-vitro toxicity of carbon nanotube/polylysine colloids to colon cancer cells

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Abstract

Single-walled carbon nanotubes (SWCNTs) are thoroughly purified and dispersed in an aqueous solution of high molecular weight poly-L-lysine (pLlys). Human intestinal epithelial Caco-2/TC7 cells are incubated with the SWCNT dispersions in pLlys, and their effects on cell viability are studied by image flow cytometry. No significant changes are observed in the cell culture wells up to pLlys concentrations of 10 μg mL^{-1}. However, high mortality is detected at pLlys concentrations of 100 μg mL^{-1}. The presence of oxygen-free SWCNTs does not modify the effects of pLlys on cell cultures at any of the tested concentrations (≤ 1 μg mL^{-1}). In addition, SWCNTs having an 8 wt.% of surface oxygen are tested with identical results. Thus, purified SWCNTs, even bearing oxygen functional groups, act as inert particles in the cell culture medium. This result supports the applicability of SWCNTs as carriers in pharmacological formulations against digestive tract diseases.

Keywords: carbon nanomaterial; polypeptide; centrifugation; purification; cell culture

1. Introduction

Poly-L-lysine (pLlys) is a polypeptide made of the essential amino acid L-lysine. The natural form of pLlys (ε-pLlys) contains 25-35 L-lysine residues, and is produced by certain microorganisms by the linkage between α-carboxyl groups and ε-amino groups [1]. Applications of ε-pLlys range from food industry to biomedicine and biosensors [1, 2]. Artificial methods for pLlys synthesis typically yield α-pLlys, since the polymerization occurs through the α-amino group, and can reach much higher molecular weights than natural ε-pLlys. Synthetic pLlys is utilized as an adhesion

promoter in cell cultures, and it is being investigated for its antineoplastic activity, anti-prion activity, and gene delivery properties [3-5].

Single-walled carbon nanotubes (SWCNTs) have been proposed for several applications in biomedicine, including their potential as nanocarriers for therapeutic and probe molecules [6]. The adsorption interactions and chemical reaction paths provided by SWCNT surfaces are expected to be excellent tools for the design of multipurpose supramolecular biostructures. However, important toxicity issues need to be understood and controlled before the application of SWCNTs to biological systems [7].

It has been previously confirmed that arc-discharge SWCNTs can induce oxidative stress in intestinal epithelial cells, and affect the contractility of ileum [8, 9]. Possible reasons for those adverse effects are not necessarily given by the SWCNTs themselves, but could be associated to metal catalyst impurities (Ni, Y), or to functional groups produced during SWCNT preparation [7]. Quite recently, we have reported the possibility of purifying and dispersing pristine or functionalized arc-discharge SWCNTs by ultracentrifugation in different aqueous media [10]. Interestingly for biomedical applications, metal catalyst impurities can be totally eliminated by that method, and the dispersion medium can be chosen among a range of surfactants, polymers, and biomolecules [10]. Long polymer chains are preferred for the stabilization of carbon nanotubes in water suspensions through excluded volume interactions [11]. Previously, SWCNTs have been covalently functionalized with pLlys as a method for further derivatization [12]. In addition, non-covalent functionalization with pLlys has been proposed as a way for the preparation of water-stable dispersions of carbon nanotubes [13, 14].

In this work, arc-discharge SWCNTs are purified and dispersed in a high molecular weight pLlys solution. Metal impurities, as well as amorphous carbon and graphitic

particles are totally eliminated from SWCNTs by the purification treatment. Toxicity of SWCNT/pLlys dispersions is studied by flow cytometry in the human intestinal epithelial Caco-2/TC7 cell line. To our knowledge, the effects of high molecular weight pLlys on colon cancer cells have not been reported so far. Since the presence of oxygenated functional groups on carbon nanotube surfaces could modify their toxicology [15], two SWCNT materials are compared, one containing a certain amount of functional groups while the other bearing no surface oxygen. The roles of pLlys and SWCNTs are clarified, at least from a chemical viewpoint, towards the preparation of pharmacological complexes against colorectal cancer.

2. Materials and methods

2.1. Preparation of SWCNT powder materials

The pristine SWCNT material was purchased from Carbon Solutions Inc., Riverside, California (AP-SWNT grade). This SWCNT material is synthesized by the arc discharge method using Ni/Y catalysts, and contains approximately 30 wt% metal catalyst residue. The average SWCNT diameter is in the range of 1.4-1.6 nm, calculated from optical absorption and Raman spectroscopy. The average SWCNT length in centrifuged dispersions is of 400-800 nm, according to atomic force microscopy measurements [16].

The pristine SWCNT powder material was air-oxidized in an oven at 350 ºC for 1h, with a process yield of 85 wt%. Air oxidation was performed for breaking the carbon shells that initially can protect metal catalyst impurities. Then, the material was refluxed in 150 mL of 3 M HCl at 150 ºC for 4 h, filtered through a 3 μm polycarbonate membrane, washed with 300 mL of water, and dried at 70 ºC for 20 h. The cumulative

yield after the acid treatment was of 56 wt%. Metal content strongly decreases with the acid treatment, and also many oxygen functional groups previously created by the air-oxidation treatment evolve as CO_2. For the total removal of surface oxygen, a fraction of the acid treated material (SWCNT-HCl) was heated at 10 ºC min^{-1} and treated at 700 ºC for 5 min under a N_2 flow in a horizontal tubular reactor. The thermal treatment yield was of 92 wt%, and the resulting powder material is hereafter called SWCNT-700.

2.2. Characterization of SWCNT powder materials

Oxygen content determination was performed in a Thermo Flash 1112 elemental analyzer by sample pyrolysis at 1080 ºC. Thermogravimetric analysis (TGA) experiments were performed in a Setaram Setsys Evolution balance. Metal contents were calculated after combustion of the samples in an air flow at 1000 ºC. The TGA residue after combustion mainly consists of metal oxides. The initial metal content can be approximated by assuming an oxygen content of 25 wt% in the TGA residue [17].

For the determination of extinction coefficients, the SWCNT-HCl and SWCNT-700 materials were dispersed in a sodium deoxycholate (DOC, Acros Organics 218591000) aqueous solution, since DOC is considered one of the best surfactants for the dispersion of SWCNTs [18], and the extinction coefficient does not depend on the surfactant [10]. In a typical experiment, 10 mL of 1 wt/vol% DOC was added to 1 mg of the SWCNT powders, and the mixture was tip-sonicated (Hielscher UP400S at 24 kHz) in an ice bath for approximately 1h. Absorbance of the resulting dispersions was measured in a Shimadzu UV-2401PC spectrometer. Extinction coefficients (ε) were calculated applying the Lambert-Beer law: $A_\lambda = \varepsilon l C$, where A_λ is the absorbance at a given wavelength excitation (λ), C is the SWCNT dispersion concentration (mg mL^{-1}) and l is

the optical pathway, which is given by the length of the quartz cuvette (1 cm). Extinction coefficients were calculated at 600 and 850 nm.

2.3. Preparation and characterization of SWCNT dispersions in pLlys

A pLlys aqueous solution with a concentration of 0.1 wt/vol% was purchased from Sigma-Aldrich (Ref. P8920, M_w = 150,000-300,000). The solution (10 mL) was added to 10 or 30 mg of the SWCNT-HCl or the SWCNT-700 powder materials respectively. The mixtures were tip-sonicated (Hielscher UP400S at 24 kHz) in an ice bath for approximately 1h. The dispersions were centrifuged at 120,000 xg for 1h in a Beckman Coulter L-100 XP ultracentrifuge provided with a SW55Ti 3671 rotor and Beckman centrifugation tubes (Ref. 326819). The supernatant was carefully decanted and the sediment was discarded. Depending on the original powder material, SWCNT-HCl or SWCNT-700, the resulting purified SWCNTs are hereafter called SWCNT-P or SWCNT-P700 respectively.

Visible-near-infrared (Vis-NIR) spectra of the dispersions were measured in 2 mL quartz cuvettes using Shimadzu UV-2401PC and Bruker VERTEX 70 spectrometers. SWCNT relative purity in the pLlys dispersions can be analyzed by means of the purity index. Purity indexes were calculated from spectral data in the window of 7750-11750 cm^{-1}, where the S_{22} band transition can be found for all the SWCNT samples studied [19]. The total area below the S_{22} curve is called a_t, while the baseline subtracted peak area is called a_s. According to Itkis et al. [19], SWCNT purity should be a function of the ratio a_s:a_t, which is called the purity index.

Conductivity (σ), electrophoretic mobility (μ), and zeta potential (ζ) were determined in a Malvern Zetasizer Nano device. The zeta potential is calculated by the Henry

equation, assuming the dielectric constant and viscosity to be those of pure water. A Crison GLP21 pH-meter was utilized for pH determinations.

Transmission electron microscopy (TEM) images were taken in a Tecnai T20 (FEI) microscope working at 200 kV and provided with a CCD Veleta (Olympus) camera. Samples were prepared by vitrification with liquid ethane in a Vitrobot (FEI). The sample supports (holey carbon grids) were previously ionized in a plasma cleaner (20% O_2 / 80% Ar) for 7 seconds. The vitrified samples were transferred to a low-temperature sample-holder, and kept at the liquid nitrogen temperature during the observation.

The purity evaluation of the purified SWCNT dispersions was completed by energy dispersive X-ray spectroscopy (EDX, Hitachi S3400N), which allows the qualitative determination of metals and other impurities with an accuracy of better than 0.1 wt.%. The SWCNT dispersions in pLlys were filtered through 0.3 μm polycarbonate membranes, and the residue was dried and analyzed directly on the filters.

2.4. Cell culture

This study was carried out in the human enterocyte-like cell line Caco-2/TC [20], kindly provided by Dr. Edith Brot-Laroche (INSERM, UMR S 872, Centre de Recherches de Cordeliers, Paris). Caco-2/TC7 cells have been used in the present study since they are an excellent human enterocyte-like model to study intestinal epithelial physiology [20, 21]. The cells were cultured at 37 ºC in an atmosphere of 5% CO_2 and maintained in high glucose DMEM supplemented with 2 mM glutamine, 100 U mL^{-1} penicillin, 100 μg mL^{-1} streptomycin, 1% non-essential amino acids, and 20% heat inactivated fetal bovine serum (Life Technologies, Carlsbad, CA, USA). The cells were passaged enzymatically (0.25% trypsin–1 mM EDTA) and subcultured in 25 cm^2 plastic culture

flasks (Sarstedt, Nuembrecht, Germany). The medium was changed 48 h after seeding and daily thereafter.

For cell viability experiments, cells were seeded in 6-well plates at a density of 2 x 10^5 cells/well, and measurements were carried out 14 days after seeding (9 days after confluence).

Caco-2 cells were incubated for 24 h with pLlys and the purified SWCNTs dispersed in pLlys. In the experiments, stock solutions of 1 mg mL^{-1} pLlys, 8 µg mL^{-1} SWCNT-P + 1 mg mL^{-1} pLlys, and 9 µg mL^{-1} SWCNT-P700 + 1 mg mL^{-1} pLlys were added to the culture medium. Five concentrations of SWCNT-P (0.08, 0.8, 8, 80 and 800 ng mL^{-1}) and SWCNT-P700 (0.09, 0.9, 9, 90 and 900 ng mL^{-1}) dispersed in their corresponding concentrations of pLlys (0.01, 0.1, 1, 10 and 100 µg mL^{-1}) were assayed. In control experiments, cells were incubated in culture medium without adding any substance.

After the incubations with the different substances, cells were detached enzymatically (0.25% trypsin - 1 mM EDTA, for 30 min) and separated using a micropipette. Individualized cells were collected by centrifugation at 900 xg for 5 min at room temperature, and the cell pellet was washed twice in PBS. Finally, cells (4-5 x 10^6 cells/sample) were resuspended in 400 µl of cold PBS and kept on ice until flow cytometry determinations.

2.5. Viability studies by image flow cytometry

The Image Stream X (ISX Amnis Corporation, Seattle, WA, USA) flow cytometer was used. Images were collected using two cameras obtaining brightfield, side scatter (SSC for cells complexity using 785 nm lasers), and signals with different band-pass filters. The channel signals were measured as intensity and location of fluorescence. The analyzed events were selected from the focused cells. The results were obtained in

biparametric graphs, representing the normalized frequency of the events and the intensity of fluorescence in all samples.

Propidium iodide staining was used to assess cellular damage induced by SWCNTs and pLlys [21, 22]. Single-cell suspensions of Caco-2/TC7 cells in PBS (2×10^6 cells/sample) were incubated with 1.87 μM propidium iodide for 3 min in the dark at room temperature. Immediately after incubation with propidium iodide, signals from cells (10^4 cells/sample) were taken using a 660 to 745 nm bandpass filter for red fluorescence. Data were analyzed using the IDEAS 5.0.252 software (Amnis Corporation).

2.6. Statistical analysis

Results of viable cells were measured as the percentage of the control (100%) in the same experiment and expressed as means ± SEM (standard error of the mean) of at least four independent experiments with consecutive passages. Differences between the concentrations for each treatment and between treatments for each concentration were analyzed using one-way ANOVA followed by Bonferroni's *post-hoc* group comparison test. Data were analyzed with GraphPad Prism5 software (GraphPad Prism, San Diego, CA), and the differences with P-values < 0.05 were considered statistically significant**.**

3. Results and discussion

3.1. Characterization of the modified SWCNT solids

Metal and oxygen contents in the modified SWCNTs are included in Table 1. Metal content in both the SWCNT-HCl and SWCNT-700 materials is lower than 10 wt.%, indicating that acid treatment decreases the metal content to approximately 1/3 of the

starting level in pristine SWCNTs. We will see that metals are finally eliminated after the subsequent step of ultracentrifugation.

According to elemental analysis results, oxygen content in the SWCNT-HCl solid is approximately 8 wt.%, which is lower than typical values for carbon nanotubes treated with oxidant acids such as HNO_3 or H_2SO_4 [10]. However, it must be considered that there is still a substantial amount of oxygen functional groups on the SWCNT-HCl solid. Therefore, a thermal treatment at 700 ºC is applied that removes most of the remaining surface oxygen. In fact, the SWCNT-700 material contains less than 1 wt.% oxygen. Direct oxygen determination was confirmed by TGA experiments under inert atmosphere (Supplementary Information).

Table 1 includes the extinction coefficients (ε) determined for the SWCNT-HCl and SWCNT-700 dispersions at 600 and 850 nm (ε_{600} and ε_{850}) respectively. These parameters are subsequently utilized to calculate the concentrations of the purified SWCNT-P and SWCNT-P700 dispersions in pLlys after ultracentrifugation. The extinction coefficients measured in the present work are somewhat lower than others previously published for similar SWCNT materials [16]. The difference could be associated to several causes: i) carbon impurities that are still present in the SWCNT-HCl and SWCNT-700 samples, and are later eliminated with ultracentrifugation; ii) effects of air oxidation and acid treatments on the SWCNT response to light; and iii) SWCNT bundling, even though no material aggregation was apparent during the measurements. In any case, the possible deviations do not substantially affect the conclusions of this work.

3.2. Characterization of the purified SWCNT dispersions

It has been shown above that the SWCNT-HCl solid contains a substantial amount of oxygen functional groups, while most of the surface oxygen was removed from the SWCNT-700 material. Oxygen functional groups contribute to the stabilization of SWCNTs in the colloidal dispersion, since they promote specific adhesion interactions with pLlys [23]. Therefore, different stabilities are observed in the SWCNT-HCl and SWCNT-700 dispersions in pLlys, particularly when they are purified by ultracentrifugation. In both cases, centrifugation yields are low, in agreement with previous observations regarding the stability of pLlys/SWCNT complexes in aqueous dispersions [13]. A purified SWCNT-P supernatant can be obtained from a starting SWCNT-HCl concentration of 10 mg mL^{-1}. However, the starting concentration must be increased to 30 mg mL^{-1} for the preparation of the SWCNT-P700 sample from the SWCNT-700 solid. For lower starting concentrations, all the solid falls to the sediment fraction, and no supernatant is collected. Likewise, it is expected that purified SWCNT dispersions with higher concentrations would be obtained by increasing the initial load.

Table 2 includes physical properties of the purified SWCNT-P and SWCNT-P700 dispersions in pLlys, which are utilized for our viability experiments. The SWCNT-P700 dispersion was diluted with fresh pLlys in order to have a nearly identical absorbance to the SWCNT-P dispersion, facilitating a direct comparison between both purified dispersions. Applying the previously determined extinction coefficients (Table 1), the SWCNT concentration in the purified dispersions was calculated to be nearly 10 μg mL^{-1}.

The natural pH value of the SWCNT dispersions in pLlys is nearly neutral, and the zeta potential (ζ) is positive, typical of cationic surfactants. The zeta potential of the SWCNT-P dispersion is higher than that of the SWCNT-P700 dispersion, probably due

to the different oxygen content in the original SWCNT solids. This fact confirms that SWCNTs in the SWCNT-P dispersion still bear some oxygen functional groups after centrifugation, which can interact with the positively charged amino groups of pLlys. The zeta potential of both purified dispersions is higher than 40 mV, thus above the classical criterion to be considered stable colloids.

The purity of the SWCNT-P and SWCNT-P700 dispersions was first assessed by vis/NIR spectroscopy (Figure 1). The SWCNT resonant bands (M_{11}, S_{22}, and S_{33}) in the purified dispersions are clearly more intense than in a dispersion containing pristine SWCNTs. The intensity increment can be quantified in a relative way by means of the purity index, which is here calculated from the S_{22} band transition. The purity indexes of the purified dispersions are 0.25 and 0.28, while it is approximately 0.10 for pristine SWCNTs. Specifically, the increase in the purity index can be associated to the removal of graphitic particles and amorphous carbon impurities during ultracentrifugation [10].

Cryo-TEM images are a powerful tool for the study of SWCNT bundling in liquid dispersions, avoiding aggregation effects due to filtration and classical TEM sample preparation [24]. In the purified dispersions, SWCNTs are mostly individualized or forming very thin bundles (Figure 1). Cryo-TEM images also show that SWCNTs are several hundreds of nanometers long.

It has been previously demonstrated that metal impurities are eliminated by ultracentrifugation in a surfactant [10]. In order to confirm metal removal from the SWCNT-P and SWCNT-P700 dispersions in pLlys, a fraction of both dispersions was filtered through polycarbonate membranes and characterized by EDX spectroscopy. In fact, metal contents in the purified materials are below the EDX detection limit ($\leq$ 0.5 wt%).

3.3. Toxicity on Caco-2/TC7 cells

The toxicity of pLlys and both the purified SWCNT-P and SWCNT-P700 dispersions in pLlys was tested. The pLlys concentration is assumed to be identical to that of the original solution (0.1 wt./vol.%), and stock SWCNT concentrations are 8 and 9 μg mL^{-1} respectively for the SWCNT-P and SWCNT-P700 dispersions. Different aliquots of the reference pLlys solution or the purified dispersions are added to cell incubation wells, resulting in final concentrations in the range of 0.01-100 μg mL^{-1} for pLlys, 0.08-800 ng mL^{-1} for SWCNT-P, and 0.09-900 ng mL^{-1} for SWCNT-P700. Therefore, Caco-2/TC7 cells were incubated for 24 h with 0.01-100 μg mL^{-1} pLlys, 0.08-800 ng mL^{-1} SWCNT-P + 0.01-100 μg mL^{-1} pLlys, and 0.09-900 ng mL^{-1} SWCNT-P700 + 0.01-100 μg mL^{-1} pLlys. The percentages of viable cells, expressed as the percentage of control (100%) and determined by image flow cytometry with propidium iodide, are summarized in Figure 2.

Incubation of intestinal epithelial Caco-2/TC7 cells with pLlys up to 10 μg mL^{-1}, SWCNT-P up to 80 ng mL^{-1}, or SWCNT-P700 up to 90 ng mL^{-1}, revealed similar percentages of cell viability to control experiments (Figure 2). However, the incubation with 100 μg mL^{-1} pLlys resulted in a significant reduction of viable cells compared to all the other concentrations tested (Figures 2 and 3; $n \geq 4$; one way ANOVA $p < 0.01$ for pLlys and $p < 0.001$ for SWCNT-P and SWCNT-P700). In the biparametric graphs (Figure 3), Caco-2/TC7 cells were selected and distributed in two populations according to their propidium iodide fluorescence intensity. The cells with low fluorescence intensity are live cells (control population), while high fluorescence levels show dead cells. The down images in Figure 3 show the morphology and propidium iodide staining of dead cells captured by the microscope in brightfield, propidium iodide, or combined brightfield/propidium iodide channels.

The results indicate that pLlys at a concentration of 100 μg mL^{-1} has a cytotoxic effect on Caco-2/TC7 cells. Only pLlys concentrations below 10 μg mL^{-1} have no significant effect on the viability of these cells, in good agreement with previous observations for other cancer cells types [3]. There were no significant differences between pLlys, SWCNT-P or SWCNT-P700 treatments at any concentration in the assayed ranges (Figure 2). Incubation with SWCNT-P up to 80 ng mL^{-1} or SWCNT-P700 up to 90 ng mL^{-1} does not modify the viability of Caco-2/TC7 cells, indicating that both types of SWCNTs do not have cytotoxic effects at the tested concentrations. Moreover, the cytotoxic effects observed at SWCNT concentrations higher than 100 ng mL^{-1} are caused by pLlys, and do not indicate SWCNT toxicity. Previous works have already suggested the necessity of evaluating the effects of the dispersion medium when SWCNT toxicity is measured [25-27].

It has to be remarked that the presence of oxygen functional groups in the SWCNT-P sample does not produce any effect at the tested concentrations, since no differences are observed from the oxygen-free SWCNT-P700 sample. Highly purified SWCNTs, at least up to concentrations of nearly 1 μg mL^{-1}, act as inert particles in the cell culture medium. While pLlys maintains its activity against colon cancer cells, SWCNTs could be utilized as carriers for other active principles or excipients in a hypothetical pharmacological complex.

4. Conclusions

Stable dispersions of highly purified arc-discharge SWCNTs in a high molecular weight pLlys aqueous solution are prepared by subsequent stages of air oxidation, acid treatment, and ultracentrifugation in the pLlys solution. Neat pLlys strongly decreases the viability of intestinal Caco-2 cells at concentrations of higher than 10 μg mL^{-1}, as

observed by image flow cytometry. However, highly purified SWCNTs, even containing a certain amount of oxygen functional groups, do not cause any effect on Caco-2 cells at the tested concentrations. Previously reported interferences of SWCNT samples on cell cultures are avoided after a careful purification process. This result supports the utilization of SWCNTs as a chemically inert drug vehicle for the treatment of intestinal tract diseases.

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Figure captions

Figure 1. Characterization of SWCNT dispersions: visible-NIR spectra of pristine SWCNT (a), SWCNT-P (b), and SWCNT-P700 (c), and a cryo-TEM image taken from the SWCNT-P.

Figure 2. Effects of poly-L-lysine (pLlys), SWCNT-P + pLlys, and SWCNT-P700 + pLlys on Caco-2/TC7 cell viability. Values are means ± SEM of ≥ 4 independent experiments.

Figure 3. Representative biparametric graphs (up) and images (down) of Caco-2 cells obtained by image flow cytometry after propidium iodide staining. Caco-2/TC7 cells were incubated with 10 or 100 μg mL^{-1} pLlys (A and B, respectively), 80 or 800 ng mL^{-1} SWCNT-P + 10 or 100 μg mL^{-1} pLlys (C and D, respectively), and 90 or 900 ng mL^{-1} SWCNT-P700 + 10 or 100 μg mL^{-1} pLlys (E and F, respectively).

Table titles

Table 1. Characterization of the modified SWCNT powder materials: Oxygen content, metal content, and extinction coefficient (ε_λ).

Table 2. Properties of the purified SWCNT dispersions in pLlys: Absorbance (A_λ), concentration (C), zeta potential (ζ), electrophoretic mobility (μ), conductivity (σ), pH, purity index, and metal content in the filtered solid.

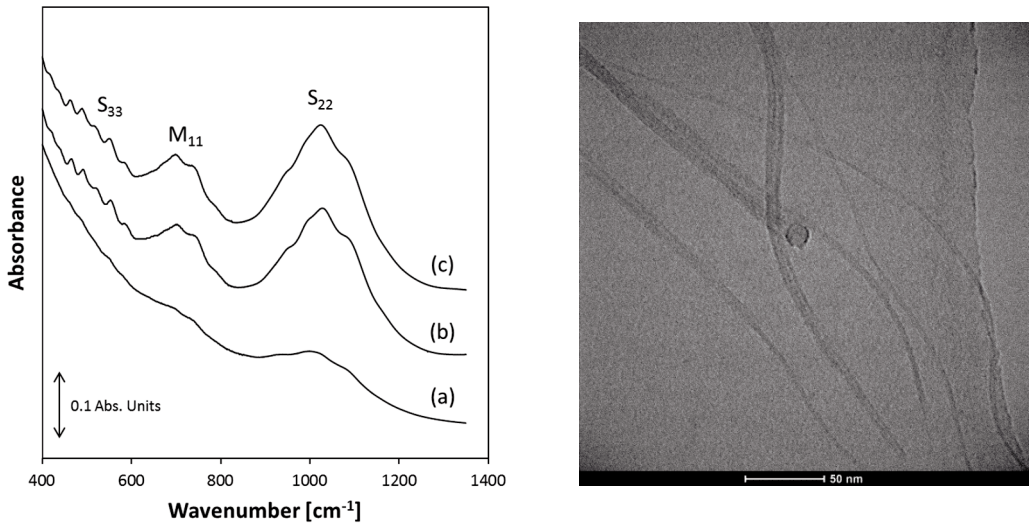
S33
M11
S22
(c)
(b)
(a)
0.1 Abs. Units
Absorbance
400
600
800
1000
1200
1400
Wavenumber [cm-1]
50 nm

Figure 1

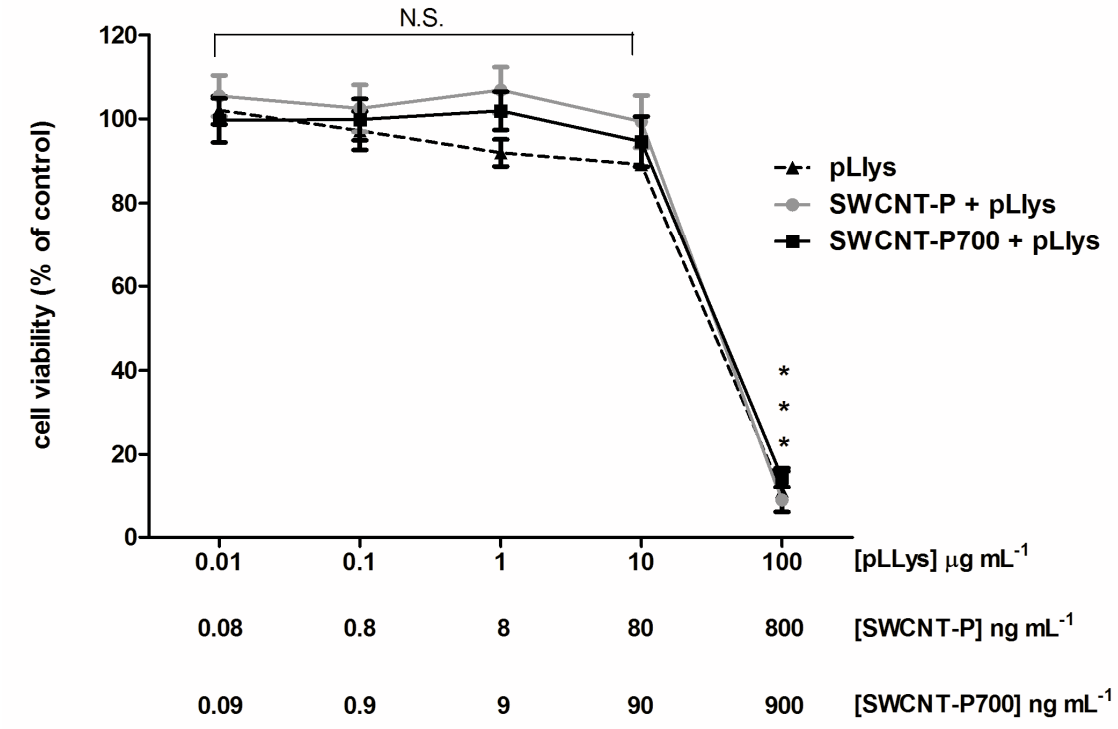
N.S.
cell viability (% of control)
120
100
80
60
40
20
0
pLlys
SWCNT-P + pLlys
SWCNT-P700 + pLlys
*
*
*
0.01
0.1
1
10
100
[pLLys] μg mL-1
0.08
0.8
8
80
800
[SWCNT-P] ng mL-1
0.09
0.9
9
90
900
[SWCNT-P700] ng mL-1

Figure 2

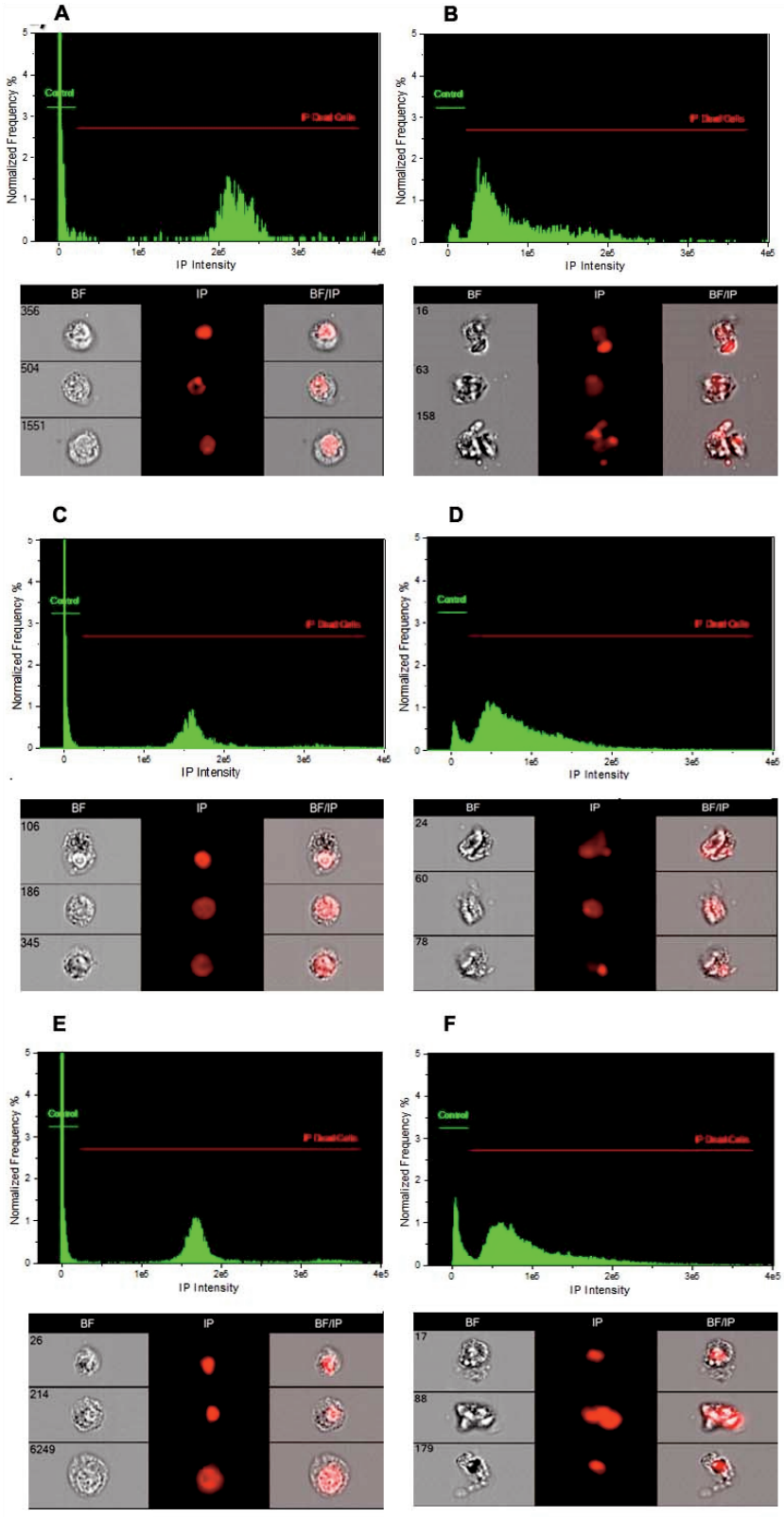
A
B
C
D
E
F
Normalized Frequency %
IP Intensity
Control
BF
IP
BF/IP

Figure 3

Table 1

	SWCNT-HCl	SWCNT-700
O [wt%]	7.63	0.80
TGA metal residue [wt%]	11.3	12.7
Metal content [wt%]	8.5	9.5
ε_{600} [mL mg^{-1} cm^{-1}][a]	23.9±1.4	20.4±1.1
ε_{850} [mL mg^{-1} cm^{-1}][a]	18.9±0.9	16.4±0.9

[a]The extinction coefficient was calculated for 0.1 mg mL^{-1} of SWCNTs dispersions in a 1 wt/vol% DOC aqueous solution

Table 2

	SWCNT-P	SWCNT-P700
A_{600}	0.191	0.184
A_{850}	0.144	0.144
C [μg mL^{-1}]	8	9
ζ [mV]	45.8±0.3	43.5±0.7
μ [μm cm V^{-1} s^{-1}]	3.59±0.02	3.41±0.06
σ [mS cm^{-1}]	0.451±0.004	0.439±0.004
pH[a]	7.3	7.3
Purity Index	0.25	0.28
Metal content [wt%]	≤ 0.05	≤ 0.05

[a]The pH of a 0.1 wt/vol% poly-L-lysine solution is of 6.9

Supplementary Material

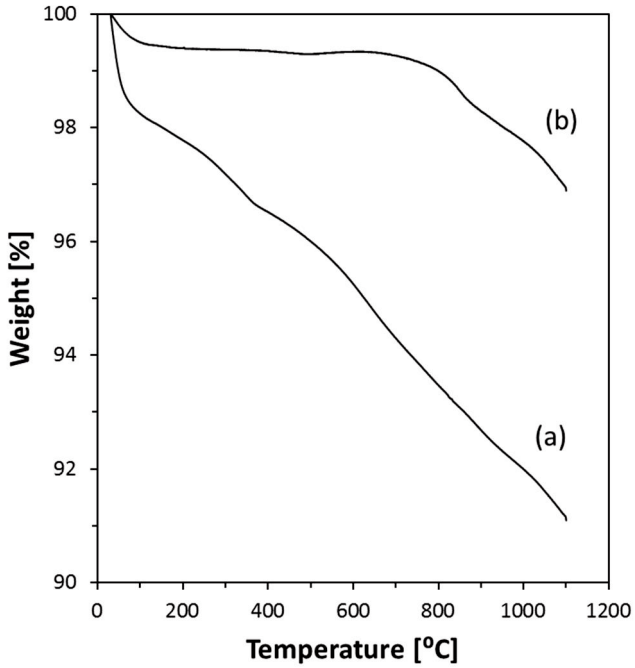

Figure S1. TGA profiles (N_2, 10 ºC min^{-1}) of the modified SWCNT powder materials: SWCNT-HCl (a) and SWCNT-700 (b).

Weight losses up to approximately 100 ºC are associated to adsorbed water moisture. Moisture content in the SWCNT-700 is lower than in the SWCNT-HCl material, since the treatment at 700 ºC eliminates most of the oxygen functional groups, which are centers for water adsorption. Therefore, the thermal treatment increases surface hydrophobicity.

TGA weight losses at temperatures higher than 100 ºC are mostly related to the evolution of oxygen functional groups as CO and CO_2. Weight losses for the SWCNT-HCl material occur steadily during the whole heating ramp. This TGA profile is indicative of chemically heterogeneous surfaces containing different types of oxygen functional groups (carboxylic acids, anhydrides, hydroxyl,…) with different thermal stabilities. The SWCNT-700 powder material does not experience weight losses until

approximately 700 ºC, since the oxygen functional groups with lower thermal stabilities were removed by the previous treatment at 700 ºC. However, certain weight losses still occur at temperatures higher than 700 ºC, in good agreement with a total oxygen content lower than 1 wt%. In any case, functional groups with high thermal stabilities also have low chemical reactivities.