Toxicity and Imaging of Chemically Modified Carbon Nanotubes

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Toxicity and imaging of chemically modified carbon nanotubes

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DSL 2011
Carbon and Oxide Nanostructures
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Advanced Carbon Nanostructures

Functionalisation and liquid crystallinity of nanotubes

Synthesis of carbon nanotubes: single, double and multiwalled nanotubes, aligned CNTs arrays, fibres, tapes and nitrogen doped CNTs

Chirality/diameter control

Carbon nanotube wires for power transmission lines

Carbon nanotube based sensors/detectors

Carbon nanotubes as wide range EM absorption coatings

Carbon nanotubes & cell interactions, bio interface

Carbon nanotube membranes for water filtration

Carbon-carbon composites for aircraft brakes (DUNLOP)

Battery, Supercapacitor, Thermoelectric devices

Align carbon nanotube multilayer composites

Carbon nanotube fillers for flame resistance
Types of CNTs used in my lab
Production methods of carbon nanotubes

- Methods
  - Arc-discharge
  - Laser ablation
  - Molten Salt
  - Chemical vapour deposition

- Key characteristics
  - Purity / Yield
  - Crystalline quality
  - Entanglements
  - Surface chemistry
  - Quantity

thermal CVD method
CNT types & structures synthesised in my group

1. Single-wall nanotube
2. Double-wall nanotubes
3. Multi-walled nanotube
4. Herringbone nanotubes
5. Nitrogen doped nanotubes

- Powder (3 reactors)
- Arrays (4 reactors)
- Films
- Fibres (3 reactors)
CNTs and N-doped CNTs arrays

CNTs arrays

50 - 500 x 10^6 nanotubes per 1 mm^2
CNTs arrays – Iron incorporation

Quartz substrate
CNTs arrays – Iron incorporation

Quartz substrate
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Quartz substrate
CNTs arrays – Iron incorporation

Quartz substrate
Off-axis electron holography

- 300 kV Philips CM300-ST
- Electron biprism used to overlap the electron wave passing through the sample with reference wave passing through vacuum
- Lorentz lens allows acquisition of holograms at higher magnification
- External magnetic fields applied with objective lens
Iron incorporation – analysis by electron holography

Chemical functionalisation
‘oxidation procedure’

MWNTs treated with a concentrated sulphuric/nitric acid mixture (proportion 3:1)

They form suspensions in water that are stable for months
Oxidised nanotubes in water, 0.01 %wt
Biomedical applications

Peptide/Nucleic acid/Drug delivery system

Glucose sensor: carbon nanotubes coated with glucose oxidase

Porous dialysis capillary

Toxicity of CNTs
Lung defence
Mucociliary clearance and macrophage phagocytosis

Clearance via the mucociliary escalator

CENTRI-ACINAR REGION
Slow clearing, delicate

Clearance by alveolar macrophage

Fast clearing, well protected

Courtesy of Ken Donaldson, Univ. Edinburgh
Macrophages can phagocytose many short fibres without problem and move them out of the lungs.

Ashed ghost of a macrophage from the lungs of a rat inhaling Code 100/475 fibre glass.
Carbon nanotubes introduced into the abdominal cavity of mice show asbestos-like pathogenicity in a pilot study.

Macrophages cannot completely phagocytose long fibres causing inflammation and impaired clearance.

1) Inflammation
2) Failure of normal motility results in an inability to clear the long fibres out of the lungs.

Proteases, oxidants, cytokines
CNTs powder form
Single photon emission tomography tracking of CNTs after intravenous administration in rats
Immune cells can deal with nanotubes

A molecular model of the myeloperoxidase enzyme 'eating' a nanotube

Myeloperoxidase is present in human immune cells called neutrophils. The myeloperoxidase enzyme uses hydrogen peroxide to produce hypochlorite and reactive radicals; a potent mixture that usually targets invading bacteria, but has now been found to degrade nanotubes and in the process release \( \text{CO}_2 \) gas.

V Kagan et al., Nature Nanotech, 2010
Carbon nanotubes used for the study

- Multi-walled carbon nanotubes (MWCNTs)  \( L = 250 \pm 120 \mu m, \ OD = 68 \pm 20 \ nm \)

- Nitrogen-doped multi-walled carbon nanotubes (N-MWCNTs)  \( L = 50 \pm 20 \mu m, \ OD = 55 \pm 20 \ nm \)

- Oxidised multi-walled carbon nanotubes (O-MWCNTs)  \( L = 0.82 \pm 0.41 \mu m, \ OD = 62 \pm 30 nm \)
Macrophages constitute a perfect model for initial toxicology studies as they are themselves a basic element of immunologic responsible for phagocytosis system and thus the most resistant to nanoparticles. Moreover, they are quite large and non-dividing cells.

Macrophages – phagocytes produced by the differentiation of monocytes. Their role is to phagocytose (engulf and then digest) cellular debris and pathogens either as stationary or as mobile cells,
Multiple approach to study the cell viability

- Viability assays:
  - Neutral Red Assay
  - Live/Dead Assay

- Imaging approach
  - SEM
  - DF-STEM
Neutral red (toluylene red, Basic Red 5, or C.I. 50040) is a phenazine-based vital stain, commonly used to stain living cells.

Relative assay measuring percentage of live cells in relation to a control sample.
Toxicity of modified CNTs to HMMs

Cells were incubated with the indicated concentrations of CNTs for 48 h.
Live/Dead Assay

1) **Dye Hoechst 33258**, the cell-permeable dye, was added 4 hrs prior to imaging to stain the nuclei of all cells.

2) **Propidium iodide** (PI), which is excluded by live cells, was added 10 minutes prior to imaging to stain the nuclei of dead cells.
Toxicity of modified CNTs to HMMs

Cells were incubated with the indicated concentrations of CNTs for 48 h.
Cellular uptake of MWCNTs

MWCNT is engulfed by one of the arm of the macrophage
Cellular uptake of $O$-MWCNTs

Incorporation of $O$-MWCNTs underneath the macrophage membrane

Macrophage in the nanotube environment
Cellular uptake of O-MWCNTs

O-MWCNT trapped within intracellular space

O-MWCNT ‘injecting’ itself into macrophage membrane

Biomaterials, Boncel et al, in press
Confocal microscopy studies

MWCNT

N-MWCNT

O-MWCNT
Conclusions

1. We used two different toxicity assays and imaging techniques to systematically and carefully determine cytotoxicity of different types of nanotubes.

2. The main property responsible for a slightly toxic action in the case of pristine CNTs is presumably their aspect ratio. This is in agreement with other research reports which have shown that longer nanotubes were more toxic than shorter.

3. Surface modification of nanotube walls results in practically non-toxicity. A lack of toxic action may occur due to the enhanced polarity and shorter dimensions of N-CNTs and O-CNTs.

4. Imaging by SEM, TEM and confocal microscopy revealed that all types of nanotubes can enter the cells.

Future requirements:
- Modelling of the nanotube transport through the cell membrane.
- Examining the type of interaction between CNT and the cell structures.
- Longer time exposures to bio systems.
Acknowledgements

Catharina Paukner, Sebastian Pattinson
Department of Materials Science & Metallurgy
University of Cambridge

Slawomir Boncel, Andrzej Gondela, Krzysztof Walczak
Department of Organic Chemistry, Biochemistry and Biotechnology,
Silesian University of Technology, Poland

Karin Müller, Jeremy Skepper
Multi-Imaging Centre, Department of Physiology, Development and Neuroscience, Anatomy School,
University of Cambridge

Funding:

THE ROYAL SOCIETY

Ministerstwo Nauki
i Szkolnictwa Wyższego
Thank You
Noorhana Yahya
ISN Vice President I
MALAYSIA

Krzysztof Koziol
ISN President
UNITED KINGDOM

Gregory Kozlowski
ISN Vice President II
UNITED STATES

Sameer Rahatekar
ISN Secretary
United Kingdom

Afza Shafie
Society Journal
MALAYSIA

Chong Fai Kait
Memberships
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Conferences
PHILIPINES
“First International Conference on Nanostructures: Synthesis, Characterisation and Application”

June 2012
University of Cambridge, United Kingdom