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Effects of acid treatment on structure, properties and biocompatibility of carbon nanotubes

Chenbo Dong[a], Alan S. Campell[a], Reem Eldawud[a], Gabriela Perhinschi[a], Yon Rojanasakul[b], Cerasela Zoica Dinu[a,*]

[a] Department of Chemical Engineering, West Virginia University, Morgantown, WV 26506, USA
[b] Department of Basic Pharmaceutical Sciences, West Virginia University, Morgantown, WV 26506, USA

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ABSTRACT

Carbon nanotubes (CNTs) are promising to be the next generation of viable tools for bioapplications. Further advances in such bioapplications may depend on improved understanding of CNTs physical and chemical properties as well as control over their biocompatibility. Herein we performed a systematic study to show how acid oxidation treatment changes CNTs physical and chemical properties and leads to improved CNTs biocompatibility. Specifically, by incubating CNTs in a strong acid mixture we created a user-defined library of CNTs samples with different characteristics as recorded using Raman energy dispersive X-ray spectroscopy, atomic force microscopy, or solubility tests. Systematically characterized CNTs were subsequently tested for their biocompatibility in relation to human epithelial cells or enzymes. Such selected examples are building pertinent relationships between CNTs biocompatibility and their intrinsic properties by showing that acid oxidation treatment lowers CNTs toxicity providing feasible platforms to be used for biomedical applications or the next generation of biosensors.

1. Introduction

Carbon nanotubes (CNTs) are nanoscale diameter materials of tubular shape and micrometer length with many interesting properties that make them viable candidates for a wide range of applications including electrical circuits [1], hydrogen storage [2], fiber optics [3], and conductive plastics [4]. In recent years, CNTs functionalization with biomolecules such as proteins [5], enzymes [6,7] or nucleic acids [8] opened up exciting bioapplications in biolabeling [9], biosensing [10], drug delivery [11], bioseparation [12] and tissue engineering [13]. However, further development of such bioapplications is hindered by: (1) CNT's limited available surface area for biomolecule functionalization [14], (2) lack of understanding of CNTs growth mechanisms in uncontaminated forms [15], (3) CNTs structural instability since larger nanotubes are prone to kinking and collapsing [16,17], and (4) CNTs cytotoxicity and associated health risks posed during their manufacturing and processing [18]. These challenges are mainly associated with the fact that as-produced CNTs form large aggregates in liquid enviroments since their hydrophobic walls are prone to van der Waals interactions [19]. Thus, in order to increase CNTs bioapplications [20] and reduce their aggregation [21] and cytotoxicity [22], it is critical to overcome their intrinsic hydrophobicity and tendency to form conglomerates in solution.

Numerous attempts have been made to overcome CNTs hydrophobicity and increase their hydrophilicity; these include gas- [23] and liquid-phase activation [24], and oxidation with strong oxidants including hydrogen peroxide [25], potassium permanganate [26], potassium hydroxide [27], and nitric and/or sulfuric acid [6,7,28]. Among these attempts, nitric and sulfuric acid oxidation is regarded as the most prevalent treatment since it is easy to implement in both laboratory and industrial settings [20]. When CNTs are oxidized with such aggressive acids, their hydrophilicity is increased by the introduction of oxygen-containing functional groups, i.e., carboxyl [29], carbonyl [26,29], and phenol groups [30]. Moreover, during such oxidation treatments amorphous carbon [31] and residual metal catalyst particles are removed, possibly resulting in reduced intrinsic toxicity of CNTs [22]. Despite the fact that wide evaluations of the effects of acid oxidation on CNTs have been carried out, systematic investigations of changes in physical and chemical properties and how such changes can be further employed for increasing CNTs biocompatibility and thus bioapplications are still lacking.

Herein we performed a systematic study of the changes in physical and chemical properties of pristine CNTs upon user-controlled

* Corresponding author at: Department of Chemical Engineering, West Virginia University, Benjamin M. Statler College of Engineering and Mineral Resources, PO Box 6102, Morgantown, WV 26506, USA. Tel.: +1 304 293 9338; fax: +1 304 293 4139.
E-mail address: cerasela-zoica.dinu@mail.wvu.edu (C.Z. Dinu).

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treatment with nitric and sulfuric acids. Further, we assessed how these changes affect CNTs biocompatibility in relation to cellular and enzymatic systems [6,7,10]. Our hypothesis was that selected biological examples will help build pertinent relationships between CNTs biocompatibility and their intrinsic properties and demonstrate how interface reactions between a biological molecule and the nanomaterial can be further used to provide systems with lower toxicity to be used for selected bioapplications as well as feasible platforms for the next generation of biosensors.

2. Materials and methods

2.1. Acid oxidation of CNTs

Acid oxidation treatment of single- and multi-walled carbon nanotubes (SW- and MWCNTs, respectively) was employed to generate a library of samples with different physical and chemical properties. Specifically, commercial SWCNTs (85% purity, Unidym Inc.) and MWCNTs (95% purity, Nanolab Inc. (PD15L5-20)) were incubated in a concentrated sulfuric (96.4%, Fisher, USA) and nitric acid (69.5%, Fisher, USA) mixture in a ratio of 3:1 (V/V). The CNTs/acids mixture (where CNTs can refer to either SW- or MWCNTs) was subsequently sonicated in an ice bath (Branson 2510, Fisher, USA) for 1, 3, or 6 h, at a constant temperature of 23 °C. When the required time elapsed, CNTs/acids mixture was diluted with deionized (di) water and filtered through a GTTP 0.2 μm polycarbonate filter membrane (Fisher, USA). Several cycles of resuspension in di water were employed to remove acidic residues or catalysts. The CNTs were isolated on the filter, subsequently dried in a vacuum desiccator and stored at room temperature for further use.

2.2. Energy dispersive X-ray analysis (EDX) of CNTs

Energy dispersive X-ray analysis (EDX) was used for quantitative elemental analysis of pristine and acid oxidized CNTs. Samples (1 mg/ml in di water) were deposited on silica wafers and dried under vacuum. The experiments were performed on a Hitachi S-4700 Field Emission Scanning Electron Microscope (USA) with a S-4700 detector combining secondary (SE) and backscattered (BSE) electron detection (all in a single unit), operating at 20 kV. Results are presented as a percent of elements relative to the most dominant element.

2.3. Scanning Electron Microscopy (SEM) of CNTs

Samples (1 mg/ml in di water of both pristine and acid treated CNTs) were dried on silica wafers under vacuum and imaged using a Hitachi S-4700 Field Emission Scanning Electron Microscope (USA) with a field emission at 10 kV.

2.4. Raman spectroscopy of CNTs

Raman spectroscopy (performed on a Renishaw InVia Raman Spectrometer, CL532-100, 100 mW, USA) allowed determination of the chemical structure and any modifications resulted from the acids oxidation of both pristine and acids treated CNTs. Briefly, CNTs deposited on glass slides (Fisher, USA) were excited through a 20× microscope objective using an Argon ion (Ar^+) laser beam with a spot size of <0.01 mm^2 operating at 514.5 nm. Detailed scans were taken in the 100–3200 cm^{-1} range; low laser energy (i.e., <0.5 mV) and exposure time of 10 s were used to prevent unexpected heating effects.

2.5. CNTs solubility measurement

The solubility of CNTs (pristine and acids oxidized) was evaluated in di water (pH 6.25) and Phosphate Saline Buffer (PBS, pH 7, 100 mM ionic strength). Briefly, CNTs were diluted in the solvent of interest to yield to a 3 mg/ml solution. The suspension was then centrifuged at 3000 rpm for 5 min; subsequently, part of the supernatant (0.8 ml) was removed and filtered through a 0.2 μm GTTP filter membrane. The filter membrane was then dried under vacuum and the amount of CNTs was weighted. The solubility of the CNTs was calculated based on the volume used for suspension and the initial starting amount.

2.6. CNTs length measurement

An atomic force microscope (AFM, Asylum Research, USA) was used to evaluate the length of pristine and acids treated CNTs. A Si tip (Asylum Research, 50–90 kHz AC240TS, USA) helped perform tapping mode in air. CNTs samples (i.e., pristine, 1, 3 or 6 h acids oxidized SW and MWCNTs) were dispersed in di water (to yield solutions of 0.1 mg/ml concentration), deposited on mica surfaces (9.5 mm diameter, 0.15–0.21 mm thickness, Electron Microscopy Sciences, USA) and allowed to dry over night under vacuum. Scan images of 10, 5 or 1 (μm × μm) areas were acquired. For each sample, at least 30 individual CNTs were counted and measured to obtain average length distribution.

2.7. Cell culture and treatment with CNTs

Non-tumorigenic human bronchial epithelial cells (BEAS-2B) were purchased from American Type Culture Collection (ATCC, USA). The cells were cultured in DMEM medium supplemented with 5% fetal bovine serum (FBS), 2 mM L-glutamine and 100-units/ml penicillin/streptomycin (all reagents were purchased from Invitrogen, USA). Cells were passaged weekly using 0.05% trypsin (Invitrogen, USA) and kept in 5% CO_2 at 37 °C.

Pristine and acids oxidized SWCNTs were dispersed in di water by sonication, filtered through the 0.2 μm GTTP filter membrane, resuspended in cellular media and sonicated at room temperature to form stable dispersions. For treatment, BEAS-2B cells were seeded overnight in a 12 well plates (Fisher, USA) at a density of 3.5E5 cells/well, and allowed to reach confluence. Subsequently, the cells were exposed to 100 μg/ml SWCNTs; 24 h post exposure, the cells were incubated with 6.5 μg/ml Hoechst 33342 dye (Molecular Probes, USA) for 30 min at 37 °C and analyzed for apoptosis by scoring the percentage of cells with intensely condensed chromatin and/or fragmented nuclei using fluorescence microscopy (Leica Microsystems, USA). Approximately 1000 cell nuclei from ten random fields were analyzed for each sample. The apoptotic index was calculated as the percentage of cells with apoptotic nuclei relative to the total number of cells. At least 3 independent trials were performed for each sample.

2.8. Functionalization of CNTs with enzyme

Soybean peroxidase (SBP, Bioresearch, USA) was covalently attached to 1, 3 or 6 h acid treated MWCNTs using 1-ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride (EDC; Acros Organics, USA) and N-hydroxysuccinimide (NHS, Pierce, USA) [32]. Briefly, 2 mg CNTs (MWCNTs) were dispersed in 160 mM EDC and 80 mM NHS (total volume of 2 ml in MES (2-(N-morpholino)ethanesulfonic acid sodium salt, 50 mM, pH 4.7, Sigma, USA) for 15 min at room temperature with shaking at 200 rpm. The activated MWCNTs were next filtered through the 0.2 μm GTTP filter membrane, washed thoroughly with MES buffer to remove any ester residues, immediately dispersed in 2 ml of 1 mg/ml SBP