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Biomedical Platforms Based on Composite Nanomaterials and Cellular Toxicity

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Abstract. Carbon nanotubes 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 describe fluorescent nanocomposites for use in biosensors or nanoelectronics. Then we describe recent results on the issue of cytotoxicity of carbon nanotubes obtained in our labs. 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. We present a tunable synthesis of Multi Wall Carbon nanotubes–Silica nanoparticles. 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). We describe the construction and characterization of supramolecular nanostructures consisting of ruthenium-complex luminophores, 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. These structures have been evaluated as potential electron-acceptor complexes for use in the fabrication of photovoltaic devices, and for their properties as fluorescent nanocomposites for use in biosensors or nanoelectronics. 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). 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 (what shows the relevance of structure and topology); oxidized CNTs are more toxic than pristine CNTs.

Carbon nanotubes (CNTs) are an example of a carbon-based nanomaterial [1], which has won enormous popularity in nanotechnology for its unique properties and applications [2]. The development of nanomaterials is currently underway in laboratories worldwide for medical and
biotechnological applications including gene delivery [3] drug delivery [4] enzyme immobilization [5] and biosensing [6]. The most commonly used materials are gold [7], silica and semiconductors. 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 [8].

A key challenge in nanotechnology is the more 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 upon new CNT–composite materials, such as CNT with a thin surface cover [9] or CNT bound to nanoparticles [10], in order to tailor their properties for specific applications. Here, reviewing the results reported in [11], we present the tunable synthesis of multi-walled CNT–silica nanoparticle composite materials. Instead of coupling prefabricated silica nanobeads to CNT, we chose to grow the silica nanobeads directly onto functionalized multi-walled CNT by reaction of tetraethyl- or tetramethyl-orthosilicate (TEOS or TMOS) with a functionalized CNT precursor, prepared by coupling aminopropyltriethoxysilane (APTEOS) to a functionalized multi-walled CNT through a carboxamide bond, using a water-in-oil microemulsion to strictly control the nanobead size.

Perhaps, the most valuable feature of our work [11] is that the architecture of the obtained assemblies of covalently coated carbon nanotubes, with silica nanoparticles of different sizes, can be largely controlled by varying the conditions in the synthesis. Thus, the length of CNT is regulated by the oxidation time and the size of the nanobeads by using microemulsion conditions that yield micelles of a particular size. Indeed, silica nanobeads were prepared in a water-in-oil microemulsion system in which the water droplets served as nanoreactors [12]. The size of the final nanospheres was mainly regulated by the dimension of the water droplets. Because the chemical properties of the silica surface are particularly versatile and silica can be doped with fluorescent [13], magnetic [14] or biological macromolecules [15], nanostructures with a wide range of morphologies suitable for different applications can be obtained, including providing an interface between living cells and biosensor arrays.

In [16] we synthesized and characterized three kinds of supramolecular nanostructures based on CNT and ruthenium-complex luminophores. In the first nanostructure ruthenium-complex luminophores were directly grafted onto short oxidized single-walled carbon nanotubes. Hence, it 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. In the second and the third nanostructures ruthenium-complex luminophores were 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.

Very little is yet known about the toxicity of CNTs, which exist in many different forms and can be chemically modified and/or functionalized with biomolecules. Pristine single-walled CNTs are extremely hydrophobic tubes of hexagonal carbon (graphene) with diameters as small as 0.4 nm and lengths up to micrometers. Multi-walled CNTs consist of several concentric graphene tubes and diameters of up to 100 nm. These pristine CNTs are chemically inert and insoluble in aqueous media and therefore of little use in biological or medical applications. Due to the hydrophobicity and tendency to aggregate, they are harmful to living cells in culture [17]. As we have seen in the above, for many applications, CNTs are oxidized in strong acid to create hydroxyl and carboxyl groups [18], particularly in their ends, to which biomolecules or other nanomaterials can be coupled [11]. These oxidized CNTs are much more readily dispersed in aqueous solutions and have been coupled to oligonucleotides, proteins, or peptides. Indeed, CNTs have been used as vehicles to deliver macromolecules that are not able pass through the cellular membrane by themselves into cells [19].
Since little is yet known about the toxicity of CNTs, particularly of oxidized CNTs, we compared in [20] 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 found that, especially for high concentration (>1 ng/cell), CB are less toxic than pristine CNTs, therefore suggesting the relevance of the structure and topology (carbon black is amorphous) on the evaluation of the toxicity of a carbonaceous nanomaterial. Moreover we found that oxidized CNTs are more toxic than pristine CNTs for both the two analyzed concentrations, although they considered better suited for biological applications. This may well be because they are better dispersed in aqueous solution and therefore reach a higher concentration of free CNTs at similar weight per volume values. We calculated that the less toxic amount of 40 µg/ml of CNTs is equal to an order of magnitude of 10^6 individual CNTs per cell in our experiments, based on an average length of 1 µm and a diameter of 40 nm, giving an average molecular mass of 5×10^9 Da.

For biotechnological uses [21], a high level of purity is required to avoid undesired toxic effects from impurities. Contaminants in SWNT can be classified as carbonaceous (amorphous carbon and graphitic nanoparticles) and metallic (typically transition metal catalysts). It is well documented that nickel, which in combination with yttrium is used as a catalyst in the production of arc-discharged nanotubes, is cytotoxic [22]. Common SWNT purification methods based on oxidation (nitric acid and/or air) have the potential disadvantage of modifying the CNT by introducing functional groups and defects. Other less rigorous purification techniques rely upon filtration, centrifugation and chromatography. Recently, electrophoresis of nitric acid-treated arc-discharged SWNT was used to separate tubular carbon from fluorescent nanoparticles [23].

As we reported in [24], 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 photoluminescence 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. 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. Thanks to these findings we have been able to design a new SWNT purification method.

References