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Highly biocompatible multi-walled carbon nanotube–chitosan nanoparticle hybrids as protein carriers

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ABSTRACT

Carbon nanotube (CNT)–organic polymer hybrids have important potential applications in the immobilization of therapeutic biomolecules. Recently developed CNT–organic polymer composites require the use of organic solvents for their preparation and have limited polymer functionalization. To address these limitations, multi-walled CNT (MWCNT)–chitosan nanoparticle (CS NP) hybrids have been synthesized in situ by an ionotropic gelation process, which is extremely mild and involves the mixture of two aqueous solutions at room temperature. The MWCNT–CS NP hybrids were characterized by atomic force microscopy and thermogravimetric analysis. Under optimal conditions the CS NP can be tethered to the MWCNT surface in high density and with relatively uniform coverage. The MWCNT–CS NP hybrids show good dispersibility and stability in aqueous solutions. In order to evaluate the potential utilization of the hybrids as protein carries the cytotoxicity to HeLa cells and protein immobilization (of bovine serum albumin (BSA), used here as a model) capacity of the hybrids were investigated in detail. The results demonstrate that the MWCNT–CS NP hybrids are biocompatible at concentrations up to 100 μg mL^{-1} for 24 h incubation. The MWCNT–CS NP hybrids improve the BSA immobilization efficiency 0.8 times and simultaneously decrease the cellular toxicity by about 50% compared with carboxylated MWCNT.

1. Introduction

Carbon nanotubes (CNT) possess unique structure-dependent physical, chemical, and mechanical properties [1]. Many aspects of the potential applications of CNT have been intensively explored, such as the synthesis of composites [2,3], catalysts [4], biosensors [5,6], and carrier materials for drugs [7] or biomolecules [8–14]. To extend and optimize the applications of CNT in these fields it is highly desirable to modify CNT with different functional groups and incorporate other nanomaterials to create new hybrid architectures [15,16]. Recently CNT have been functionalized with various inorganic nanoparticles, such as Au, Pt, Ag nanoparticles or quantum dots [17–21]. The results suggest that CNT-based hybrids have unique properties, leading to advanced catalytic systems, highly efficient fuel cells, tunable electronic or optoelectronic devices, and ultrasensitive chemical sensors/biosensors [18,20,22,23]. On the other hand, many works have reported functionalization of CNT with organic polymers through covalent, noncovalent or wrapping methods [24–30]. Most of these efforts were devoted to improving the reactivity, solution process ability, and biological performance of CNT. However, the studies on the CNT–organic nanoparticle hybrid architectures are poorly developed comparatively.

We here report a simple strategy to prepare highly biocompatible multi-walled CNT (MWCNT)–chitosan nanoparticle hybrids as novel protein carriers. Chitosan (CS), a natural biopolymer, has found wide applications in a variety of areas, including biomedicine, pharmaceuticals, metal chelation, sensors, and other industrial applications [30–34], due to its biocompatibility, biodegradability, low toxicity, good film-forming characteristics, and anti-infection activity. With the development of nanoscience and nanotechnology CS nanoparticle (CS NP) systems have received a great deal of attention because of their nano-size, large surface area, and good biocompatibility [35]. Moreover, unlike most other natural polymers, CS is a polymer with a positive charge in aqueous solution. These characteristics favor the employment of CS NP in a wide range of biomedical applications, including drug [36] or gene [37] delivery systems. Many recent attempts have been made to create CS NP systems through emulsion cross-linking [38], reverse micellar extraction [39], coacervation [40], solvent evaporation [41], spray drying [42], or thermal cross-linking [43]. These methods have intrinsic advantages, along

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with some limitations. Although CNT–CS particle composites have been prepared by electrostatic interaction between CS particles and mixed acid-treated CNT [44], several synthetic issues remain in the reported method. For example, the emulsion cross-linking process may introduce toxic organic materials into the CS particles. Moreover, the number of functional groups on the CNT surface is limited by the use of a chemical oxidation treatment, resulting in few CS particles tethered to the CNT surface. In this paper we report an approach for in situ synthesis of MWCNT–CS NP nano-hybrids by an ionotropic gelation process [45]. As shown in Fig. 1, a simple polyanion such as sodium tripolyphosphate (TPP) can interact with CS to generate CS NP and simultaneously couple to the MWCNT surface. This approach may offer several interesting features for the synthesis of CNT-based organic nanohybrids: (i) the synthetic process is carried out under exceptionally mild conditions without involving high temperature, sonication, or toxic organic cross-linking agents; (ii) in situ synthesis will simplify the process of preparation of MWCNT–CS NP hybrids; (iii) the size and amount of CS NP on the MWCNT surface may be tailored by changing the preparation conditions. The morphology and the number of CS NP tethered on MWCNT were characterized by atomic force microscopy (AFM) and thermogravimetric analysis (TGA). In order to evaluate the potential utilization of MWCNT–CS NP hybrids as protein carries the dispersive property in aqueous solution, cytotoxicity to HeLa cells, and protein immobilization capacity (bovine serum albumin (BSA), used here as a model) of the hybrids were investigated in detail.

2. Material and methods

2.1. Chemicals and instruments

MWCNT were purchased from Shenzhen Nano-Technologies Port Co., Ltd. (China). The detailed parameters are as follows: outer diameter 10–20 nm; length 5–15 μm; purity >95%; amorphous carbon <2%; ash ⩽0.2 wt.%; special surface area 180–190 $m^2 g^{-1}$; bulk density 5–7 mL g^{-1}. CS (deacetylation degree ⩾90%) and acetic acid were supplied by Sinopharm Chemical Reagent Co., Ltd. TPP was obtained from Tianjin Hengxing Chemical Preparation Co., Ltd. BSA was purchased from Jiehui Biotechnology Co., Ltd. (Changsha). Fluorescein isothiocyanate (FITC)-labeled BSA were obtained from Sigma–Aldrich. HeLa cells were provided by the Cancer Research Institute of Xiangya Medical College. RPMI-1640 medium (Gibco) containing 10% heat-inactivated newborn calf serum (Invitrogen Corp.), 100 IU mL^{-1} penicillin, 100 μg mL^{-1} streptomycin, trypsinase (0.25% + 0.02% EDTA), and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were obtained from Amresco. Phosphate-buffered saline (PBS), pH 6.5 and 7.4, was used in the experiments. All other reagents were of analytical grade or better. Milli-Q ultrapure water (>18 MΩ cm, Millipore Co., Ltd.) and fresh prepared solutions were used throughout.

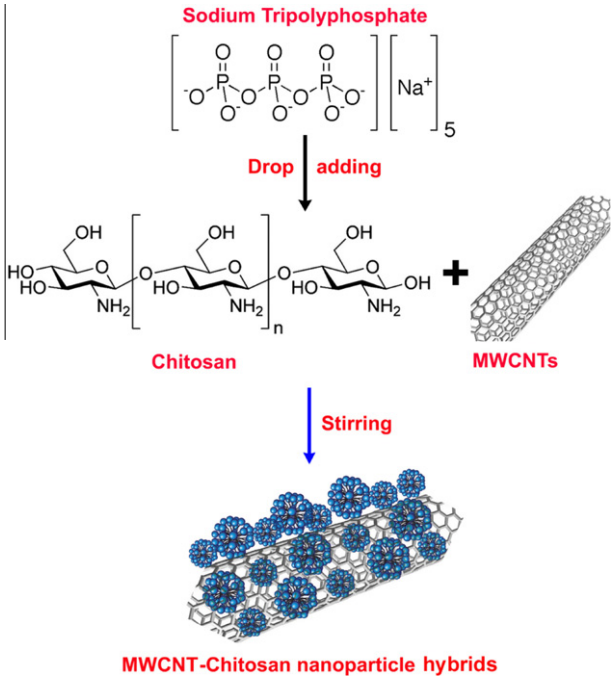

Fig. 1. A schematic demonstrating the synthesis of MWCNT–CS NP hybrids.

The morphologies of the carboxylated MWCNT, CS NP, and MWCNT–CS NP hybrids were characterized by AFM (PicoPlus, Molecular Imaging Co., USA). The AFM experiments were operated under a.c. tapping mode. The TGA experiments were performed in a Netzsch STA409PC/PG system at a heating rate of 10 °C min^{-1} under a nitrogen atmosphere. The proliferation morphologies of HeLa cells were observed with an inverted optical microscope (Olympus IM). The MWCNT–BSA–FITC, CS NP–BSA–FITC, and MWCNT–CS NP–BSA–FITC complexes were characterized by fluorescence microscopy (Nikon Eclipse Ti-S). Ultraviolet–visible (UV–vis) spectra were recorded in a UV-2450 UV–vis spectrophotometer (Shimadzu Co., Kyoto, Japan).

2.2. Preparation of CS NPs and MWCNT–CS NP hybrids

The preparation of CS NP was based on the ability of CS to undergo a liquid–gel transition due to ionic interaction with TPP [45]. Typically, 2 mL of TPP aqueous solution (at different concentrations) was dropwise added to 5 mL of 2 mg mL^{-1} CS in acetic acid solution with magnetic stirring at room temperature. The concentration of acetic acid was in all cases 1.75 times that of CS. The mixture was further stirred for 1 h. The obtained CS NP were purified by centrifugation (13,000 rpm, 30 min) and rinsed with ultrapure water three times for future use.

The in situ synthesis of MWCNT–CS NP hybrids is schematically shown in Fig. 1. First, 500 mg of as-received MWCNT were purified by refluxing at 80 °C in concentrated HCl for 12 h, followed by filtration with a Millipore VC membrane (pore size 0.22 μm) and a careful rinse with copious ultrapure water till the filtrate was neutral. Then, 300 mg of purified MWCNT were refluxed in mixed acids (concentrated H_2SO_4 + HNO_3, 75:25 vol.%) at 80 °C for 24 h [46]. The suspension was cooled and diluted with ultrapure water, followed by filtration and rinsing as above. The residual impurities in the carboxylated MWCNT were not further analyzed [47] and the carboxylated MWCNT were collected and stored in a desiccator for future use. The in situ synthesis of MWCNT–CS NP hybrids is similar to that of CS NP. Briefly, 2 mL of TPP aqueous solution (at different concentrations) was dropwise added to 5 mL of 2 mg mL^{-1} CS in acetic acid solution containing MWCNT with magnetic stirring at room temperature (hereafter, unless otherwise specified, the MWCNT are referred to as carboxylated MWCNT). The mixture was further stirred for 5 h. The MWCNT–CS NP hybrids obtained were purified by centrifugation (13,000 rpm, 30 min) and rinsed with ultrapure water three times for future use.

2.3. Immobilization of BSA–FITC on MWCNT–CS NP hybrids

To immobilize BSA–FITC on the surface of MWCNT–CS NP hybrids 6.0 μL of 1 mg mL^{-1} BSA–FITC aqueous solution was added to 94 μL of 0.5 mg mL^{-1} ultrasonicated MWCNT–CS NP aqueous suspension, followed by 2 h reaction at room temperature with magnetic stirring in a heliophobe environment. Thereafter the excess BSA–FITC was separated by centrifugation (13,000 rpm,

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