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Synthesis and characteristics of Fe-filled multi-walled carbon nanotubes for biomedical application

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Abstract. Multifunctional nanocontainers can be produced based on partially filled Fe-multi walled carbon nanotubes (MWCNTs). Using thermal decomposition ferrocene filled nanotubes can be grown aligned on substrates. The encapsulated metal nanowires have diameters of 5-30nm and a length up to few microns. They consist of single-crystalline of α - and γ -Fe- phases. Using heat treatment, it is possible to transform γ -Fe into α -Fe. With the aid of wet chemical methods the nanotubes can be opened and additionally filled with an agent, e.g., therapeutic agents (carboplatin) or other metals (copper). Initial studies do not show a high toxicity over a period of 440 days. These materials can be used for drug delivery and hyperthermia. The specific absorption rate (SAR) is greater than $100\text{W}/(\text{g}\cdot\alpha\text{-Fe})$ in a magnetic field of $18\text{kA}/\text{m}$ ($f = 250\text{kHz}$).

1. Introduction

Fundamental discoveries of various species of carbon nanotubes (CNTs) have stimulated research into their applications including the field of human medicine [1-4]. The success of these applications depends significantly on the physical, chemical and biological properties of the CNT materials and their supplements. Here, we describe novel types of functionalized and partially ferromagnetic filled multi-walled CNTs (MWCNTs) with various possibilities for future application in human medicine. These structures represent multi-functional nano-scaled containers for possible use in various medical treatments e.g. drug delivery and hyperthermia. Such a container is schematically illustrated in figure 1. The main component is a MWCNT, partially filled with Fe as a ferromagnetic material, with an external diameter of 20-60nm and a length of 100-1000nm. The shell is made up of a pre-defined number of graphene layers (5-20). The material can be synthesized by a chemical vapour deposition (CVD) process using $\text{Fe}(\text{C}_5\text{H}_5)_2$ (ferrocene) as starting material and oxidized silicon wafer as substrate. After the CVD-growing procedure and an opening process, the MWCNTs can be filled with one or more agents e.g. therapeutic agent or other metals using wet chemical methods.

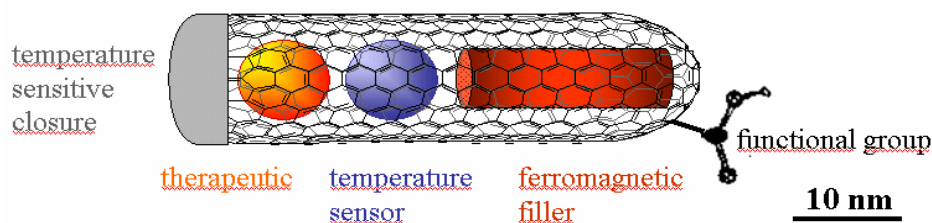


Figure 1. Schematic representation of the composition of a nanocontainer

2. Experimental

For the synthesis of partially filled and functionalised (ff)-MWCNTs, two different equipment are available. In the first apparatus the ff-MWCNTs are synthesized by catalytical decomposition of ferrocene in a quartz tube reactor inside a dual zone furnace as described by Leonhardt [5]. The main parameters are the sublimation temperature of the ferrocene in the first furnace ($T_{\text{subl.}} = 150^{\circ}\text{C}$), the carrier gas argon (120sccm) and the deposition temperature in the second furnace ($T_{\text{reac.}} = 830^{\circ}\text{C}$). The processes are carried out at atmospheric pressure and on oxidized Si-substrates, precoated with a thin Fe-layer.

The second CVD-equipment [6] consists of a continuously moving band evaporator and a hot wall reactor. It is equipped with a tape on which the oxidized and Fe-precoated Si-substrates are fixed. This tape can be moved through the hot zone of the reactor. The ferrocene is solved in cyclopentane and falls onto the moving band. In the first part of the band evaporator the solvent evaporated at a temperature of 45°C . In the second part of the process the ferrocene completely sublimates at 270°C and the final vapour can be transported in the CVD-reactor. At 900°C the precursor decomposes and ff-MWCNTs are deposited on the precoated substrates. The final step has the distinct advantage in the ability of continuous production of filled MWCNTs for high scale application.

The characterisation of the produced CNT material was performed by different techniques: i) analytical scanning (SEM, XL 30, Philips), ii) transmission electron microscopy (TEM; TECNAI F30 with GiF 200), iii) thermal gravimetric analysis (TGA, RUBOTHERM), iv) alternating gradient magnetometry (AGM).

Animal experiments were performed with different doses of ff-MWCNTs in solution and two ways of administration. The Fe-filled MWCNTs were injected into the mice intraperitoneally or intravenously. Histological and TEM studies were applied using standard techniques.

3. Results and discussion

3.1.1. Morphology, structure and chemical composition of the samples

By using both equipment, in dependence on the deposition conditions vary the length, structure, filling grade and the magnetic properties of the CNTs. For high resolution TEM (HRTEM) studies, MWCNTs were separated from the substrate and dispersed onto a copper grid. Figure 2(a) illustrates a typical TEM image of a partially Fe-filled MWCNT. The single-crystalline Fe-fillings have diameters of 5-30nm and lengths in the μm -range, depending on the technological parameters in both equipment. In most cases, the base-centered cubic (bcc) phase of iron ($\alpha\text{-Fe}$) is the main component. Sometimes, $\gamma\text{-Fe}$ (fcc) was detected. For biomedical application, the ferromagnetic bcc phase is the more important material. Using heat treatment, it is possible to transform completely the $\gamma\text{-Fe}$ into the $\alpha\text{-Fe}$. All nanowires are tightly wrapped by the C-shell, i.e., no gap between the two different materials was found.

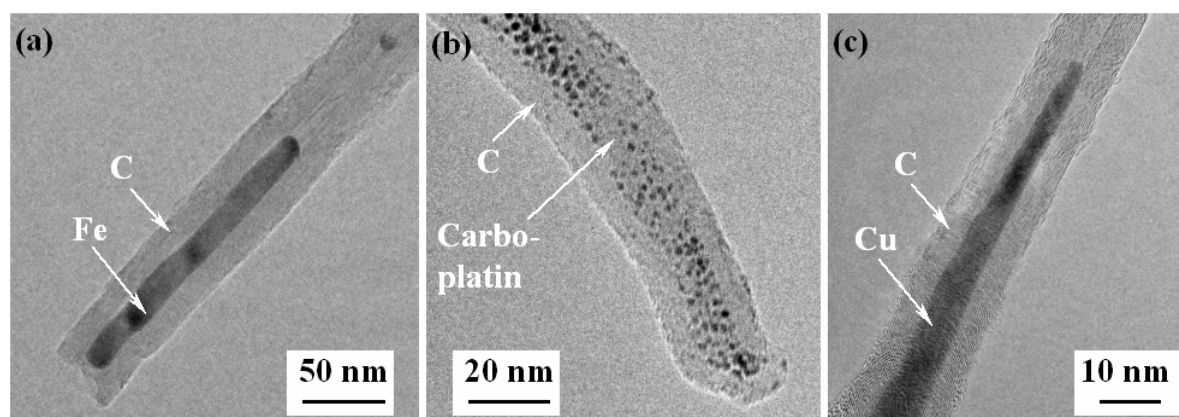


Figure 2. High resolution TEM images of (a) an opened MWCNT partially filled with Fe, (b) a MWCNT filled *ex-situ* with carboplatin and (c) a Cu-filled MWCNT.

3.1.2. *Ex-situ* filling with different agents (therapeutics and temperature sensors)

Figure 2(b) shows a MWCNT partially filled with a therapeutic agent (carboplatin). The *ex-situ* filling process i.e. a filling after the synthesis of partially filled MWCNTs and after the opening of the CNTs by an oxidation process, is based on the capillarity of nanotubes. By using a wet chemical technique, thermal treatment of a suspension of organic materials (therapeutic agents) and opened MWCNTs in a suitable solvent a partially filling can be reached. Filling with other organic materials and therapeutic agents is being investigated [7].

Materials which have temperature-sensitive NMR peaks and known shifts of these peaks with high temperatures can be used to measure the temperature inside the MWCNT. Copper could be such a material. MWCNTs can be filled by gas phase reaction with salts (copper (II) acetylacetonate, Cu jodide or PdBr_2). Subsequent hydrogen treatment at a pre-selected temperature will then reduce the compounds. Figure 2(c) shows a Cu-filled MWCNT.

3.1.3. Magnetic characteristics and heat generation

The magnetic characteristics i.e. saturation magnetization M_{sat} and coercivity H_c depend not only on the synthesis conditions and the γ -/ α -Fe ratio, but also on the degree of alignment, the diameter and the length of the MWCNTs on the substrate. It was possible to demonstrate values of $\mu_0 H_c > 130\text{mT}$ (aligned Fe-filled MWCNTs on substrates) [6]. This is significantly higher than the bulk material $\mu_0 H_{c\text{Fe}}$ of 0.09mT.

The measurements always reveal a uniaxial magnetic anisotropy with the natural easy axis parallel to the tube axis. For biomedical applications, the Fe-MWCNTs have to be removed from the substrate. Thereafter, the Fe-MWCNTs are no longer aligned and a random spatial tube orientation is produced. After transfer into cells, magnetic characteristics can no longer be determined for the spatial alignment. The coercivity is significantly lower than in the aligned arrangement. First experiments using the Fe-filled MWCNTs for hyperthermia were successful. Using calorimetric experiments the specific absorption rate (SAR) and specific power loss (SPL) were determined. Figure 3 shows the measured values (symbols) and the calculated dependence (line) based on the quasi static hysteresis loops.

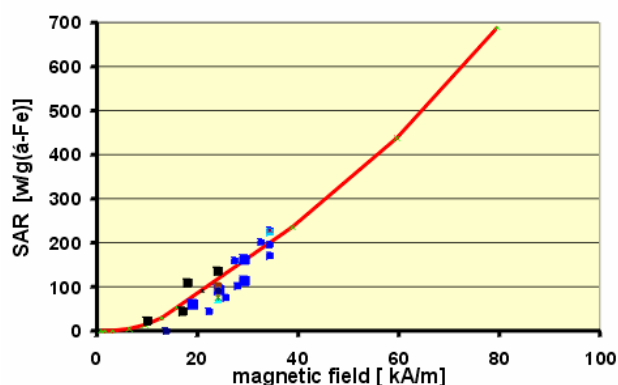


Figure 3. Variation of specific absorption rate (SAR_{Fe}) of Fe-MWCNTs with magnetic field.

The SAR-value was $> 100W/(g-\alpha-Fe)$ in a magnetic field of $18kA/m$ ($f = 230kHz$). Up to now, it was not been possible to measure significant SAR in magnetic fields $< 5kA/m$. The reason is the single domain status of the Fe-nanomagnets, determined by their geometric dimensions. To change the switching behaviours, the geometric dimensions have to be changed.

3.1.4. *In vitro and in vivo Delivery and toxicity studies for ff-MWCNTs*

Biocompatibility is a major concern when introducing a therapeutic agent inside the human body. The main goal of these studies was to investigate the accumulation and distribution of the containers within the body. In addition, potential *in vivo* toxic effects after single or multiple administrations were assessed *via* histopathological investigations.

Recent results of both TEM and light microscopy studies indicate that ff-MWCNTs aggregate to form clusters, independent of the localization site *in vivo* [4]. The aggregation can even be observed macroscopically after sonication and during injection. The clusters detected by TEM range from 0.5 to several microns. Thus, they reach different tissues by diffusing through capillaries. The structure of these clusters is identical to those found in cell culture experiments. Based on the calculations, intracellular clusters may consist of several hundreds to thousands individual ff-MWCNTs. Remarkably, the tendency to accumulate in different tissues and the formation of such large clusters did not appear to have any adverse influence on the health and survival of the animals. All animals ($n = 20$) injected once or more times with doses of approximately $100-400\mu g$ ff-MWCNTs per injection survived more than one year yet without any indication of toxic or other adverse events. Analysis of the body weight indicated no adverse influence of the administration of ff-MWCNTs on the health condition.

In several animals injected once with one of two types of ff-MWCNTs tested the CNTs were detected within the cell types of different tissues and organs including lung, heart, liver, and colon [4]. Several animals were sacrificed 6 weeks after the first injections and analyzed by an experienced pathologist. In the majority of cases, ff-MWCNTs were found to be cell-associated at a period of 6 weeks after the first injection. In several microphotographs, the detection of ff-MWCNTs was associated with the presence of macrophages. No formation of giant cells or granuloma was detected, indicating the absence of inflammation. The unrestricted localization of bundles and clusters of ff-MWCNTs was not associated with encapsulation of the accumulated tubes within the different tissues and organs. In lung tissue specimens, the clusters were found near the alveolae and beside microvessels. Interestingly, after several cases of intraperitoneal administration MWCNTs were also detected in the lung but in significantly lower amounts and numbers than with intravenous administration [4].

The histological characteristics of the accumulated ff-MWCNTs within tissues and organs demonstrate the biocompatibility of this compound over several weeks without dramatic toxicity. The comparable patterns of accumulation after different durations post-injection treated animals show that

the injected ff-MWCNTs can distribute and accumulate within hours and then persist within these depot tissues without encapsulation or inflammatory reactions.

4. Conclusions

This work describes the production and properties of partially filled MWCNT as main component of a multifunctional nanocontainer. The influence of the technological parameters on the phase composition, the magnetic characteristics, and the morphology were studied. The most important filler is the ferromagnetic α -Fe. A special annealing treatment of the samples causes a distinct increase in the quantity of α -Fe. An *ex-situ* filling with therapeutic drugs and other agents is possible.

The magnetic characteristics (saturation magnetization M_{sat} , coercivity H_c) depend not only on the synthesis conditions and the γ -/ α -Fe ratio but also on the degree of alignment, the diameter and the length of the MWCNTs on the substrate. It was possible to demonstrate values of $\mu_0 H_c > 130\text{mT}$ (aligned Fe-filled MWCNTs on substrates). This is significantly higher than the bulk material $\mu_0 H_{c\text{Fe}}$ of 0.09mT.

In cell culture experiments an efficient delivery of the MWCNTs into human cancer cell lines was detected. In a pilot animal study (mice), the biocompatibility was tested. The Fe-filled MWCNTs were injected into the mice intraperitoneally or intravenously. One mouse was injected 5 times over a period of 3 months with a total of $> 1\text{g}$ Fe-filled MWCNTs/kg body weight. In a follow-up study, treated and untreated animals were observed over a period of more than one year. All treated animals survived and showed no abnormalities. This suggests a general biocompatibility of the nanocontainers for the applied doses after both routes of administration in solution.

Initial experiments using the Fe-filled MWCNTs for hyperthermia were successful. Using calorimetric experiments the specific absorption rate (SAR) and specific power loss (SPL) were respectively determined. The SAR was $> 100\text{W}/(\text{g}\cdot\alpha\text{-Fe})$ in a magnetic field of 18kA/m ($f = 230\text{kHz}$). These results indicate that partially filled MWCNTs can be used as multifunctional nanocontainers in biomedicine [1,2].

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