Toxicity Studies with Nanoparticles and Detection Methods for Pathology Evaluation

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Published Detection Methods

- Electron microscopy (TEM, SEM)
- Atomic force microscopy (AFM)
- dynamic light scattering (DLS)
- D-ray photoelectron spectroscopy (XPS)
- powder X-ray diffraction (XRD)
- Fourier transform infrared spectroscopy (FTIR)
- Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF)
- Ultraviolet-visible spectroscopy
- Rutherford backscattering spectrometry (RBS)
- Dual polarisation interferometry
- Nuclear magnetic resonance (NMR)

Learning from fiber-related pathology

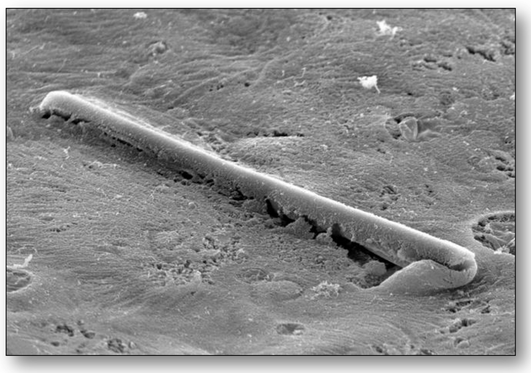


Fig. 1: Glas fiber in respiratory tract partly covered by surfactant (hamster). Electron microscopy: Marianne Geiser, Dept. Anatomy, University Bern

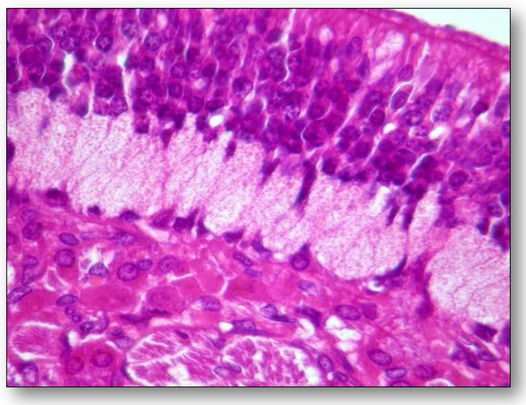


Fig. 2: Example nasal cavity: Intracellular material (carrier for hair spray) (Weber K, Carthew P, Schuler D: Polymers as Inhalation Carriers. Classic Examples in Toxicologic Pathology (4th Edition) Eds: Drommer W, Karbe E, Germann P

Introduction

Particles are considered small objects behaving as a whole unit with respect to its transport and properties. They are classified according to their diameters into

- Coarse particles: 2’500 - 10’000 nm
- Fine particles: 100 - 2’500 nm
- Nanoparticles (ultrafine particles): 1 - 100 nm

Furthermore, nanoclusters have at least one dimension of 1 - 10 nm and nanopowders consist of agglomerates of nanoparticles or nanoclusters.
Nanoparticles including nanotubes are currently under special considerations for their toxicologically relevant reactions. Hereby, a major issue is traceability in cells and tissues that is related to their size, elasticity and mechanical behavior. Especially the latter is of a big concern, e.g. nanotubes may be extremely flexible causing coils that may settle in cells, or nanoparticles with the potential of bio-adhesion that causes entering into mucus overlaying cell layers, getting endocytosed etc.

Materials and Methods

A positive control study was performed by inhalation. Fifteen RccHan™: WIST male rats were treated for 5 continuous days by inhalation at Vivotecnia S.L. Tres Cantos (Madrid) / Spain. The animals were treated daily for 6 continuous hrs per day. Control animals (group 1) received air only. Animals from groups 2 and 3 were treated with carbon nanotubes (CNT) at a concentration of 1 and 10 mg/m³, respectively. The test item was evaluated as free test item, on inhalation filters, as well as in tissues by different methods. Changes in functional and enzymatic parameters (alkaline phosphatasis (AP), gamma glutamyl transferase (GGT), lactate dehydrogenase (LDH)) were observed in bronchoalveolar lavage fluid (BALF). Respiratory organs underwent routine histology, i.e. paraffin embedded sections were stained by hematoxylin and eosin (HE).
Hyperspectral nanoscale analysis was applied to trace even minimal quantities within macrophages and in connective tissue in the previously evaluated sections of animals from inhalation studies. This was supported by EDX scanning and laser scanning microscopy on unstained paraffin sections, laser scanning microscopy on Epon embedded material, and by a new method called ‘dried thin tissue sections. Furthermore, energy dispersive X-ray spectroscopy analysis was performed on all available material. In addition, low temperature ashing was applied to regain nanomaterials in ashes to establish possible methods for quantifications.

Results and Discussion

The detection of nanoparticles may be very difficult and time consuming. According to the particle type, different sophisticated methods or a combination thereof may be applied. Most of these technologies have been approved for particles other than CNT (Figures 1, 2).
The CNT used in the internal validation study was characterized by the supplier using RAMAN analysis. This technique however was not applicable for the BALF and tissues distribution due to the composition of the used CNT by C >98%. Similarly, all further trials with EDX did not reveal convincing results by the same reason (Figures 8-11). For other types of CNT, i.e. those that are composed by C and other elements, the technology could be used as a powerful tool. The test item was morphologically characterized by LEXT (Figures 12-14). First results were obtained by a dose-dependent increase in lung weight and increased AP, GGT and LDH in BALF (Figure 3). BALF analysis revealed the presence of CNT-laden macrophages (Figures 4, 15). The particles destroyed partially overloaded macrophages (Figures 15-17).
Hyperspectral nanoscale analysis however, was useful to trace even minimal quantities within macrophages and in connective tissue in the previously evaluated sections of animals from inhalation studies stained by HE (Figures 5-7). However, this technology is time consuming due to the fact that C is absorbing light, and hence, detection can be performed only at high energy charging intensities.

Conclusion

There is no single detection method to evaluate the distribution of nanoparticles within tissues. Electron, laser scanning microscopy, and hyperspectral nanoscale analysis are most promising tools. Depending on the composition, EDX analysis may be a useful tool.

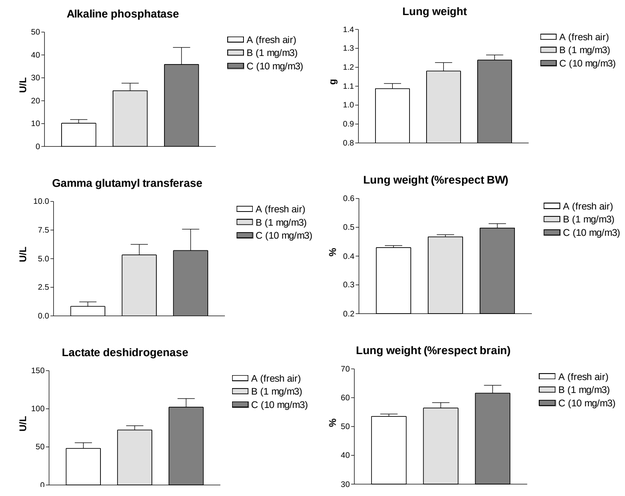


Fig. 3: Internal validation study by inhalation: Results on lung weights (left lung) and enzyme release in BALF

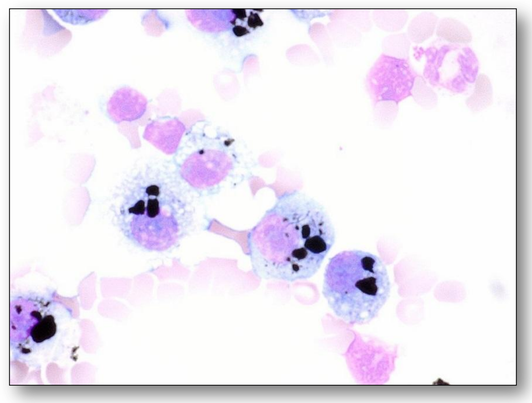


Fig. 4: Macrophages in BALF containing nano-Tubes (modified May-Grünwald).

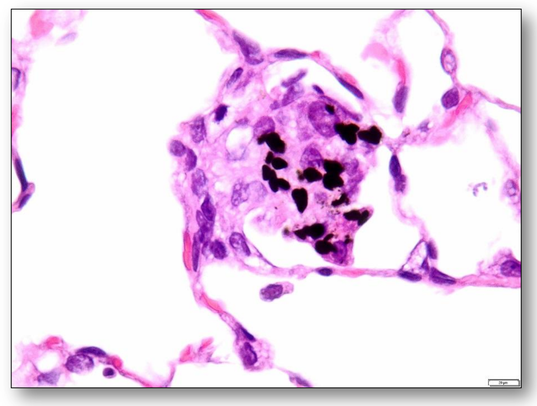


Fig. 5: Agglomeration of nanotube-laden macrophages in alveoli (HE).

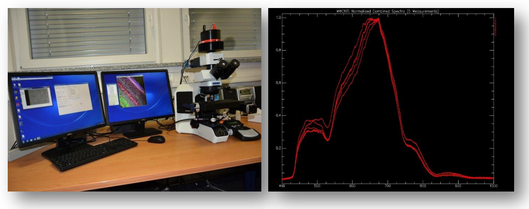


Fig. 6: Hyperspectral Nanoscale Analysis. Microscope with Cytoviva illumination system, quarzlamp, photometer and digital camera. On right side: Collective spectrum of carbon nanotube.

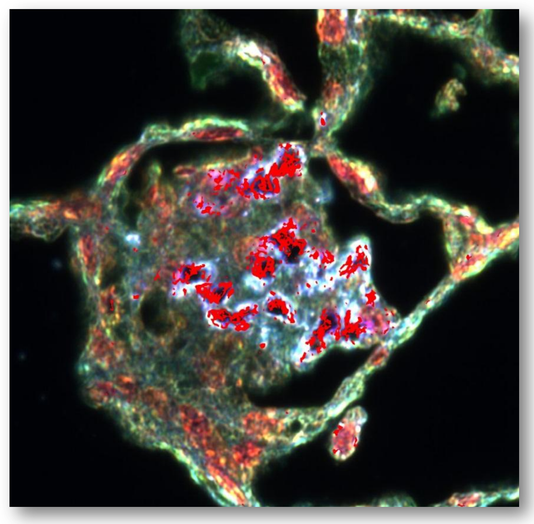


Fig. 7: Carbon nanotube detection by nanoscale hyperspectral analysis in tissue (false color picture).

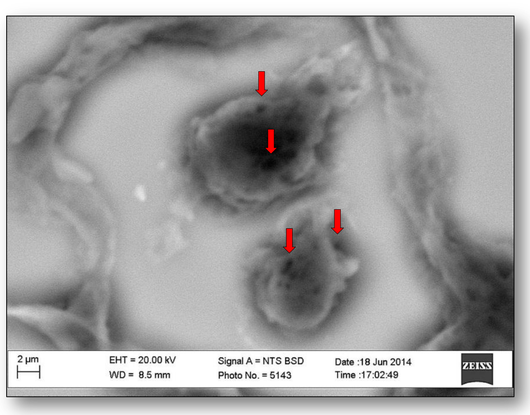


Fig. 8: Unstained paraffin section. Evaluation by EDX.

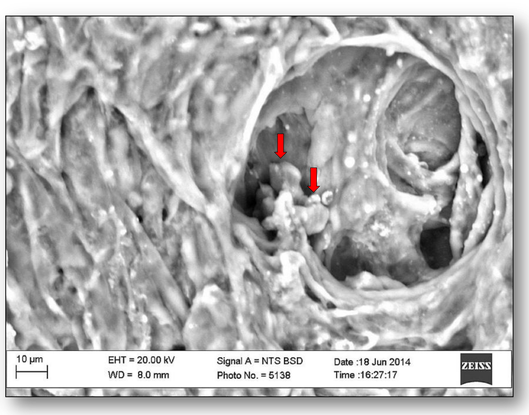


Fig. 9: Thin dried section. View into endterminal sac. Evaluation by EDX.

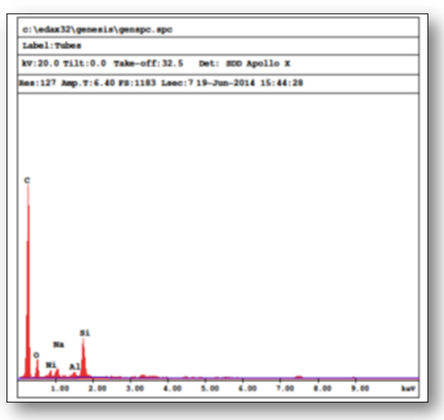


Fig. 10: C is major component (>98%), hence no detection possibilities by EDX.

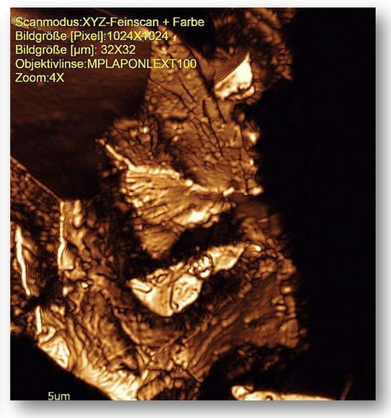
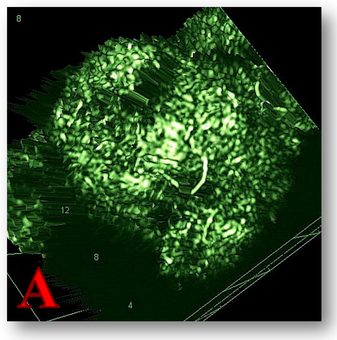


Fig. 11: Ashes. Ashing at 130 °C, for 48 Hrs. LEXT OLS2000. Original magnification x8545.



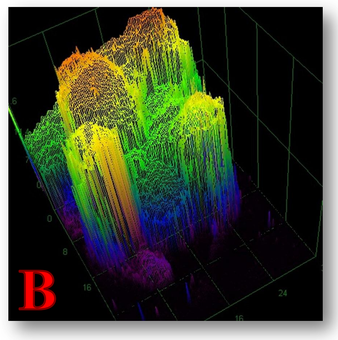


Fig. 12; Laser scanning microscopy (LEXT OLS2000): Characterization of test item. A) Original magnification x 17090. B) 3D-surface modelling of agglomeration from Nanotubes in wire model. Original magnification x 17090.

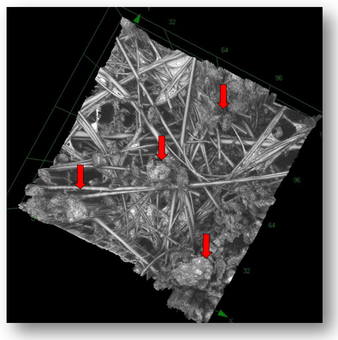


Fig. 13: LEXT OLS2000): Nanotubes on inhalation Filter. Original magnification x 17090.

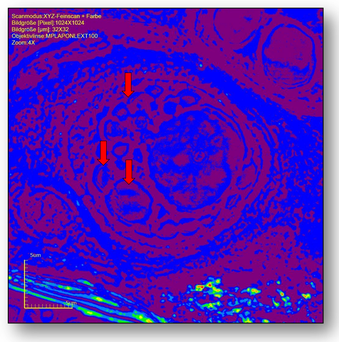


Fig. 14: LEXT OLS2000): Nanotubes within cells. Detection on 300 nm Epon embedded material. Original magnification x 17090.

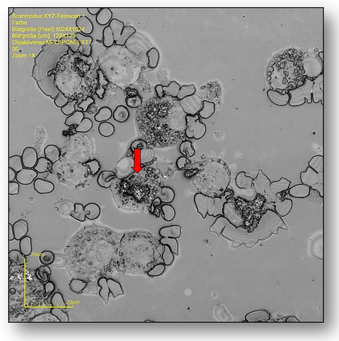


Fig. 15: LEXT OLS2000): Macrophages in BAL containing nanotubes. Original magnification x 2136.

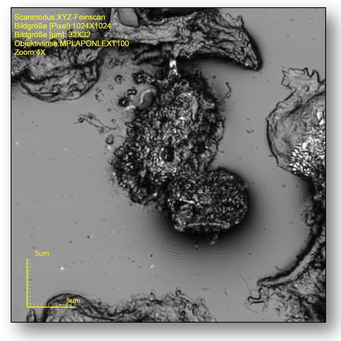


Fig. 16: LEXT OLS2000): Alveolar macrophage laden with nanotubes. Original magnification x 8545.

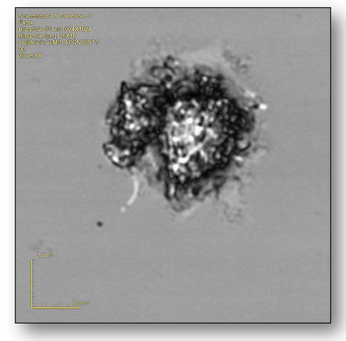


Fig. 17: LEXT OLS2000): Alveolar macrophage laden with nanotubes. Original magnification x 17090.