Synthesis, characterization and cytotoxicity of phosphoryl choline-grafted water-soluble carbon nanotubes

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\textbf{ABSTRACT}

Carbon nanotubes (CNTs) were rendered water-soluble by grafting on phosphoryl choline (PC). The modified CNTs were characterized utilizing Fourier transform infrared spectra, X-ray photoelectron spectra, thermogravimetric analysis, nuclear magnetic resonance, transmission electron microscopy, ultraviolet/visible absorbance spectra and dynamic laser light-scattering. The results show that the target products are easily dispersed in water and remain dispersed for at least three months. This study showed that both CNTs and CNT-PC induce no cytotoxicity on clonal pheochromocytoma cells (PC12) and human colon carcinoma cell lines (Caco-2). The grafted PC group confers water solubility and keeps the cell-compatibility of CNTs.

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1. Introduction

Carbon nanotubes (CNTs) have potentially important roles in the emerging field of biological nanotechnology. However, the applications of CNTs have been severely limited due to poor solubility in water. One way in which to dissolve, or at least disperse, CNTs in water is to add water-soluble side moieties, such as carboxyl group \[1\], hydroxyl group \[2\], amino group \[3\], polyethylene glycol \[4-7\], poly(allylamine) \[8\], poly(vinyl alcohol) \[9-12\], and many others \[13-16\]. With surface-initiated atom transfer radical polymerization, phosphocholine polymeric structures were also been coated onto the single-wall CNTs and obtained the aqueous dispersion of the functionalized CNTs \[17\]. Different from the existing chemical structures used in hydrophilic modifications of CNTs, we report here the synthesis of water-soluble, multi-walled CNTs with the biocompatible phosphoryl choline (PC) side-group, a moiety of the lecithin that is a major component of all biological membranes, along with glycolipids, cholesterol and proteins. The zwitterionic character of the PC group gives it a strong hydrophilicity, and it is the polar moiety in the amphiphilic lecithin molecule. By grafting this group onto the CNTs surface, it is possible to confer hydrophilic properties to CNTs that make it water-soluble.

During the last decade, there have been numerous studies of the potential applications of CNTs, especially in the biomedical field as biosensors, drug and vaccine delivery vehicles, tissue engineering constructions and many other novel biomaterials \[18\]. However, before such materials can be incorporated into new and existing biomedical devices, the toxicity and biocompatibility need to be thoroughly investigated \[19\], especially the former, which is of fundamental
importance for any bio-application. The biological and environmental safety should be considered carefully whatever the application. The earliest study dates from 2001 [20] and research since then has been focused mainly on lung toxicity [20–24], skin irritation [25] and cytotoxicity [20,26–30]. In vitro cytotoxicity evaluation is a convenient method for toxicity studies on CNTs, and the cell lines that have been used include immortalized human epidermal keratinocytes (HaCaT) [26], human epidermal keratinocyte (HEK) cells [27], alveolar macrophages [20,28], human embryonic kidney (HEK293) cells [29] and neutrophils [30]. These studies regarding parameters of CNTs such as dose, particle size, refined or unrefined, single-wall or multi-wall, etc., and the results are often inconclusive and/or contradictory. Furthermore, although various chemically modified CNTs have been used in many fields, the degree of toxicity has not been studied in depth and there is a need for further scientific evaluations and studies [19]. In this work, water-soluble PC-grafted CNTs (CNT-PC) were synthesized, which could avoid the problems caused by aggregation of CNTs. The cytotoxicity of CNT-PC was evaluated against clonal pheochromocytoma cells (PC12) and human colon carcinoma cell lines (Caco-2).

2. Experimental

2.1. Materials

CNTs with a diameter of 10–30 nm and a length of 5–15 μm were purchased from Shenzhen Nano-Technologies Port Co., Ltd. (Shenzhen, China) which were prepared via chemical vapor deposition (CVD) with La–Ni catalyst and the purity is no less than 95% with 3–5% amorphous carbon. 2-Chloro-1,3,2-dioxaphospholane 2-oxide (COP) was synthesized as described by Lucas et al. [31] and Yasuda et al. [32]. All other solvents and reagents used in the synthesis of water-soluble CNT were purchased from Sinopharm Chemical Reagent Co., (Shanghai, China) and used as received, except N,N-dimethylformamide (DMF), which was dried with 4A molecular sieve for at least 24 h and vacuum-distilled before use.

PC12 and Caco-2 cell lines were obtained from the Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences (Shanghai, China). Dulbecco’s modified Eagle’s medium (DMEM, with 4500 mg/l-glucose and l-glutamine) was purchased from Sigma and prepared by dissolving in ultrapure water with 3.7 g of sodium bicarbonate, 3.6 g of 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (Hepes), 100 mg of penicillin and 100 mg of streptomycin. The culture medium was filtered through a microporous membrane (pore size 0.22 μm) and stored at 4 °C. Neonatal bovine serum (NBS) was purchased from Hangzhou Sijiqing Biological Engineering Materials Co., Ltd. (Hangzhou, China), heat-inactivated for 30 min at 56 °C and then stored at −20 °C. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Fluka) and the tetrazolium salt 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium (WST-1, Sigma) was, respectively, dissolved in phosphate-buffered saline (PBS, pH 7.4) at a concentration of 5 mg/ml, filtered through a microporous membrane (pore size 0.22 μm) and then stored at 4 °C in darkness. Trypsin was purchased from Sino-American Biotechnology Co., (Luoyang, China), dissolved in D-Hanks solution at a concentration of 0.25% mass percent. The other chemicals used in the cytotoxicity studies, such as dimethyl sulphoxide (DMSO), NaCl, NaHCO₃, etc., were all analytical reagent grade.

2.2. Instrumentation

Fourier transform infrared spectra (FTIR) were recorded on a Nicolet Nexus 870 FTIR spectrometer (Thermo Nicolet, USA) from 4000 to 400 cm⁻¹ with a resolution of 4 cm⁻¹ at room temperature on a KBr pellet with ~1% sample concentration. The X-ray photoelectron spectra (XPS) of CNT-PC were recorded on a VG Scientific ESCALab MK-II spectrometer (West Sussex, England) equipped with a monochromatic Mg-Kα X-ray source. The ultraviolet/visible absorbance spectra (UV/Vis) were recorded on a Shimadzu UV3100 spectrometer (Shimadzu, Japan) with a scanning rate of 480 nm/min and 2 nm/scan step in a 1 cm quartz cell with reference to distilled water. Thermogravimetric analysis (TGA) was done with a PE Pyris 1 thermogravimeter (PerkinElmer Life and Analytical Sciences, Inc., MA, USA) in a N₂ atmosphere at a heating rate of 20 °C/min from room temperature to 500 °C. Nuclear magnetic resonance (NMR) spectra were obtained with a Bruker ARX500 NMR spectrometer (Bruker, Germany) with ²H₂O as the solvent. Transmission electron microscopy (TEM) images were captured using a JEOL 100MS electron microscopes (JEOL Ltd. Tokyo, Japan) operated at 80 kV. Multimodal size distributions of CNT-PC were determined with dynamic laser light-scattering (DLS) on a Brookhaven ZetaPlus Zeta potential/ particle size analyzer (Brookhaven Instruments Corp., NY, USA) at 660 nm wavelength. The cells used in cytotoxicity evaluations were cultivated in a CO₂ incubator (Heraeus model BB16; Heraeus Instruments, Hanau, Germany) and the evaluations were carried out with a PE Envision microplate reader (PerkinElmer Life and Analytical Sciences, Inc., MA, USA) at a wavelength of 570 nm.

2.3. Preparation of samples

The water-soluble CNTs were synthesized as illustrated by Fig. 1. A typical process includes carboxylation, acylation, hydroxylation, linking the COP group and opening the ring of the COP moiety.

2.3.1. Carboxylation of CNTs

In a typical procedure, 2 g of commercial CNTs were ultrasonicated in 100 mL of a 1:3 (by volume) mixture of concentrated nitric acid and sulfuric acid to purify and oxidize for 24 h at room temperature. After centrifugation, the clear brown supernatant was decanted. The residual solid was dispersed repeatedly in water and centrifuged until the wash water was neutral and the residual solid was then vacuum-dried at 60 °C to yield 1.9 g of CNT-COOH.

2.3.2. Chloroacylation and amidation of CNTs

In a 100 mL round-bottom flask, a 1.8 g sample of CNT-COOH was dispersed in 30 mL of SOCl₂ by ultrasonication for 3 h and then refluxed for 24 h. The unreacted SOCl₂ was removed with a rotary evaporator to yield CNT-COCl. The flask was...
immediately immersed in cold water and 30 mL of ethanolamine (EA) was added dropwise, dispersed by ultrasonication and then stirred for 24 h at ambient temperature. The black reactants were poured into acetone to precipitate the products, which were collected by centrifugation. The solid was washed in anhydrous ethanol at least 3 times to remove the excess EA, then vacuum-dried to yield 1.8 g of CNT-EA.

2.3.3. Preparation of CNT-PC
A 1.4 g sample of CNT-EA was placed into a 100 mL round-bottom flask then 50 mL of anhydrous DMF and 3 mL of triethylamine were added. The flask was immersed in an ice-salt bath at –10 °C and the CNTs dispersed by ultrasonication for 30 min. While stirring, 10 mL of DMF containing 5 g of COP (IR 2990, 2920, 1860, 1475, 1315, 1225, 1033, 928, 870, 830, 609, 523 cm⁻¹; ¹H NMR (CDCl₃) 4.55, 4.62 ppm; ¹³C NMR (CDCl₃) 66.7 ppm; calculated for C₂H₄ClO₃P, C 16.86%, H 2.83%, elemental analysis found C 16.83%, H 2.85%) was added dropwise. By controlling the drop rate, the temperature was kept below 5 °C and COP was added over about 30 min. Then the ice-salt bath was removed and the temperature of the reactor was allowed to reach room temperature and react for 3 h. Then the system was centrifuged and the separated solid was washed 4 times in anhydrous ethanol and then vacuum-dried. The dried intermediate product was dispersed by ultrasonication in 50 mL of 33% trimethylamine solution containing NBS, and bubbled to form a single-cell suspension, then diluted and counted. The viable cells were moved and trypsin/D-Hanks solution was added. After 2–3 min the digestion was terminated by adding DMEM culture medium containing 15% NBS in a culture flask and incubated for another 4 h at 37 °C. Supernatants were removed, 150 μL of DMSO was added and the plates were shaken for 10 min to completely dissolve the dark blue formazan crystals. The absorbance of the solution at 570 nm was recorded with a PE microplate reader. Every experiment was performed at least in triplicate.

As a comparison, WST-1 assay was also used to detect a loss in viability of cells. The cells were incubated for 48 h with CNTs and CNT-PC in an identical experimental system as was used for the MTT assay. Photometric quantification was performed at 450 nm in a microplate reader. Calculation was identical to that of the MTT test.

3. Results and discussion

3.1. XPS spectroscopy

The XPS of CNT-PC was recorded to confirm the linkage between CNTs and the PC group (Fig. 2). The scan (Fig 2B) shows the presence of carbon, oxygen, nitrogen and phosphorus elements. The latter two could not be observed in raw CNTs (Fig 2A), it was their presence that indicated the successful introduction of PC groups provided by EA and COP. For CNT-PC, the O1s XPS spectrum (Fig. 2C) with a binding energy of 532.5 eV is the contribution of oxygen atoms, but is indistinguishable for different linkages: for oxygen, the peak width is obviously broader than that of carbon, while the shift of binding energy is limited to a narrow range. The binding energy at 400 and 403.0 eV on the N1s XPS spectrum (Fig. 2D) cor-

Fig. 1 – Synthesis of water-soluble CNT-PC.
respond to the nitrogen in amide bonds and quaternary amine groups, respectively. The C1s XPS spectrum (Fig. 2E) shows two different binding energies for carbon atoms: one is from CNTs to the saturated methylene carbon at 285 eV, and the other one, the hump at 284–290 eV, arises from the overlap of three different carbon atoms, O=C–NH at 288.4, C–O at 286.8, and C–N+ at 286.3 eV. A distinct peak for phosphorus 2p at 134 eV (Fig. 2F) confirms the presence of the phosphorus-containing bond in the modified CNTs.

3.2. FTIR spectroscopy

Fig. 3 shows the FTIR spectra of raw CNTs and the modified CNTs according to the steps shown in Fig. 1. For the raw CNTs, the curve is almost a straight line. For CNT-COOH, carboxylation in nitric acid and sulfuric acid provides a typical absorption peak at 1700 cm⁻¹ associated with the carboxylic group and also provides the absorbance at around 1100 cm⁻¹ corre-

Fig. 2 – XPS spectra of CNTs and CNT-PC. A, a survey scan of CNTs; B, a survey scan of CNT-PC. For the sample CNT-PC; C, the O1s region, 26.89%; D, the N1s region, 3.94%; E, the C1s region, 68.12%; F, the P2p region, 1.05% (atomic percentage).

Fig. 3 – FTIR spectra of CNT and modified CNTs.
sponding to the other side-groups. On the CNT-EA curve, the introduction of EA is reflected in a huge absorption peak at 3420 cm\(^{-1}\) related to the hydroxyl group (OH) and imine group (NH), while the double bonds at 1640 and 1560 cm\(^{-1}\) associated with the transformation of the carboxylic group to an amide group. For the CNT-PC spectrum, the absorbance at 940 cm\(^{-1}\) could be assigned to a tertiary amine group while the peak at about 1200 cm\(^{-1}\) was due to the synergetic effect of the phosphate and tertiary amine groups, the peak at 1540 cm\(^{-1}\) was contributed by P-O-alkyl and amide groups. Although we could not assign every peak with confidence for the complexity of CNTs, the transformation of the FTIR spectra indicated the modification procedure according to Fig. 1 and confirmed the successful synthesis of CNT-PC.

3.3. \(^1\)H NMR and \(^{31}\)P NMR spectra of CNT-PC

Fig. 4A shows the \(^1\)H NMR spectra of CNT-PC with tetramethylsilane (TMS) as the internal standard and the assignments are indicated in the figure. The incisive peak at 4.70 ppm is the resonance of H\(_2\)O, the peak at 3.18 ppm is the resonance of the methyl hydrogen linked to the quaternary amine nitrogen atom that comes from the trimethylamine in the ring-opening reaction of the COP moiety, while the peak at 3.39 ppm corresponds to the methylene hydrogen atoms that were introduced from EA and COP. The presence of the methyl and methylene groups confirmed the structure of the target products.

For the PC group, the phosphorus is a characteristic element whose presence can be confirmed by \(^{31}\)P NMR spectra as shown in Fig. 4B. According to the \(^{31}\)P NMR spectra, there are two kinds of phosphorus-containing structures: one is the cyclo-COP moiety, which shows the chemical shift at about 13.1 ppm with reference to triphenylphosphine (TPP), and the other one is the phosphoryl group coming from the ring-opening transformation of COP, which shows the chemical shift at about 0.23 ppm. According to Fig. 1, the cyclo-structure of COP should be opened in the fifth step. However, although the reaction time is prolonged and the temperature is increased compared to the normal conditions of the ring-opening reaction of COP, a part of the COP original moiety remains, which is probably because of steric hindrance by CNTs. The support of this inference can also be found on \(^1\)H NMR spectrum, if the ring-open transformation of COP is 100%, the intensity of "b" peak (8C) should be less than "a" peak (9C), but a higher "b" peak can be seen (Fig. 4A), which may also imply the incomplete transformation of COP.

![Fig. 4 – NMR spectra of CNT-PC in \(^2\)H\(_2\)O. A, \(^1\)H NMR ppm \(\delta\) from TMS; B, \(^{31}\)P NMR ppm \(\delta\) with reference to TPP.](image-url)
We also tried to acquire the $^{13}$C NMR spectra of CNT-PC. However, because the short grafted groups were close to the CNTs surface, their resonances were shielded by the paramagnetism of CNTs and are not seen in the $^{13}$C NMR spectra [33–35].

3.4. TGA of CNTs and modified CNTs

The TGA curves of raw CNTs and modified CNTs were recorded and are shown in Fig. 5. In the total heating process, raw CNTs lost about 1.3% by weight. From the curves of modified CNTs, the hydrophilicity of samples increases with the grafting process. The weight losses of CNT-COOH, CNT-EA and CNT-PC before 150 °C were about 1.4%, 1.8%, and 4.6%, which was contributed mainly by the saturated water absorption. At higher temperatures, the weight loss can be attributed to the thermal decomposition of side-groups. Up to 500 °C, the value was 10.9%, 13.6%, and 16.9%. It may be concluded that weight loss increases with increased size of the side-group grafted onto CNTs.

3.5. Water dispersibility of CNT-PC

The insoluble raw CNTs become “soluble” and are easy to disperse homogenously in water after modification with PC. As shown in Fig. 6, CNTs aggregated soon after ultrasonication and precipitated completely when centrifuged (all including CNT-PC are centrifuged directly in glass vials) at 2500g for 5 min. However, dispersions of CNT-PC at concentrations of 0.1, 1, and 10 mg/mL were stable after centrifugation and when left for at least 10 days. In fact, the samples are still stable after about three months, and there is just a little precipitate in the 10 mg/mL sample.

The stable dispersion of CNT-PC can be attributed to the hydrophilic properties of the PC group. The strong solvation of water on the PC moiety makes the side-group extend into the solution, which stabilizes CNTs in water.

Actually, there are many ways to help CNTs dispersing in water and the modification of CNTs with grafting water-soluble substance is one of the most effective methods. We choose PC moiety as the modifier to accomplish the mission is base on the character of its well known biocompatible and zwitterionic properties. Zhao et al. [7] reported the synthesis of water-soluble SWCNT with zwitterionic poly(m-aminobenzene sulfonic acid) graft copolymer and found the water solubility is about 5 mg/mL [6]. As similar but smaller zwitterionic group, PC moiety provides CNTs higher water solubility as high as more than 10 mg/mL.

3.6. TEM images of CNTs and modified CNTs

CNTs and the modified CNTs were observed by TEM and the negative images were shown in Fig. 7. Although the samples were dispersed by ultrasonication before they were dropped onto the copper grids, the CNTs and CNT-COOH samples showed the presence of many aggregates (Fig. 7a and b). There was far less aggregation in the CNT-EA sample but there was some (Fig. 7c). However, most of the CNT-PC sample (Fig. 7d), was dispersed homogenously and it was very difficult to find any flocculation. Moreover, following modification, CNTs were noticeably shortened: in fact the length was reduced from micrometers to nanometers. Besides the effect of side PC groups, the shortening also enhanced the water dispersibility of modified CNTs, especially CNT-PC, and the average length was just of the order of hundreds of nanometers. From TEM, the average diameter of CNT-PC was also widened compared to the raw CNTs sample, which was attributed to the encapsulation of the nanotube core by PC groups with a water of hydration shell.

![Fig. 5 – TGA curves of CNTs and modified CNTs recorded in a N2 atmosphere at a heating rate of 20 °C/min.](image)

![Fig. 6 – Raw CNTs and CNT-PC dispersed in water. A, 1 mg/mL raw CNTs dispersed in water; B, C, and D, CNT-PC dispersed in water at concentrations of 0.1, 1 and 10 mg/mL, respectively. a, Immediately after ultrasonication with an ultrasonic cell disrupter; b, directly centrifuged in the glass vials at 2500g for 5 min after ultrasonication; and c, left for 10 days after ultrasonication.](image)
3.7. **DLLS determination**

DLLS was used to investigate the water dispersibility of CNT-PC and the results are shown in Fig. 8. For CNT-PC, the determination gives the average effective diameter of 200.1(±1.0) nm with polydispersity of 0.154 ± 0.022. Although the calculation of the diameter by DLLS was based on the assumption of global particles and the method is not appropriate for high aspect ratio structure of tube, the stable results in three runs at least promoted the homogenous disperse of CNT-PC in water. Under the same conditions, the CNTs/water dispersion system gives a mean of 22,416 nm with a standard error of 20,475 nm, values that are seriously outside the 3000 nm limits of the instruments used. Obviously, these high values and the lack of reproducibility between runs were due to the rapid aggregation of CNTs in water. The contrast between the DLLS results for CNTs and CNT-PC confirm the good dispersibility of CNT-PC in water.

3.8. **UV/Vis absorbance of CNT-PC dispersion**

The homogenous dispersion in water facilitates determination of the properties of modified CNTs in solution. Fig. 9 shows the relationship between the absorbance and the concentration of CNT-PC dispersed in water, and the inset shows the absorbance spectra at concentrations of 1.5–96 µg/mL. The maximum absorbance at about 260 nm is due to the aromatic structure of CNTs and the enhancement of absorption makes the solution progressively darker with increased concentration, as shown in Fig. 6.
The absorbance \( (A) \) of CNT-PC dispersed in water increased in direct proportion to the increased concentration \((c)\), conforming to Lambert–Beer’s law. For concentrations of CNT-PC of 1.5–96 \( \mu g/mL \), this can be written as:

\[
A = 0.02822(\text{mL} \cdot \mu g^{-1} \cdot \text{cm}^{-1}) \times 1(\text{cm}) \times c(\mu g \cdot \text{mL}^{-1})
\]

The agreement with Lambert–Beer’s law confirmed the homogenous dispersion of CNT-PC in water.

### 3.9. Cytotoxicity of CNTs and CNT-PC: MTT assay

The cytotoxicity induced by CNTs and CNT-PC was determined by the MTT assay, which is based on mitochondrial dehydrogenase activity. For a living cell, the succinate dehydrogenase in mitochondria may reduce exogenous MTT and form insoluble blue formazan crystals, while a dead cell, by definition, does not have such activity. The quantity of forma-
zanz crystals formed is directly proportional to the quantity of living cells, which can be determined by dissolving the formazan with an organic solvent, e.g., DMSO, and measuring the absorbance.

We chose PC12 and Caco-2 as model cell lines to evaluate the cytotoxicity of CNTs and CNT-PC. PC12, from the adrenal medulla of rat, is a kind of typical neuroendocrine cell widely used in studies of nerve cell differentiation, ion channels, and receptor and neurotransmitter release. PC12 is also one of the most important cell lines used in neurotoxicity studies. Caco-2 has the typical characteristics of intestinal epithelial cells, whose marker enzyme’s functional expression and permeability is similar to that of the small intestine. This cell line is widely used as an in vitro model in studies of transport and metabolism of small intestine epithelial cells, drug screening and absorption mechanisms. Here, the cytotoxicity of CNT-PC and CNTs against these model cell lines were studied, which allows us to predict the likely effects of the CNTs on the nervous system and the digestive system. In Fig. 10, the amounts of living cells after exposure to CNTs and CNT-PC were normalized according to the control and are represented as the relative amounts vs. the control as a percentage.

3.10. Cytotoxicity of CNTs and CNT-PC: WST-1 assay

From the results in MTT assay of CNTs and CNT-PC incubated with PC12 and Caco-2 cells, it looks like that CNTs shows cytotoxicity on Caco-2 at extremely high concentration. However, it is well known that single-walled CNTs do interact with MTT formazan crystals [36] which may induce the distortion. Then, whether the interaction is also exit for multi-walled CNTs used in our system? As a comparison, we investigated the WST-1 assay at the same conditions. The Formazan from WST-1 is water-soluble; therefore, no organic solvents extraction is necessary. Photometric quantification was performed at 450 nm and the results were calculated as the percentage vs. the control. From Fig. 11, it is obviously that both CNTs and CNT-PC do not show obvious reduction in viability on any dosages and both cell lines. From WST-1 assay, it is correct to say that both CNTs and CNT-PC show non-cytotoxicity against Caco-2 and PC 12 cell lines.

Combining the results of MTT and WST-1 assay, at least for Caco-2 and PC12 cell lines, our results suggest that there is no cytotoxicity for CNTs. And the modification product CNT-PC also shows good cell-compatibility. The PC group on CNTs is a moiety of phospholipids that exists widely in living organisms. The biocompatibility of PC is well established and this was one of our motivations for grafting it onto CNTs, expecting it to confer both water solubility and biocompatibility. The results presented here confirm our expectations.

4. Conclusion

CNT-PC with excellent water dispersibility was prepared and characterized for potential biomedical applications. By MTT and WST-1 assays, the cytotoxicity was evaluated using PC12 and Caco-2 cell lines as an in vitro model. In MTT assay, the suspicious cytotoxicity of CNTs at high dosage (1000 µg/mL) was observed. Considering the interaction between hydrophobic CNTs and MTT formazan and combining the results in both assays, our results support the opinion that CNTs are non-cytotoxic. For CNT-PC, the side-group not only offered the water solubility but also provide the good cell-compatibility.

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