Aryl-derivatized, water-soluble functionalized carbon nanotubes for biomedical applications

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ABSTRACT

The functionalization of very-thin multi-walled carbon nanotubes (VT-MWNTs) with an aniline derivative, via the protocol of in situ generated aryl diazonium salts results, upon acidic deprotection of the terminal BOC group, on the formation of the water-soluble positively charged ammonium functionalized VT-MWNTs-NH3+ material. The new materials have been structurally and morphologically characterized by infra-red (ATR-IR) spectroscopy and transmission electron microscopy (TEM). The quantitative calculation of the grafted aryl units onto the skeleton of VT-MWNTs has been estimated by thermogravimetric analysis (TGA), while the quantitative Kaiser test showed the amine group loaded onto VT-MWNTs-NH3+ material. The aqueous solubility of this material has allowed the performance of some initial toxicological in vitro investigations.

1. Introduction

Since their discovery, carbon nanotubes (CNTs) have attracted considerable attention due to their unique chemical and physical properties as well as their promise in the area of materials chemistry [1,2]. Additionally, CNTs have been explored for possible applications in nanobiotechnology [3], as biosensors [4] and as drug and gene delivery systems [5–8]. However, CNTs must first be functionalized [9–11] and thus adequately dispersed in physiological media in order to be able to interact with cells and lead to cellular internalization in the absence of toxicity. In fact, it is known that CNTs accumulate in the cytoplasm and can even reach the nucleus, without being cytotoxic (in concentrations up to 10 mM) [12]. Thus, CNTs can potentially act as carriers that transport and deliver other bioactive components into cells [13].

An effective way of functionalization of CNTs introduced by Tour and coworkers [14–24] consists of the thermal reaction of in situ generated aryl diazonium compounds with CNTs, thus effecting the introduction of a plethora of aryl groups onto the sidewalls of the CNTs. The great advantages of this method are the high degree of functionalization, the solubilization enhancement and the variety of the substituted aryl compounds that can be used. Considering all the above, our aim is to functionalize very-thin multi-walled carbon nanotubes (abbreviated as VT-MWNTs) with the aniline derivative (3) according to Fig. 1. By this procedure, we can obtain hybrid material (2) en-route toward water-soluble functionalized VT-MWNTs to be biologically evaluated.

2. Experimental

2.1. General methods

All solvents and reagents were purchased from commercially available sources and used without further purification unless otherwise stated. Purified VT-MWNTs (diameter: 5–15 nm, purity >95%) were purchased by Nanocyl and used as received. Transmission electron microscopy images were collected with a Philips TEM 208 instrument at an accelerating voltage of 100 kV. Mid-infrared spectra in the region 550–4000 cm−1 were obtained on a Fourier Transform infra-red (FT-IR) spectrometer (Equinox 55 from Bruker Optics) equipped with a single reflection diamond attenuated-total-reflectance (ATR) accessory (DuraSamp1IR II by SensIR Technologies). The thermogravimetric analysis was performed using a TGA Q500 V20.2 Build 27 instrument by TA in a nitrogen inert atmosphere.

2.2. Aryl functionalized VT-MWNTs-NHBOC (1)

In a typical experiment, 10 mg of VT-MWNTs suspended in 20 mL of 1,2-dichlorobenzene (ODCB) and sonicated for 20 min. Then, a solution of the aniline derivative (3) (0.5 mmol) in 10 mL of acetonitrile is added. After degassing the reaction mixture and

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Functionalization scheme of VT-MWNTs with in situ generated aryl diazonium salts and formation of water-soluble cationic ammonium functionalized VT-MWNTs-NH$_3^+$ (2).

3. Results and discussion

The aniline derivative (3), possessing the polar oligoethylene chain, was synthesized in two steps from 4-nitro benzoyl chloride and the mono-tert-butoxycarbonyl (BOC) protected ethylene glycol diamine [25]. The functionalized VT-MWNT material (1) is soluble in several solvents like chloroform, methanol and DMF, while the ink-colored water soluble functionalized VT-MWNT material (2) is stable for several weeks, that is without observing any significant precipitation.

The structure confirmation of the modified VT-MWNTs-NHBOC (1) is conducted by ATR-IR spectroscopy, where the presence of the added functional groups onto the skeleton of VT-MWNTs is verified. Thus, the characteristic C–H stretching and bending modes are observed. Importantly, in the ammonium functionalized VT-MWNTs (2), where the BOC protecting group is cleaved, the strong carbonyl vibration at 1699 cm$^{-1}$ is disappearing.

Transmission electron microscopy (TEM) is a meaningful means to probe the morphological characteristics of the functionalized VT-MWNTs. A representative TEM image of the soluble functionalized VT-MWNTs-NHBOC (1) is shown in Fig. 2. Evidently, VT-MWNTs of high purity with mean diameter of approximately 7 nm are identified, while the functionalized material does not suffer any degradation during the reaction conditions applied.

Thermogravimetric analysis (TGA) measurements of the ammonium functionalized material VT-MWNTs-NH$_3^+$ (2) allowed the quantitative evaluation of the aryl moieties grafted onto the skeleton of VT-MWNTs (Fig. 3). In this frame, intact VT-MWNTs demonstrate excellent thermal stability up to 700 °C, while above that temperature start to slowly degrade. The VT-MWNTs-NH$_3^+$ material (2) shows a two-step weight loss of approximately 67% to occur in the temperature range of 250–700 °C. The first thermal decomposition at the range 250–350 °C is attributed to the lost of aryl side chain, namely the ethylene glycol ammonium unit, while further thermal loss up to 700 °C is attributed to the decomposition of the benzamido-moiety coated the surface of VT-MWNTs. Thus, the percentage of the organic matter attached onto the skeleton of VT-MWNTs is calculated as 15 aryl units per every 100 carbon atoms of the VT-MWNTs.

The concentration of the terminal amino functions present on VT-MWNTs-NH$_3^+$ material (2) is calculated with the aid of the quantitative Kaiser test. Thus, it is predicted the presence of 331 μmol of free amino groups per gram of VT-MWNTs-NH$_3^+$ (2).
Before any further biological investigation can take place, it is imperative to assess the limitations presented by the functionalized VT-MWNTs. The MTT assay is a well-established toxicological assay to assess cell viability based on the activity of mitochondrial enzymes [26–29]. Hence has been used by several groups to assess the toxicity of carbon nanotubes [30–33]. This assay is based on the conversion and cleavage of the tetrazolium salt MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) into the purple formazan product by mitochondrial reductase of only living and metabolically active cells, observed as the purple color of the formazan that is measured at 570 nm. As can be seen from Fig. 4, the cell viability of MCF-7 is around 70–85% after 24 h treatment with the VT-MWNTs-NH3⁺ (2) at all concentrations ranging from 6.25 to 200 μg/mL. Moreover, in control experiments, the high-cell viability expected with the improved solubility of VT-MWNTs-NH3⁺ (2) indicates the suitability of using carbon nanotubes as components for biological experimentation, and agrees with previous observations that increasing the degree of functionalization of carbon nanotubes reduces their toxicity [31] and improves their biocompatibility [34]. These initial studies also indicate that the more aryl functionalities grafted on the surface of the nanotubes, the more improved the in vitro toxicity profile of carbon nanotubes and thereby their in vivo pharmacological profiles will be.

4. Conclusions

In summary, the functionalization of VT-MWNTs is achieved via in situ generated aryl diazonium salts of aniline derivative (3). The cleavage of the BOC protecting group liberates positively charged ammonium units at the side chain of the grafted aryl moieties onto the skeleton of the VT-MWNTs. The charged ammonium units as well as the hydrophilic nature of the ethylene glycol chain in (2) induce aqueous solubility. Spectroscopic characterization as well as thermal gravimetric analysis and electron microscopy measurements probed the structural and morphological characteristics of the new functionalized VT-MWNTs materials. Initial toxicological investigations indicated that further studies of such materials are warranted also confirming the general rule being formulated that functionalization of nanotubes improves dramatically the toxicity profile of this class of nanomaterials.

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