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The study of the interaction of human mesenchymal stem cells and monocytes/macrophages with single-walled carbon nanotube films

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1 Introduction

Due to their unique properties, the application of single-walled carbon nanotubes (SWCNT) is explored for many areas including biomedical engineering, drug delivery systems and medical chemistry. Thus the addressing of toxicological issues of SWCNT is of interest with regard to safety and the evaluation of ecological and health risks as an important prerequisite for any application of this material.

Despite the general high interest in the topic, little literature data exists concerning the biological properties and biotoxicity of carbon nanotubes up to now [1]. The studies are mostly focused on inhalation toxicology [2–4] or on effects of skin exposure [5, 6]. There are also studies on implanted carbon nanotubes [7] as well as *in vitro* studies concerning the influence of SWCNT on different cell cultures (endothelial cells [8], macrophages [9] or osteoblasts [10]).

Most of the studied carbon nanotube samples up to now are complex mixtures containing a large variety of impurities. Thus in many cases the observed toxicity may arise from by-products of carbon nanotube synthesis rather than from the carbon nanotube material itself. In the present study we tested high quality SWCNT films with regard to cellular metabolic activity, showing that such a high quality material occasions only a slight decrease in metabolic activity. According to Flauhaut et al. [8] the reduction of cell metabolic activity by less than 25% should not be regarded as a toxic effect of SWCNT.

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In this study we cultured two cell types on SWCNT films – human mesenchymal stem cells (adherent cells) and monocytes/macrophages (suspension cell line). Mesenchymal stem cells are thought to be multipotent cells. They are present in adult marrow, can replicate as undifferentiated cells and have the potential to differentiate to lineages of mesenchymal tissues, including bone, cartilage, fat, tendon, muscle and marrow stroma [11] as a response to different stimuli. Thus they can serve as a model for a large variety of cells. Macrophages can be derived from monocytes that normally circulate in the peripheral blood. In response to cytokines or other signals, monocytes will leave the circulation and enter a tissue where they can differentiate into macrophages. Macrophages then perform many functions including phagocytosis and the destruction of invading pathogens, release of cytokines and inflammatory mediators, and presentation of antigens [12]. Macrophage interaction with materials (implants, particulate debris) exposed in the body is thought to involve their activation, and the secretion of a variety of soluble mediators such as radicals (e.g. superoxide anions), cytokines (e.g. TNFα) and eicosanoids (prostaglandin E_2) [13] at the implantation site. These molecules can mediate immune and inflammatory processes as well as modulate cell differentiation and proliferation.

2 Materials and methods

Mesenchymal stem cells (MSC) (primary cells) were obtained from healthy Caucasian bone marrow donors at the Bone Marrow Transplantation Center of the University Hospital Carl Gustav Carus, Dresden. MSC were isolated and cultured according to previously reported method [14]. Cells were maintained in DMEM (Biochrom) supplemented with 10% heat inactivated fetal bovine serum (Biowest), penicillin and streptomycin. Monocytes/macrophages (THP-1) cells were purchased from the DSMZ and maintained in RPMI-1640 (Biochrom) supplemented with 10% heat inactivated fetal bovine serum (Biowest), penicillin and streptomycin.

Cells were plated on tissue culture plastic (polystyrene-PS, Nunc) covered with a SWCNT film. The SWCNT, which were available from our previous work [15] were suspended in ethanol and sonicated for 10 min. Either 5 µl or 25 µl drops were placed onto the PS support and left to dry, which resulted in a coverage greater than approximately 75% of the well area with dried SWCNT residue. The carbon fiber (CF) discs (Sigratherm PE 715, SIGRI) were cut out of a thin carbon paper sheet in such a way that they fitted into the wells of a 24 well plate well.

Metabolic activity tests based on the activity of the mitochondria (MTS – CellTiter 96 AQueous One Solution Cell Proliferation Assay – Promega) were performed according to the provided protocol (briefly, the absorbance of soluble formazan formed by metabolically active cellular dehydrogenases was measured). The results are presented as difference in % between control (cells on PS) and cells on SWCNT or CF, respectively. For statistical analysis the ANOVA was used. Cell number was estimated using trypan blue staining, and found to be identical for cells on PS and on SWCNT films at the time point when the metabolic activity tests were conducted.

Raman spectra were measured on a T-64000 spectrometer (Instruments, SA) interfaced to a microscope Olympus BH2 (the laser power impinging on the sample was in the range of 1–5 mW). Spectra were excited by Ar^+ laser at 2.41 eV (Innova 305, Coherent). The UV-VIS-NIR spectra were recorded on a double-beam Shimadzu 3100 spectrometer. The spectra were measured from SWCNT films deposited on optical glass. Optical densities were normalized against the clean glass substrate of the same quality.

3 Results and discussion

3.1 Characterization of SWCNT material

To exclude the influence of impurities in SWCNT on the results in our study, we checked the quality of the material by Raman and UV-VIS-NIR spectroscopy (not shown). The ratio of D/TG (D: disorder mode, TG: tangential mode) lines in Raman spectra is often taken as a criterion of the purity of samples, and the D/TG value of 0.009 confirms the high purity of the SWCNT used. The UV-VIS-NIR spectra

showed three well resolved peaks, which again indicate the high purity of the SWCNT (For a more detailed report on the characteristics of the SWCNT samples see Ref. [16]). Furthermore, we measured the Raman spectra of SWCNT also after experiments with cells where we have found the TG mode to be slightly shifted compared to pristine SWCNT. However, the same shift of TG was observed if the SWCNT films were treated only with cell culture medium. This indicates that there is a charge transfer between medium and SWCNT films. On the other hand the interactions with the cell proteins do not lead to the change of electronic structure of SWCNT.

3.2 Metabolic activity of cells cultured on SWCNT films

The function of mitochondria (energy producing machinery within the cell) is usually examined as one of the first markers of affected cell behavior. To evaluate the biocompatibility of this material we thus determined the metabolic activity of dehydrogenases as an indicator of mitochondrial function in different cell types plated on SWCNT films. Figure 1 shows the reduction in activity for mesenchymal stem cells (MSC) and monocytes/macrophages (THP-1) after 48 h incubation on SWCNT film compared to cells cultured on polystyrene (PS). The metabolic activity was measured at three different cellular densities for both cell types used, as a result of their different size and character. The middle bar gives the value for the optimal cell number used as a standard in experiments with these particular cells. We also tested cell number above and below the optimum to evaluate the dependence of toxicity on cell density, which is shown in the left and right bars, respectively. In all cases, the decrease of cell metabolic activity is relatively low. This is in agreement with results obtained by Fiorito [9]. The maximum decrease of metabolic activity (25%) was observed at the lowest concentration of the cells e.g. 6000 for MSC (subconfluent) and 15000 for THP-1 (sub-saturated). For higher cell numbers the metabolic activity is reduced to a lesser degree: a decrease of 15% was found for MSC at 15000 (confluent) and of 5% for 78000 of THP-1 (saturated). It indicates that the cells at higher density in well were not affected as much as cells at lower density. In the case of adherent cells, this may be due to the fact that at higher cell numbers the SWCNT films limited surface area is fully covered by part of the cells, leaving another part untouched, though the uptake of released particles cannot be excluded.

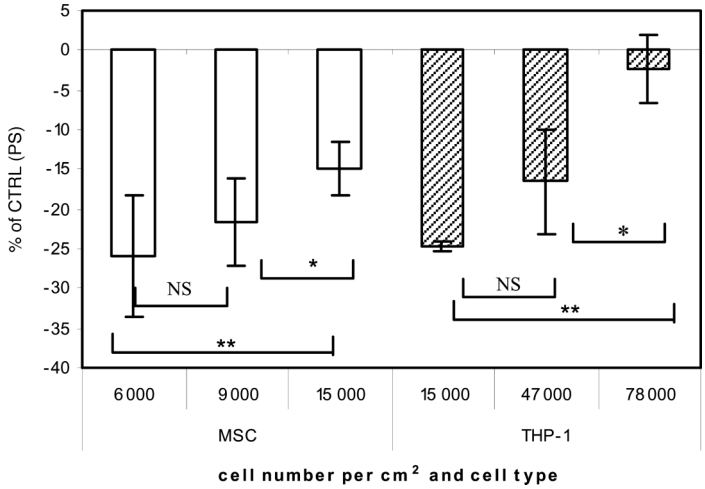

Fig. 1 Metabolic activity of mesenchymal stem cells (MSC) and monocytes/macrophages (THP-1) after 48 h incubation on SWCNT film (7.8 μg/cm^2) correlated to the metabolic activity of cells on tissue-culture polystyrene (PS). Cells were cultured in 96-well plates at different cell densities. The graphs represent means of 3 independent experiments performed in triplicate ± S.E.M. * Significant difference $p < 0.05$; ** significant difference $p < 0.01$.

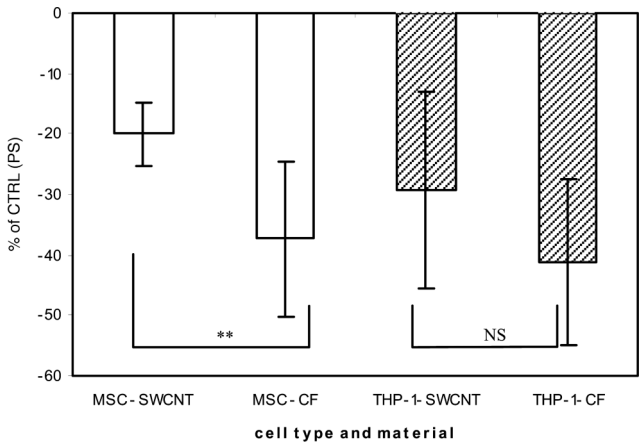

Fig. 2 Metabolic activity of mesenchymal stem cells (MSC – 10 000 cells/cm^2) and monocytes/macrophages (THP-1 – 25 000 cells/cm^2) after 48 h incubation on SWCNT (6.25 µg/cm^2) and carbon fibers in 24-well plates. The graphs represent means of 3 independent experiments performed in triplicate ± S.E.M. ** Significant difference $p < 0.01$.

3.3 Metabolic activity of cells cultured on SWCNT and carbon fibers (CF)

In order to study the interaction of different cell types with other carbon materials, we plated mesenchymal stem cells and monocytes/macrophages on carbon fibers. Figure 2 shows the metabolic activity of MSC and THP-1 after 48 h incubation on SWCNT and carbon fibers compared to on tissue culture plastic. It is obvious that the decrease of metabolic activity was much stronger for carbon fibers than for SWCNT films. In the case of carbon fibers, the activity was reduced by approximately 40% for both cell types, while the reduction of metabolic activity on SWCNT film was only about 20% for MSC and 30% for THP-1 cells. Without further investigation we cannot decide whether this apparent higher toxicity of carbon fibers is caused by the micro-structure of the CF surface or by the release of constituents of the carbon fiber paper.

4 Conclusions

SWCNT and carbon fibers reduce the metabolic activity of both investigated cell types in different ways, as the reduction is more pronounced for carbon fibers. The high quality SWCNT films are apparently not toxic for the cell types under the conditions used. The observed reductions of metabolic activities can either be occasioned by the surface morphology of the carbon material to which the adherent cells in our system respond, or due to particles released from SWCNT films which may be taken up by both cell types.

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