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Comparative in vivo biocompatibility study of single- and multi-wall carbon nanotubes

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Abstract

Carbon nanotubes are expected to be of use in both genetic engineering and biomaterials engineering. In each of these potential areas of application, nanoparticles are introduced into a living organism either in the form of active biomolecule carriers or as a result of the degradation process of an implant. In the present study we focus on the in vivo behavior of two types of carbon nanotubes (single- and multi-wall nanotubes). Raman and Fourier transform infrared spectroscopy, thermogravimetric analysis and differential scanning calorimetry techniques are used to characterize the materials before introducing them into the living system. The nanotubes were implanted into the skeletal rat muscle. A comparative analysis of the tissue reaction to the presence of the two types of carbon nanotubes was made. It was observed that multi-wall carbon nanotubes were found to form large aggregates within the living tissue, while distinctly smaller particles consisting of single-wall nanotubes were easily phagocytosed by macrophages and transported to local lymph nodes.

Keywords: Carbon nanotubes; Biocompatibility; In vivo investigation

1. Introduction

Carbon nanotubes (CNTs) are the objects of research in an increasing number of works. Due to their unique mechanical, physical and chemical properties, it is expected that they will find use in a range of medical techniques [1–5]. CNTs comprise a carbon fibrous form consisting of one (single-wall carbon nanotubes – SWNTs) to tens of concentric tubes (multi-wall carbon nanotubes – MWNTs) of carbon elements with adjacent graphene sheets separated by 0.34 nm. Their diameters range from two to several nanometers. Due to their unique form and set of physical, chemical and biological properties, they are becoming more and more attractive for use in medical applications. At present, CNTs are used as drug carriers [6,7], in gene therapy [8–10], as membrane elements, as materials for tissue engineering [11,12] as scaffolds for cells [13,14] and in neuronal growth [15–17] using the effect of electrostimulation, specifically in compositions with polymers. In vitro experiments show that carbon nanotubes can be used to mimic neural fibers for neuronal growth. The surface of carbon nanotubes can additionally be subjected to chemical processing to create electrically charged sites, capable of fixing various biomolecules [18–20]. On the other hand, CNTs are very mobile within the living body and easily migrate through biological membranes, skin, hair follicles and the respiratory and alimentary tracts, and diffuse through biological tissue and cellular membranes [21,22]. These features make CNTs useful materials as drug and gene carriers. Their mobility within living systems is a highly advantageous feature in diagnostics, gene transport and drug delivery devices. Attempts have been made to design CNTs with specific shapes and properties in order to produce a new class of biocompatible composite implants [12,23,24]. Polymeric matrices modified with CNTs present greater biocompatibility in contact with cell cultures than do pure polymers. However, a few studies have reported reactions between living tissue

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and CNTs. Taking into consideration the significant increase in the industrial use of nanomaterials, deeper knowledge about such possible reactions in living systems is required. An increasing number of companies are involved in manufacturing and developing various nanoparticulates and nanotechnologies. Thus, more questions relating to the safety and potential hazards due to their application are arising. In this respect, studies on what happens with nanotubes after delivering the drug or as a result of implant degradation in the living body, and determination of how CNTs react with tissue, are needed [12,25–29]. An essential aspect of the use of CNTs is the functionalization of their surface [30–32]. Previous studies on carbon fibrous biomaterials have revealed that the chemical state of the surface of CNTs may strongly influence tissue response [30,33]. Chemically treating the surface of CNTs can alter their susceptibility to form agglomerates or to disperse in an environment, as well as to evoke an interaction with the cells responsible for inflammation. AS well as the chemical state of the material surface, the shape and size of particles may also affect cells' response to a foreign body [34]. The influence of catalytic particles, like Fe, Ni, V and Y, applied during the synthesis of CNTs (catalytic residues) on the toxicity of CNTs has also been reported [12,29]. However, the amount of impurities necessary to influence the pathogenic activity of CNTs has not still been determined. Some results have shown that CNTs containing from 1 to 5 $\mu g\ ml^{-1}$ metallic impurities evoked no cytotoxic effect on a mesothelioma cell line, although the results have often been divergent or controversial [34]. In our study we analyse the in vivo biocompatibility of two types of CNTs differing in their surface chemical state and structure.

2. Materials and methods

The CNTs examined in this study were from NanoCraft, Inc. of Renton (USA). Multi- and single-wall CNTs were synthesized using arc-discharge evaporation of graphite rods. The SWNTs were 2–3 nm in diameter and 30–50 nm in length, with a 19° closed end, and called a carbon nanohorn. They were grown in the presence of iron as catalyst. The concentration of Fe catalyst determined by atomic absorption spectrometry (ASA) using an electrothermal technique (spectrometer Model 3110, Perkin–Elmer Co.) was about 1.8 wt.%.

MWNTs were 5–20 nm in diameter and 300–2000 nm long. The structure of these nanotubes contains 99.5% carbon elements in sp^2 form. The residual metal particles were not detected in this material.

The surface areas of the MWNTs and SWNTs were about 20 and 220 $m^2\ g^{-1}$, respectively. Both types of nanotubes were used in the experiments without any additional purification or treatment. SWNTs in the form of nanohorns, rather than normal nanotubes, are expected to become a low-cost raw material for practical use in medicine. The details of these samples in terms of their structure and functionality are presented in the next section.

Both types of nanotubes were characterized by Fourier transform infrared (FTIR) and FT Raman spectroscopy. The FTIR spectra of the samples were recorded by means of a Bio-Rad FTS60v spectrometer. The transmission FTIR spectra were registered in the range of 4000–400 cm^{-1} using KBr pellets. The FT Raman spectra were collected in the range of 1700–1100 cm^{-1} on a BioRad FT Raman accessory spectrometer (FTS 6000) with a Ge detector. The samples were excited at 1064 nm with a diode pumped Nd-YAG Spectra Physics laser. The spectra were measured at a resolution of 4 cm^{-1}. After spectra normalization, the areas of two bands of CNTs existing in the Raman spectra were fitted to a Lorenzian line shape and the area ratio of the G and D bands was calculated.

An STA (simultaneous thermal analysis) type SDT 2960 thermogravimetric analyser (TA Instruments Co.) was used to determine the differences in both types of nanotubes. The samples (5 mg) were placed in a platinum crucible and heated in air at 10 °C min^{-1} to 1000 °C. Differential scanning calorimetry (DSC) with a type DSC 2010 calorimeter (TA Instruments Co.) was used in order to determine the differences between single- and multi-wall CNTs. The samples (3 mg) were placed in a aluminum crucible and heated in air at 5 °C min^{-1} to 500 °C.

Measurements of the particles size (agglomerates) before implantation were conducted in water by the dynamic light scattering (DLS) technique (Malvern Zetasizer Nano ZS) in the range from 0.6 to 6 μm, with the laser light source of wave length $\lambda = 520$ nm. Larger particles formed during agglomeration were determined by stereoscopic microscopy (Alpha, Vision Engineering Ltd.) aided with a Pixel-Fox computer image analyser.

2.1. Animal experiments

The experiments were performed according to the EU ISO 10993-6 guidelinesand the study protocol was approved by the I Local Bioethics Committee in Krakow, Poland (No. 25/2007).

2.1.1. Implantation

Before implantation, the specimens of nanotubes were sterilized at 200 °C for 1 h. The nanotubes were implanted under sterile conditions into the gluteal muscle of adult hooded Oxford (HO/Krf) inbred rats. Animals were anesthetized and the skin at the site of surgery was shaved and disinfected with iodine (Cefarm Lublin, Poland). A small incision was made in the skin and the underlying muscle to create a 4 mm deep pouch. Equal portions (4 mg) of SWNTs or MWNTs were inserted into the bottom of the pouch. The muscle and skin wounds were closed with 5/0 PDS II (polydioxanone) monofilament absorbable sutures (Ethicon Ltd., UK). All animals survived the surgery. No wound healing complications were observed after the surgery or during the whole experiment. Before and after surgery the animals were maintained under standard conditions with free access to food and water.

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