Effects of single-walled carbon nanotubes on primary mixed neuro-glial cultures from chicken spinal cord and dorsal root ganglia

C. Hirsch*, L. Belyanskaya*, S. Weigel[§], U. Tobler*, H.F. Krug*, P. Wick*

*Empa, Swiss Federal Laboratories for Materials Testing and Research, Laboratory for Materials-Biology Interactions, CH-9014 St. Gallen, Switzerland
[§]Technical University of Munich, Chair of Zoology, Freising-Weihenstephan, Germany

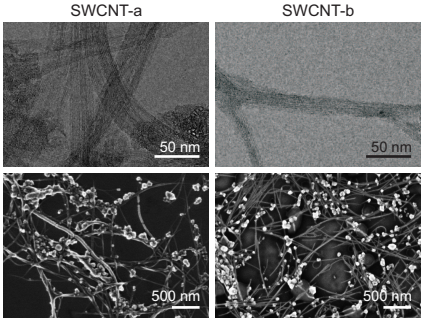
Introduction

The unique physical and chemical properties of carbon nanotubes (CNTs) suggest enormous potential for many areas of research and application. The increasing use of CNTs in consumer products and medical applications lays emphasis on the importance of understanding their potential toxic effects on human health and the environment. Even though it has been demonstrated that nanosized carbon particles can be taken up by olfactory neurons and are translocated to the brain, thus making neurotoxicity of CNTs an important issue, there is still only limited knowledge about possible neurotoxic effects of CNTs.

In this study we analyzed the effects of single-walled CNTs (SWCNTs) with different degrees of agglomeration on primary mixed neuro-glial cultures as well as on glia-enriched cultures. The cells were isolated from embryonic chicken ventral spinal cord (SPC) or dorsal root ganglia (DRG). This allowed us to differentiate between effects on cells derived from a central (CNS) or peripheral nervous system (PNS) tissue, respectively. Electrophysiological properties of neurons from both areas of the nervous system were assessed by whole-cell patch-clamp recordings.

Materials

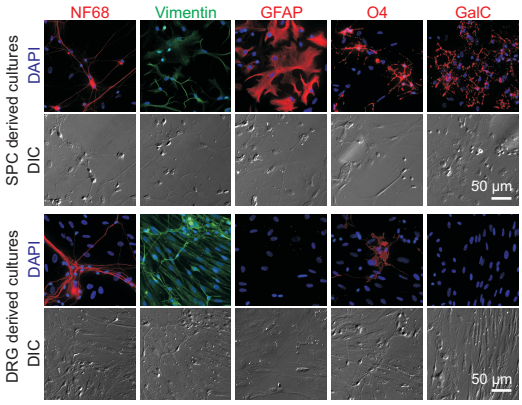
Arc discharged SWCNT raw material was purchased from Yangtze Nanotechnology, Shanghai, carefully characterized (Wick et al. 2007) and suspended in 20 µg/ml polyoxyethylene sorbitan monooleate (PS80).



TEM (upper panel) and SEM (lower panel) images of agglomerated SWCNTs (SWCNT-a) and less agglomerated SWCNT bundles (SWCNT-b).

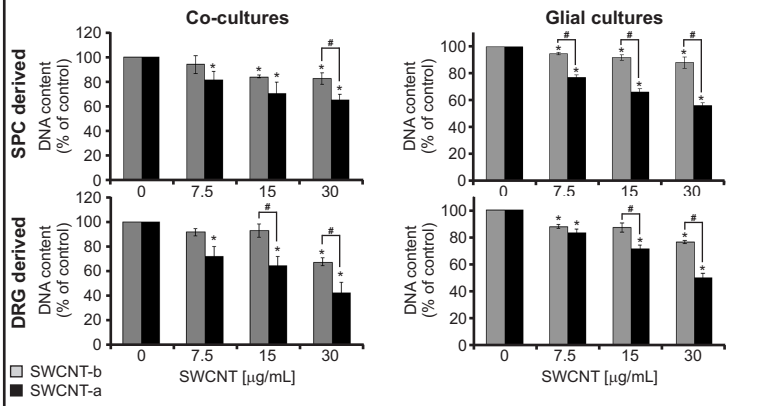
Primary cell culture:
Mixed neuro-glial cultures from ventral SPC and DRG were grown on Poly-D-Lysin/Laminin coated coverslips or cell culture plates in modified minimal essential medium (MMEM).

Culture composition was analyzed by **indirect immunofluorescence** using antibodies against NF68 (neurons), Vimentin (glial cells), GFAP (astrocytes), O4 (Oligodendrocytes, sub-population of Schwann cells) and GalC (Oligodendrocytes). Nuclei were counterstained with DAPI (blue).



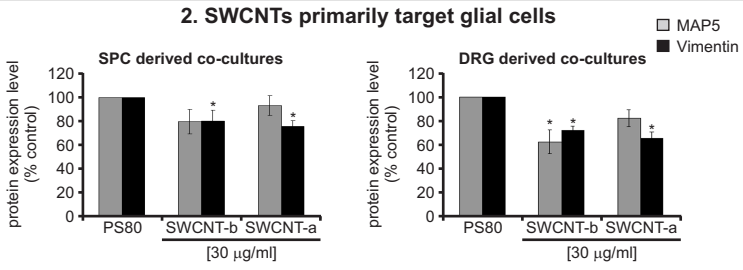
Results

1. SWCNTs reduce the total DNA content of primary mixed neuro-glial cultures as well as of glia-enriched cultures



*P<0.05 compared to control (0 µg/ml SWCNT); #P<0.05 comparing SWCNT-b and SWCNT-a treatment; n=5
DNA assay: Cell culture density after six days of SWCNT exposure was assessed using the Hoechst 33258 assay.

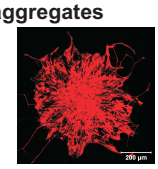
2. SWCNTs primarily target glial cells



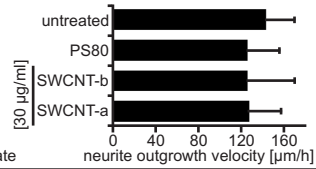
*P<0.05: compared to control (0 µg/ml SWCNT); n=4
ELISA: Cytotoxic effects of SWCNTs on neurons or glial cells were studied after six days of exposure using MAP5 and Vimentin specific enzyme linked immunosorbant assays, respectively.

3. SWCNTs do not influence the velocity of neurite outgrowth from SPC derived reaggregates

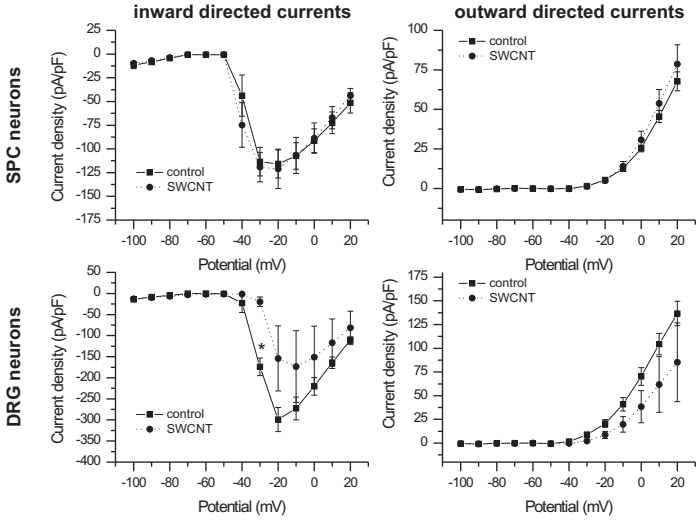
Neurite outgrowth was measured according to Osterwalder, T. et al.



SPC derived reaggregate



4. SWCNTs affect electrophysiological properties of DRG but not SPC derived neurons



Electrophysiological properties were assessed by whole-cell patch-clamp recordings.

Conclusions & Outlook

1. SWCNTs significantly reduce the total DNA content of mixed neuro-glial cultures; this effect is independent of cell origin but more pronounced with increasing agglomeration of SWCNTs.

2. While SWCNTs primarily target glial cells in DRG as well as spinal cord derived cultures, only DRG derived MAP5 positive neurons are affected by SWCNT-b.

3. SWCNTs do not influence the neurite outgrowth of spinal cord derived neurons.

4. In accordance with (2.) and (3.) electrophysiological analysis reveal no influence of SWCNTs on SPC derived neurons, but a change in the resting membrane potential and the ionic conductance of DRG neurons.

In summary, our findings suggest that SWCNTs can have acute adverse effects on glial cells and - depending on the tissue type they originate from - also on neuronal cells, even though we cannot rule out that neurons might be affected secondarily.

These results necessitate further investigations to address the detailed mechanisms of nanoparticle induced toxicity in glial cells and neurons.

References:
Wick et al., 2007, Toxicol Lett. 168 (2): 121-31
Osterwalder et al., 2008, Biomaterials, submitted

Contact: cordula.hirsch@empa.ch