

# IN VIVO BIOCOMPATIBILITY ASSESSMENT OF CARBON NANOTUBES

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## Abstract

The work deals with *in vivo* testing of single and multi wall carbon nanotubes. The microstructure of both types of carbon nanotubes was examined by Scanning Electron Microscopy (SEM), Fourier Infrared Spectroscopy (FT-IR). The nanotubes were implanted into skeleton muscle of rat and comparative analysis of the tissue in the vicinity of the material was made. The histological and histochemical analyses of tissue response to the materials revealed the distinct differences in tissue response, depending on the type of nanotubes.

**Keywords:** Carbon nanotubes, Bioactivity

## Introduction

Application of nanotechnology in various aspects of Medicine like diagnostics, therapeutics, controlled drug or gene delivery systems, tissue engineering requires specific nanodevices and nanomaterials. Carbon nanotubes and bulk carbon materials with engineered surface in nanoscale have attracted more and more interest as potential compatible components of biomaterials for different domains of medicine. Studies on biological properties of carbon nanotubes, particularly *in vitro* experiments, are carried out in many laboratories. However, few data exist regarding the reaction of living tissue with this form of nanoparticles. Taking into consideration a significant increase of such materials in industrial scale, deepen knowledge relevant to possible reactions in living system in contact with such particles is required.

Previous study on carbon fibrous biomaterials revealed that chemical surface state of carbon nanotubes may strongly influence tissue response. Due to the surface chemical groups nanotubes may form agglomerates as well as evoke specific interaction with phagocytes responsible for inflammation. In our study we analyzed *in vivo* behavior of two types of carbon nanotubes differing in surface chemical state and structure.

## Materials and methods

Carbon nanotubes were received from NanoCraft, Inc. of Renton (USA). Their geometrical parameters are, as follows:

- Single-wall Carbon Nanotubes, SWNTs. Nanotubes were 2 to 3 nm in diameter and 30 to 50 nm in length with a 19 degree closed end called horn.
- Multi-wall Nanotubes, MWNTs, were used as received. They consisted of nanotubes and nano-onion like and nano-polygonal particles. The nanotubes were 5 to 20 nm in diameter and 300 nm to 2000nm long

Both types of nanotubes were characterized by FTIR spectroscopy. The transmission FT-IR spectra were recorded in the range of 4000-400  $\text{cm}^{-1}$  using KBr pellets method. Microstructure of carbon nanotubes was analyzed by SEM, Nova NanoSEM 200, FEI Co.

## *In vivo* experiments

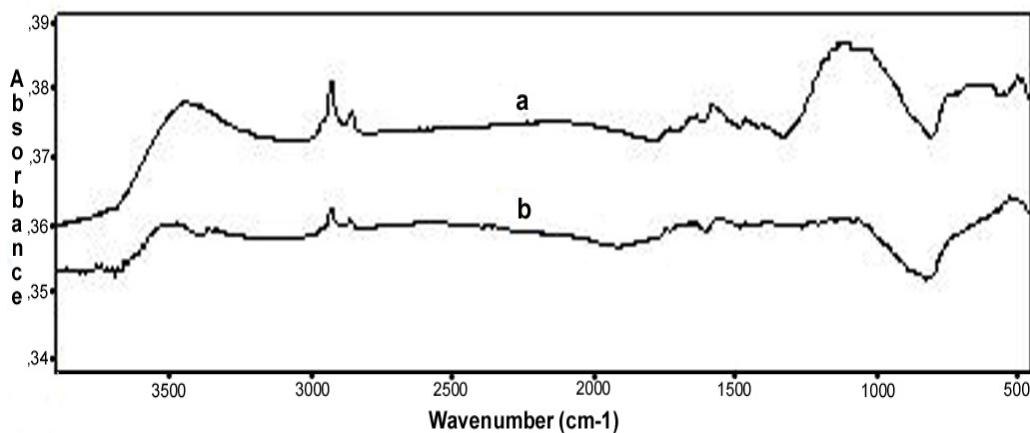
Carbon nanotubes were implanted into the skeletal muscle of rats. At 7, 30, 90 day from implantation the tissue specimens containing the implanted material were retrieved, and the prepared samples were frozen in liquid nitrogen and next cut into 8 $\mu\text{m}$  thick slides. To estimate the processes of tissue regeneration histological and histochemical reactions were carried out on the obtained slides.

## Results

### *Spectroscopy study*

FT-IR analysis provides information about presence of covalent functionalities in carbon materials (Figure 1). The major differences of IR spectrum are related to the bands attributed to carbon – oxygen and hydrogen bonds existing in

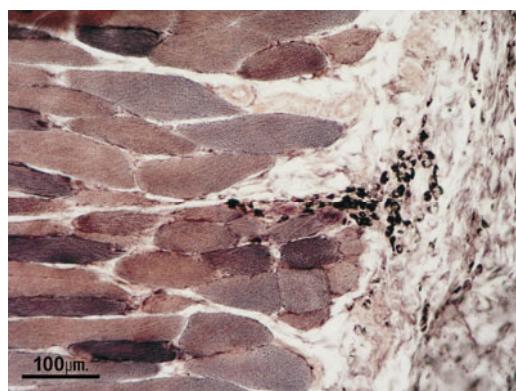
the structure of carbon nanotubes. A broad envelope of band in the range 800- 1200 cm<sup>-1</sup> is coming from C-O-H stretching vibration. Such groups make the SWNT -surface more hydrophilic than the surface of multi-walled carbon nanotubes (MWNT) which have probably hydrophobic character.



**Figure 1.** IR spectrum of SWNT (c- initial, a- after base line correction) and MWNT (d- initial, b- after base line correction)

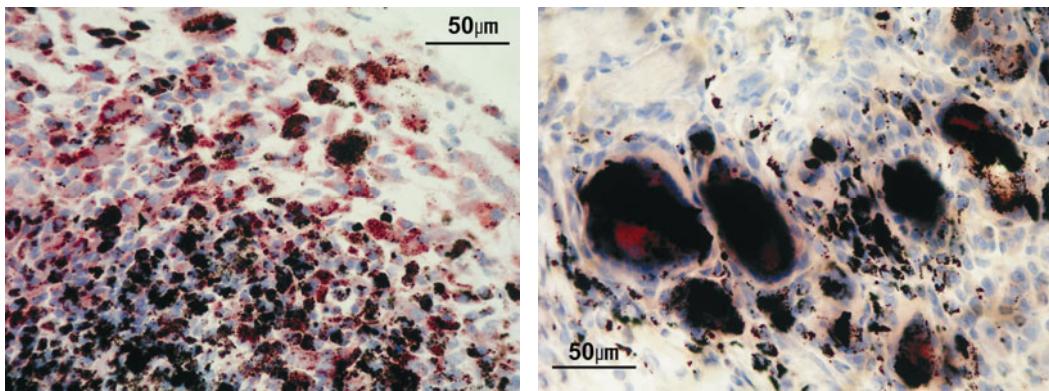
#### *In vivo experiments*

Our preliminary work indicates that both multi and single carbon nanotubes induce relatively fast tissue regeneration. Study on single and multi -walled CNTs revealed that enzymatic activity in muscle tissue surrounding carbon sample was proper, numerous regenerating muscle fibres and blood vessels were observed on the border and inside the sample (Figure 2). Muscles fibers with different activity of metabolic enzyme were observed in contact with carbon nanotubes.



**Figure 2.** Cross-section area of the muscle with nanotubes. Histochemical reaction: oxidase cytochrome C (OCC). Different activity of OCC enzyme in muscle fibers, regenerating muscle and blood vessels in contact with carbon nanotubes Magnification 20x.

Various forms of nanotubes in the site of implantation were observed: SWNTs were found in the form of well phagocitosed small clasters uniformly dispersed in the tissue (Figure 3), while MWNTs cumulated into large size aggregates (Figure 4). These aggregates were accompanied by abundant foreign body giant cells.



**Figure 3,4.** Cross-section areas of muscle containing SWNT and MWNT nanotubes.  
Histochemical reaction: acid phosphatase. SWNT in the form of small round particles. An irregular shape of MWNT agglomerates is seen. Magnification 40x.

## Conclusion

*In vivo* analysis indicated that two types of carbon nanotubes behaved in different way in tissue environment. SWNT were well dispersed in muscle tissue and easily phagocitosed by macrophages, probably due to more hydrophilic nature of SWNTs surface. With the time they were found in local lymph nodes. On the contrary, MWNT were found to form complex shape agglomerates surrounded by numerous multinucleated giant cells. Phagocytosis process was minimal and slow. Results of our *in vivo* experiments encourage to further careful study to assess the potential of carbon nanoforms in tissue regeneration process, and to explain the role of a such specific component in stimulating process of tissue remodeling.

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