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Single walled carbon nanotube reactivity and cytotoxicity following extended aqueous exposure

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Oxidized SWCNTs in pH neutral fresh and saline water showed no reduction in surface oxidation with time, yet exposure of these nanotubes to saline and NOM reduced human cell toxicity markedly.

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ABSTRACT

Globally carbon nanoparticles are increasingly utilized, yet it is not known if these nanoparticles pose a threat to the environment or human health. This investigation examined 'as-prepared', and acid cleaned carbon nanoparticle physicochemical characteristics (by FTIR, TEM, FESEM, UV–VIS and X-ray microanalysis), and whether these characteristics changed following 2.5–7 yr exposure to pH neutral saline or fresh water. To determine if these aqueous aged nanotubes were cytotoxic, these nanotubes were incubated with human epithelial monolayers and analyzed for cell viability (vital staining) and ultrastructural nanoparticle binding/localization (TEM, FESEM). The presence of Ni and Y catalyst, was less damaging to cells than CNT lattice surface oxidation. Extended fresh water storage of oxidized CNTs did not reduce surface reactive groups, nor lessen cell membrane destruction or cell death. However storing oxidized CNTs in saline or NOM significantly reduced CNT-induced cell membrane damage and increased cell survival to control levels.

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1. Introduction

The current commercial incorporation of various types of engineered nanoparticles into household, personal and industrial products is estimated to increase to 58,000 tons in 2011–2020 (Maynard et al., 2004), which can result in nanoparticles in surface and ground water following product use, disposal and decomposition (Murr et al., 2004a,b; Murr and Soto, 2005; Soto et al., 2008; Nowack and Bucheli, 2007; Mueller and Nowack, 2008; Helland et al., 2007). The water that we depend on for drinking, bathing and food production may be at risk, and global concerns about environmental nanoparticle contamination may be warranted (Service, 2004). The fate of carbon nanotubes (CNTs) in environmental compartments may differ depending on CNT specific properties (surface chemistry, electrical properties and oxidative potential) and the physical and chemical conditions of the specific environmental compartments (such as redox potential, pH, temperature, UV light or synergistic effects with toxins) (Helland et al., 2007; Oberdorster et al., 2005, 2006; Scheringer, 2008). Fullerene carbon nanoparticles (n-C_{60}) have been found to aggregate in weak salt solutions (ionic strengths >0.001 M) forming large aggregates capable of settling out of suspension, adhering to other particles or media, or becoming otherwise immobilized, thus reducing their toxicity (Brant et al., 2005). Similarly, SWCNTs have been shown to form micrometer range aggregates in aqueous environments (Cheng and Cheng, 2005), which showed no change in size or distribution with increasing salinity or temperature. However Chen et al. (2004) found that SWCNTs changed aggregate characteristics with pH change and SWCNT post synthesis treatments utilized in industrial processing (e.g. acid cleaning, surfactants).

Both 'as-prepared' carbon nanoparticles and industrially acid cleaned carbon nanotubes are used widely in commercial products, which following disposal can enter the environment through direct contact with surface and ground water (via drains, sewers), and indirectly through degradation in landfills (Maynard, 2006). This investigation examines both 'as-prepared' and acid cleaned carbon nanotubes following aqueous exposure in fresh and saline water. We examined (1) the physicochemical changes at time zero (when the carbon nanomaterial was first suspended in fresh or saline water), and following 2.5–7 yr aqueous exposure; and (2) how

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aqueous aging of carbon nanotubes (CNTs) in saline and ~18 MΩ water over time can affect interactions with living human cells; and (3) whether these early interactions between the CNTs and cells can be modified to reduce toxicity by pretreatment with PBS or NOM.

Using an environmental fate model, Mueller and Nowack (2008) reported that of the 3 types of nanoparticles that they studied in all of the environmental compartments, carbon nanotubes (CNTs) were least present in their Swiss exposure model. This is not the case for largest island (Long Island) adjoining the US mainland, with a population greater than 2.75 million people (O'Connell et al., 2005), and the sole natural source of drinking water for much of Long Island constitutes three subsoil water-bearing layers (e.g. the Upper Glacier Aquifer, the Magothy Aquifer and Lloyd Sands). Here the populace has been using and discarding many products containing loosely bound carbon nanoparticles (e.g. creams, ointments, cosmetics, paints, filters, lubricants, household supplies, nano-Tex fabrics, electronics, camping and sports equipment, carbon nanoparticle hardened soles on boots and shoes, sensors, fabric coatings, electronic components, etc.) (The Project on Emerging Nanotechnologies, 2005). In addition, there are numerous industries and colleges/research institutions synthesizing and consuming large amounts of CNTs daily for research and manufacturing on this same small landmass. Following usage and disposal via drains, or packaging as solid municipal waste (ultimately residing in landfills, sewers and surface waters), this environment may present unique conditions. Mueller and Nowack (2008) stated that they had not included nanoparticles leaching from landfills in their model, yet in our landfills the possibility of carbon nanoparticle leaching from landfills and run-off from sewers and storm drains all have to be considered, in light of previous reported water contamination problems and contamination plumes (Halpin, 2008). With nanoparticles already in our environment, our interest in studying CNT fate and interactions with human cells under aqueous conditions serves an urgent need to understand whether CNTs in this pollution sensitive environment pose any threat to human safety and the island environment.

2. Methods

2.1. Carbon nanoparticles

2.1.1. 'As-prepared' Carbolex and acid-air oxidized Carbolex samples

Commercially prepared Carbolex nanotubes purchased in 1999 from Carbolex, Inc. (Lexington, KY) were used for all of the experiments in this study. Carbon nanotubes (CNTs) are frequently cleaned to remove metal catalyst and carbon debris (the carbon black, non-tubular graphene and amorphous carbon), using acids (Colomer et al., 1999; Park et al., 2006), or acid–peroxide (piranha) solutions with sonication and heat (Liu et al., 1998). This process used in manufacturing and in research laboratories' attaches highly reactive oxygen containing groups on the nanotube sidewalls and open ends (Ziegler et al., 2005; Kuznetsova et al., 2001; Mawhinney et al., 2000). Since both of these forms of CNTs could be found in the environment following disposal of commercial products or via manufacturing waste disposal, we examined two types of acid cleaned Carbolex CNTs (acid-air oxidized Carbolex SWCNTs having some Ni, Y, and carbon debris removed (Colomer et al., 1999; Park et al., 2006); and acid/peroxide cleaned SWCNTs (Liu et al., 1998; Panessa-Warren et al., 2008) with all Ni, Y and most of the carbon contaminants removed), as well as the original 'as-prepared' Carbolex.

'As-prepared' Carbolex material was used directly from the manufacture's shipping container. Acid treated air-oxidized Carbolex nanotubes, were prepared, and both types of carbon nanomaterial were suspended in either sterile, milli-Q water (~18 MΩ) fresh water, or phosphate buffered (PBS) saline (0.2 M monobasic sodium phosphate with 0.2 M dibasic sodium phosphate with 4.5% saline at pH 7.2) using brief sonication (1.5–3 min) 2×s with intermittent vortexing (30 s, 3×). Sonication was carefully monitored (preventing heating of the nanotubes and using short sonication times) to prevent damage to the carbon lattice sidewalls of the nanotubes during mixing and suspension (Hoffman, 2008).

2.1.2. Acid/peroxide (A/P) cleaned Carbolex

Both industrially and for research, carbon nanoparticles are often cleaned and cut using acids and peroxide (Ziegler et al., 2005; Liu et al., 1998), especially in the manufacture of commercial products. Since these nanoparticles, and nanoparticle-containing products, through disposal and leaching can enter the surface water, sewers and eventually the environment, acid/peroxide (H_2SO_4/H_2O_2) cleaned carbon nanotubes were included in this investigation. The preparations were made by treating "as-prepared" Carbolex with a mixture of concentrated sulfuric acid and 30% aqueous hydrogen peroxide with sonication at 60 °C (Liu et al., 1998; Panessa-Warren et al., 2008). The cleaned nanotube preparations were neutralized with sterile deionized water and titrated with KOH to pH 4–5. The cleaned CNTs were examined by TEM, FESEM and X-ray microanalysis to determine CNT number, size and morphology, the type and amount of metal catalyst particles, and the presence of non-tubular graphene, graphite, carbon black or any debris. This cleaning method reduced the number of total CNTs, making it necessary to determine final concentrations of the stock solutions (mg/ml) by UV–VIS.

2.1.3. Electron microscopy and X-ray microanalysis

For TEM, aqueous CNT suspensions (1 μl droplets) were placed onto formvar-carbon coated copper grids and examined unstained at 80–100 KV on a Philips 300 or JEOL 1200 TEM. To enhance delineation of amorphous carbon, CNT bundles and aggregates, some grids were stained with 2% aqueous uranyl acetate. For FESEM 1–2 μl droplets of CNT suspension was placed on either conductive silicon wafers or SEM mounts and examined coated with a thin Pt film (3 nm) with a turbo pumped K575XD Emitech sputter coater (Emitech Products, Inc., Houston, TX). These uniform, polycrystalline Pt coatings, (Panessa-Warren et al., 2007), assured a continuous structureless surface without decoration artifacts. Samples were imaged at 5–15 KV with a JEOL 6500F field emission SEM.

CNTs (2 μl droplets) were placed on cleaned graphite or silicon planchets for X-ray microanalysis, and analyzed in raster mode at 20 KV, spot size 12, 100x, and raster size 300 μm × 250 μm using a Princeton Gamma Tech energy dispersive Li x-ray detector. For higher sensitivity CNT samples were placed on beryllium planchets and analyzed using the aforementioned conditions with a Bruker AXS 133 eV X Flash Detector 4030 (Bruker, Berlin, Germany). Spectra were taken in 3–4 areas/sample using ZAF software measuring the elements of interest as wt%, and the means and standard deviations calculated.

2.2. Chemical characterization

Chemical characterization was done at the Center for Functional Nanomaterials at Brookhaven National Laboratory (Upton, NY). Carbolex nanoparticles were analyzed for concentration by UV–VIS (Perkin-Elmer Lambda 35 Spectrophotometer) (200–1100 nm), and CNT surface reactive groups (Bellucci, 2007) by Fourier Transform Infra-red Spectroscopy, FTIR (Thermo-Nicolet 6700 FTIR spectrometer equipped with a liquid N_2 cooled MCT-A detector). The small sample size and low CNT concentration necessitated the use of a single-reflection Horizontal Attenuated Total Reflectance (HATR) Smart Miracle accessory for FTIR data collection employing a ZnSe crystal with constant liquid nitrogen purging. Purified carbon nanoparticle samples (~50 μl) in milli-Q water (~18 MΩ) were dried via a nitrogen stream for 1 h before measurement. Carbon nanoparticle samples in phosphate buffered saline (PBS) were similarly prepared. FTIR spectra were processed following background, water and carbon monoxide subtraction.

2.3. CNT aqueous aging experiments in PBS and in ~18 MΩ water

The Carbolex suspensions were tested when the samples were freshly made (time zero), after which CNT suspensions were sealed in low potassium glass vials, or in 50 ml centrifuge tubes and placed in secondary containment vessels at 4–8 °C, or in a plexiglass cabinet at 21 °C. All stock solutions were measured by UV–VIS to verify concentrations (especially following acid/peroxide cleaning which reduced the number of nanotubes). The stock solutions were used to make CNT dilutions corresponding to 0.12 mg/L (10 μM) concentration; and a concentration of 1.2 mg/L (100 μM) (molality based on the gram molecular weight of carbon). In this way the carbon content of each experimental dose was normalized for each sample tested based on carbon content. The CNTs were sonicated (2×, 1.5–5 min) and vortexed (3×, 1.5 min) to disperse and suspend the nanoparticles in ~18 MΩ water or buffered saline (PBS) prior to use. All samples were analyzed by TEM, FESEM, UV–VIS and FTIR.

2.3.1. Cytotoxicity experiments using epithelial cell monolayers

To determine the cytotoxicity of saline- or ~18 MΩ water aged CNTs, a human epithelial cell cytotoxicity model was used (Panessa-Warren et al., 2006, 2008). Nanoparticles in contaminated water can disperse via aerosolization, wind dispersal following water evaporation, or during bathing, eating and drinking. Therefore nanoparticles entering the back of the throat via inhalation or ingestion can travel to the trachea or esophagus. In this case, epithelial cells lining the respiratory tract and alimentary canal are a first line of contact for inhaled or ingested nanoparticles. This study utilized human lung muco-epithelial cells grown in vitro to examine the 'first responses to nanoparticle contact', and cytotoxicity. Human lung NCI-H292 epithelial cell monolayers were grown as 12 mm diameter sheets (monolayers) on coverslips as continuous polarized, epithelial layers (95–97% confluency), with tight junctions joining the cells together (ViroMed Laboratories, Minneapolis, MN, USA). Each monolayer was grown in 1.5 ml low serum (3–7%) culture medium to which