Biomedical Applications of Carbon Nanotubes and the related Cellular Toxicity

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ABSTRACT

We present a tunable synthesis of Carbon nanotubes-Silica Nanoparticles Composites and illustrate their potential uses in Biomedicine. We also compare the cytotoxicity of pristine and oxidized Multi Walled Carbon nanotubes on human T cells - which would be among the first exposed cell types upon intravenous administration of Carbon nanotubes in therapeutic and diagnostic nanodevices.

1. INTRODUCTION

Nanotechnology is the manufacture and science of materials with at least one dimension in the nanometer scale. Many nanomaterials have novel chemical and biological properties and most of them are not naturally occurring. Carbon nanotubes (CNTs) are an example of a carbon-based nanomaterial [1], which has won enormous popularity in nanotechnology for its unique physico-chemical properties and applications [2] in commercial, environmental and medical sectors which will dramatically affect occupational and public exposure to CNT-based nanomaterials in the near future.

We begin with illustrating the potential use of Carbon nanotubes-Silica Nanoparticles Composites in Biomedicine. Silica nanoparticles have been widely used for biosensing and catalytic applications due to their large surface area-to-volume ratio, straightforward manufacture, and the compatibility of silica chemistry with covalent coupling of biomolecules. Carbon nanotubes–composite materials, such as those based on Carbon nanotubes bound to nanoparticles, are suitable, in order to tailor Carbon nanotubes properties for specific applications.

Here, we present firstly a tunable synthesis of Multi Walled Carbon nanotubes–Silica nanoparticles [3]. The control of the nanotube morphology and the bead size, coupled with the versatility of silica chemistry, makes these structures an excellent platform for the development of biosensors (optical, magnetic and catalytic applications).

Then we illustrate the realization of supramolecular fluorescent nanostructures useful for a large variety of applications ranging from biosensors to electronics, especially in case of use of pristine full-length CNT that are characterized by intact π-electronic structure [4].

Then, we report on our recent results about the isolation and spectroscopic characterization of fluorescent materials present in pristine and acid treated single wall carbon nanotubes [5].

Finally, we compare the toxicity of pristine and oxidized Multi Walled Carbon nanotubes on human T cells - which would be among the first exposed cell types upon intravenous administration of Carbon nanotubes in therapeutic and diagnostic nanodevices. Our results suggest that carbon nanotubes indeed can be very toxic and induce massive loss of cell viability through programmed cell death at sufficiently high concentrations (>1ng/cell) [6]. We conclude that careful toxicity studies need to be undertaken particularly in conjunction with nanomedical applications of Carbon nanotubes.

2. NANOCOMPOSITES IN BIOMEDICINE

Many nanomaterials have novel chemical and biological properties and most of them are not naturally occurring. Of course, CNTs are no exception. Their inclusion, aimed at improving the quality and performance of many widely used products, as well as their potential use for applications in biology and medicine, is likely to dramatically affect the occupational and public exposure to CNT-based nanomaterials in the near future. For bio-medical applications of CNTs, the main property of relevance is fluorescence, which occurs when a substance absorbs a light wavelength and emits a different one. CNTs absorb and emit light in the near infrared spectrum. This is useful to develop nanotube-based optical biosensors for the detection of specific targets inside the human body, e.g. tumor cells, by wrapping the tubes in a protein that can link only to the targeted cells. Silica nanoparticles have been widely used for biosensing and catalytic applications due to their large surface area-to-volume ratio, straightforward manufacture, and the compatibility of silica chemistry with covalent coupling of biomolecules [7].

A key challenge in nanotechnology is the precise control of nanoparticle assembly for the engineering of particles with the desired physical and chemical properties. Much research is currently focused on CNT as a promising material for the assembly of nanodevices based on new CNT–composite materials. Recently, we presented the tunable synthesis of MWCNT–silica nanoparticle composite materials; this process involves growing the silica nanoparticles directly onto functionalized MWCNTs using a water-in-oil microemulsion to strictly control the particles size [3].

Under strong oxidizing conditions, CNTs were cut into short and rather straight pieces having carboxylic acid groups both at their tips and at imperfections on their walls. Nanoparticles in the composite were covalently linked to the CNT only at locations functionalized with triethoxy-silane groups, while the bare graphitic wall of non-oxidized CNTs did not support any composite formation. The presence of carboxylic acid groups greatly facilitated the dispersion of nanotubes in aqueous solutions, as well as their further functionalization. As a result, we covalently coated CNTs with nanoparticles of different sizes.

The architecture of the obtained assemblies can be controlled by varying the conditions in the synthesis. Thus, the length of the CNT is regulated by the oxidation time and the size of the nanoparticles by using micro-emulsion conditions that yield micelles of a particular size. The chemical properties of the silica surface are particularly versatile and silica can be doped, hence yielding nanostructures with a wide range of morphologies and properties suitable for different applications.

Refining our approach for creating novel nanostructures and combining it with procedures for isolating discrete products, we expect to be able to combine different nanostructures into higher order assemblies, that could be useful for a variety of purposes, including the possibility to provide an interface between living cells and biosensor arrays.

3. SUPRAMOLECULAR NANOSTRUCTURES

We synthesized three supramolecular nanostructures based on CNT and ruthenium-complex luminophores [4]. The first nanostructure consisted of short oxidized SWCNT covalently decorated by ruthenium-complexes that act as light-harvesting antennae by donating their excited-state electrons to the SWCNT. This nanocomposite represents an excellent donor-acceptor complex, which may be particularly useful for the construction of photovoltaic devices based on metallo-organic luminophores.

The second and the third nanostructures consisted of metallo-organic luminophore-doped silica nanobeads covalently linked to short oxidized SWCNT or hydrophobically adsorbed onto full-length MWCNT. In these nanocomposites, the silica network prevented the fluorescence quenching because excited-state electrons could not be readily donated to the CNT conduction band.

Because the physical and chemical properties of the silica nanobeads are so versatile, and the π-electronic structure of the CNT can be kept intact by using a non-destructive modification of the nanotube structure, we consider these nanocomposites to have a promise for a variety of applications ranging from the biosensors to electronics.

4. FLUORESCENT NANOPARTICLES

In ref. [5], we isolated, fractionated by molecular weight, and characterized fluorescent nanoparticles (FP) from pCNT and oxCNT received from several suppliers. These FP were responsible for the photoluminescence of electric arc-produced CNT in the visible range and were likely composed of impurities that were present in the graphite rods used for the production of the CNT. Spectroscopic analysis of the samples revealed some common supplier-independent features, specifically that the FP derived from the pCNT exhibited a violet-blue photoluminescence, whereas the FP derived from the oxCNT exhibited photoluminescence ranging from blue to yellowish-green. In contrast, the molecular weight dependency for both the pristine and oxidized CNT-derived fractions was strongly related to the specific supplier. This can be explained by differing fabrication processes leading to different physical and chemical aggregation of the impurities present in the graphite rod.

We recorded HRTEM images and electron disperse X-ray (EDX) analysis of the FP isolated from the CNT (Carbon Solutions, Inc.)-derived molecular weight fractions. The FP derived from the pCNT exhibited a narrow range in width, whereas the FP derived from the oxCNT were larger, had a broader width range, and formed hydrophilic aggregates in water. Moreover, EDX analysis of the fractions from the oxCNT-in-water supernate suggested that their FP were superficially oxidized and/or coated by a thin carbon layer.

5. CELLULAR TOXICITY ISSUES

We studied in [6] the important issue of the toxicity of CNTs, in connection to their many different forms and taking into account the fact that they can be chemically modified and/or functionalized with biomolecules. Oxidized CNTs are more readily dispersed in aqueous solutions and have been coupled to oligonucleotides, proteins, or peptides. Since little is yet known about the toxicity of both pristine and oxidized CNTs, we compared these two types of CNTs in a number of functional assays with human T lymphocytes, which would be among the first exposed cell types upon intravenous administration of CNTs in therapeutic and diagnostic nanodevices. We compared the toxicity of pristine and oxidized MWCNT on human T cells and found that CNT can be very toxic at sufficiently high concentrations (larger than 1 ng/cell).

The cytotoxicity of Carbon nanotubes does depend on many other factors than concentration, including their physical form, diameter, length, and the nature of attached molecules or nanomaterials: carbon black, for instance, is less toxic than pristine CNTs (which shows the relevance of structure and topology); oxidized CNTs are more toxic than pristine CNTs. Further careful toxicity studies are clearly needed, in conjunction with biomedical applications of CNT.

6. CONCLUSIONS

Carbon nanotubes, a form of carbon that did not exist in our environment before being manufactured possess unique chemical, physical, optical, and magnetic properties, which make them suitable for many uses in industrial products and in the field of nanotechnology, including nanomedicine. We showed that it is of the uttermost importance to explore the yet almost unknown issue of the toxicity of this new material. Our results suggest that carbon nanotubes indeed can be very toxic and induce massive loss of cell viability through programmed cell death at sufficiently high concentrations (>1ng/cell) [6].

We described a novel tunable approach for the synthesis of carbon nanotube–silica nanobead composites [3]. The control of nanotube morphology and bead size coupled with the versatility of silica chemistry makes these structures an excellent platform for the development of biosensors, or for optical, magnetic and catalytic applications.

We also constructed and characterized supramolecular nanostructures consisting of ruthenium-complex luminophores, which were directly grafted onto short oxidized single-walled carbon nanotubes or physically entrapped in silica nanobeads, which had been covalently linked to short oxidized single-walled carbon nanotubes or hydrophobically adsorbed onto full-length multi-walled carbon nanotubes [4]. These structures were evaluated as potential electron-acceptor complexes for use in the fabrication of photovoltaic devices, as well as for their properties as fluorescent nanocomposites for use in biosensors or nanoelectronics.

Fluorescent nanoparticles were isolated from both pristine and nitric acid-oxidized commercially available carbon nanotubes that had been produced by an electric arc method. The pristine and oxidized carbon nanotube-derived fluorescent nanoparticles exhibited a molecular-weight-dependent photo-luminescence in the violet-blue and blue to yellowish-green ranges, respectively. The molecular weight dependency of the photoluminescence was strongly related to the specific supplier. We analyzed the composition and morphology of the fluorescent nanoparticles derived from pristine and oxidized nanotubes from one supplier. We found that the isolated fluorescent materials were mainly composed of calcium and zinc [5]. Moreover, the pristine carbon nanotube-derived fluorescent nanoparticles were hydrophobic and had a narrow distribution of maximal lateral dimension. In contrast, the oxidized carbon nanotube-derived fluorescent nanoparticles were superficially oxidized and/or coated by a thin carbon layer, had the ability to aggregate when dispersed in water, and exhibited a broader distribution of maximal lateral dimension.

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