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Materials Science and Engineering C

Effect of iron-doped multi-walled carbon nanotubes on lipid model and cellular plasma membranes

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A B S T R A C T
The aim of the present work was the study of the interaction of multi-walled carbon nanotubes filled with iron (Fe-MWCNTs) with bimolecular lipid model membrane (BLM) and cellular plasma membrane (PM). The findings demonstrate that the Fe-MWCNTs adsorb on the BLM surface with possible partial build up in the hydrophobic area of fatty acid residues of lipids and increase its specific conductivity and capacity. Furthermore, upon interaction with the PM, the Fe-MWCNTs form channels which allow the flow of water to the cells and the externalization of phosphatidylserine from the inner to the outer PM leaflet.

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1. Introduction
Carbon nanotubes (CNTs) [1] exhibit unique physical properties [2,3]. In particular, they are chemically and thermally stable, possess high mechanical strength, thermal and electrical conductivity and have a large specific surface area. It has been found that pristine CNTs – being intrinsically nonmagnetic – reveal a considerable giant magnetoresistance (GMR) effect [4]. It is quite obvious that modification of CNTs (intercalation or filling the internal cavities with different elements) should lead to significant differences in their electronic structure and properties. Although metallic impurities inside CNTs do not contribute as a source of the magnetic signal, they can play a role as a mechanism for enhancing the intrinsic ferromagnetic response of carbon. Functionalized CNTs exhibit unique biological activity both in vitro and in vivo [5,6], opening up a wide range of possibilities for their use in nanomedicine. In particular, the novel paradigm of treating cancer with magnetic hyperthermia using multi-walled CNTs (MWCNTs) filled with iron (Fe-MWCNTs) can be considered. Recent results on the study of temperature dependences of magnetic susceptibility of Fe-MWCNTs [7] indicate that such nanostructures are magnetically solid ferromagnets at room temperature. Incorporating iron nanoparticles into the MWCNTs will allow for the management of their mobility by an external magnetic field. Application of the electromagnetic field will make the heating of iron nanoparticles kept by the tumor possible, destroying cancer cells as a result of thermolysis [8]. However, for potential applications in nanobiotechnology, the study of the interaction mechanism between the Fe-MWCNTs and the lipid matrix of the cell membrane is required. Model bimolecular lipid membranes (BLMs) and cell plasma membranes (PMs) were used in this study. The influence of Fe-MWCNTs, present in the water suspension at low concentrations (10⁻⁶–10⁻⁷ mg/ml), on the specific conductivity and capacity of a BLM model was examined.

2. Materials and methods
Chemical vapor deposition was used to produce MWCNTs filled with iron [7]. According to the electron-microscopic results, the inner diameter of the MWCNT is 5–8 nm, whereas the outer diameter is up to 80 nm, and the length is up to 1 μm. The MWCNTs contain iron clusters with average diameters of 10–15 nm and lengths of up to 140 nm.

Water suspension of MWCNTs filled with iron was prepared as follows. The Fe-MWCNT samples were first washed with diluted HCl (0.1 mol/l) to remove iron located on the outside of the nanotubes. After centrifugation, the nanotubes were washed several times with de-ionized water. The air-dried samples were heated at 100 °C for 0.5 h under vacuum to degas absorbed carbon dioxide and water. To...
produce water suspension in a typical experiment, 100 mg of Fe-MWCNTs was stirred in 50.0 ml water under argon for 48 h, and sonicated for 1 h. More prolonged stirring was required to allow a fraction of the solid Fe-MWCNTs to detach in the water solution. The mixture was filtered through a membrane (pore size of 1.2 μm). The Fe-MWCNTs were collected on the membrane. The filtrate possessed a brown color and contained a small amount (0.1 mg/ml) of short Fe-MWCNTs. The partial solubility of Fe-MWCNTs in water is due to some surface carboxyl groups on the Fe-MWCNTs. The content of carboxyl groups (<3 wt.%) was recorded by X-ray photoelectron spectroscopy. The highest concentration of Fe-MWCNTs in water suspension was 1 mg/ml.

The state of Fe-MWCNTs in water was monitored using an AFM technique (“Solver Pro M” system; NT-MDT, Russia). Samples were deposited onto cleaved mica substrates by precipitation from an aqueous solution droplet. AFM measurements were performed after complete evaporation of the solvent. Sample visualization was carried out in semicontact (tapping) mode. NSG10 (NT-MDT) probes were used.

The planar model of the BLM was prepared according to the Mueller method [9] from phosphatidylcholine (23 mg/ml in n-decane) in an electrolyte solution (0.1 M KCl, t = 23 ± 1 °C). The formation of the BLM was controlled by visual (MBS-2, Russia) and potentiometric methods. The area of the BLM in the experiment was ~3.6 × 10⁻⁸ m². Electrical parameters of the BLM (the specific conductivity G and capacity C) were measured by means of non-steady cyclic current–voltage characteristics [10]. The signal registration was performed 10 min after the introduction of Fe-MWCNTs in the BLM electrolyte washing solution (pH ≈ 6.5). The obtained data are presented in the form of relative changes in the conductivity G/Gₒ and capacity C/Cₒ, where Gₒ = 51.19 ± 2.34 × 10⁻⁴ S·m² and Cₒ = 0.69 ± 0.04 × 10⁻² F/m² are the electrical parameters of the non-modified BLM (control). The values of G/Gₒ and C/Cₒ were determined using a system of equations proposed in [10].

Human cervical adenocarcinoma HeLa cells were cultured in the RPMI 1640 culture medium supplemented with 4 mM L-glutamine, 10 mM HEPES buffer, 100 U/ml penicillin and 100 μg/ml streptomycin (all reagents were obtained from PAA, Pasching, Germany). RPMI 1640 was further supplemented with 10% (v/v) heat inactivated fetal calf serum (FCS) (Gibco-BRL, Eggenstein, Germany). Cell viability was assessed by a trypan blue exclusion test. Cells were cultured in 5% CO₂ at 37 °C. For the detection of phosphatidylserine (PS) exposure on the cell surface, staining with FITC conjugated Annexin V (AnV-FITC, Böhringer, Mannheim, Germany) was performed. Cells grown on a 24 × 24 mm slide chamber were washed twice with 1 × Ringer’s solution and placed in the reaction medium — 200 μl of Ringer’s solution with 100 ng of AnV-FITC [11]. Fluorescent microscopy was performed by preparing temporal slides for analysis on a Zeiss Axiolmager A1 upright microscope equipped with a Zeiss AxioCam.

Fig. 1. AFM image of MWCNTs filled with iron on mica (tapping mode).
3. Results and discussion

The AFM picture in Fig. 1a demonstrates threadlike objects corresponding to the individual Fe-MWCNTs with diameters of 10–80 nm and lengths of up to 5 μm. These objects have thickenings (Fig. 1b), which, in our opinion, correspond to iron nanoparticles inside the MWCNT. In some AFM images the presence of individual spots larger than 80 nm size was seen. These are apparently aggregates (bundles) of the Fe-MWCNT in water suspension.

A small decrease in BLM conductivity, in the background of the increasing value of its capacity, was observed at low concentrations of Fe-MWCNTs (10⁻⁴–10⁻² mg/ml). A further increase of the concentration of these metal nanoparticles leads to an increase in the value of BLM saltatory conductivity with its subsequent breakdown (Fig. 2a). The resulting concentration dependence can be explained as follows. At low concentrations, an adsorption of Fe-MWCNTs on the BLM surface (with possible partial build up in the hydrophobic area of fatty acid residues of lipids) takes place. Interaction of the above nanomaterial with the cell surface membrane leads to a partial deformation of its structure (compression), as demonstrated by a reduction in the thickness of the BLM with increasing concentration of the Fe-MWCNTs (Fig. 2b). Increasing the concentration of the investigated nanoparticles on the surface of the BLM further leads to their intercalation into the membrane structure and its destruction.

To prove this hypothesis, we incubated HeLa cells with Fe-MWCNTs at a concentration of 0.05 μg/ml. It was taken into account that this incubation was performed in slide chambers, and the local concentration of the sample was considered to be rather high. The exposure of PS on the outer surface of the PM was studied by specific binding of this phospholipid with annexin V [12]. It is known that the PM is asymmetrical and PS is located in the inner PM leaflet [13]. A loss of PM asymmetry during cell death (apoptosis or necrosis) leads to PS exposure and this test is utilized as a specific marker of cell death by both immune cells using their receptors and in the diagnostic purposes [14]. Time-lapse microscopy of HeLa cells treated with Fe-MWCNTs revealed an increased exposure of PS on the cell surface as early as 1 min after contact with sample, with PS expression being prominent after 7 min of exposure (Fig. 3). Moreover, upon exposure to Fe-MWCNTs, HeLa cells markedly increased their volume, most likely due to their swelling. Application of high resolution DIC microscopy demonstrated the exposure of PS in the places of contact of long Fe-MWCNTs (> 3 μm, visible under 1.4 NA DIC optics, total magnification ~1600 times) with the PM (Fig. 4). Thus, the Fe-MWCNTs are probably capable of intercalating into the PM and forming a channel which allows the income of water (judged by cell swelling) and externalization of PS from the inner to the outer layer of the cell membrane.

4. Conclusion

It was shown that multi-walled iron-filled carbon nanotubes with a diameter of 10–80 nm and a length of up to 5 μm, present in the water suspension in the concentration range of 10⁻⁴–10⁻² mg/ml [1] interact with a bimolecular lipid model membrane, increasing its specific conductivity and capacity, and (2) interact with cellular plasma membrane, forming the channels which allow the income of water to the cells and externalization of phosphatidylerine from the inner to the outer plasma membrane leaflet.

In summary, the demonstrated potential of the cell membrane to incorporate Fe-MWCNTs is of high potential interest with respect to future application of these nanosystems in the magnetic hyperthermia therapy of cancer [15].

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References
