Interactions of ingested tungsten oxide (WO_X) nanofibers with a model digestive gland tissue studied by SEM/EDX and FTIR

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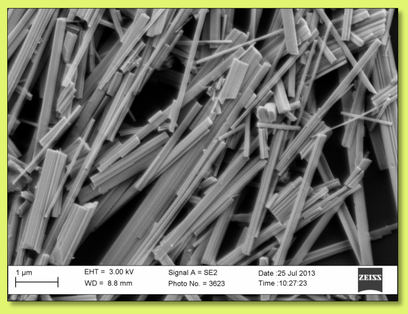
INTRODUCTION

The aim of our work was to study deviations in molecular composition in digestive gland tissue of tungsten oxide nanofibers (nano-WO_x) fed model organism, terrestrial invertebrate *Porcellio scaber* (Isopoda, Crustacea) and to reveal mechanism of nano-WO_x effect on digestive gland cells *in vivo*. Fourier Transform Infrared (FTIR) imaging is based on absorption of infrared light by the vibrational transitions in covalent bonds and intensities provide quantitative information, while frequencies give qualitative information about the nature of these bonds, their structure, and their molecular environment. The FTIR analyses were supplemented by toxicity (feeding parameters, weight change and survival) and cytotoxicity (digestive gland cell membrane stability assay) analyses. We also study interaction of ingested nano-WO_x with epithelial cells by scanning electron microscopy (SEM) and by energy dispersive x-ray spectroscopy (EDX).

RESULTS

Tungsten oxide nanofibers used in the study



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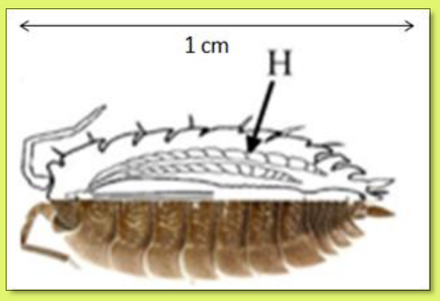
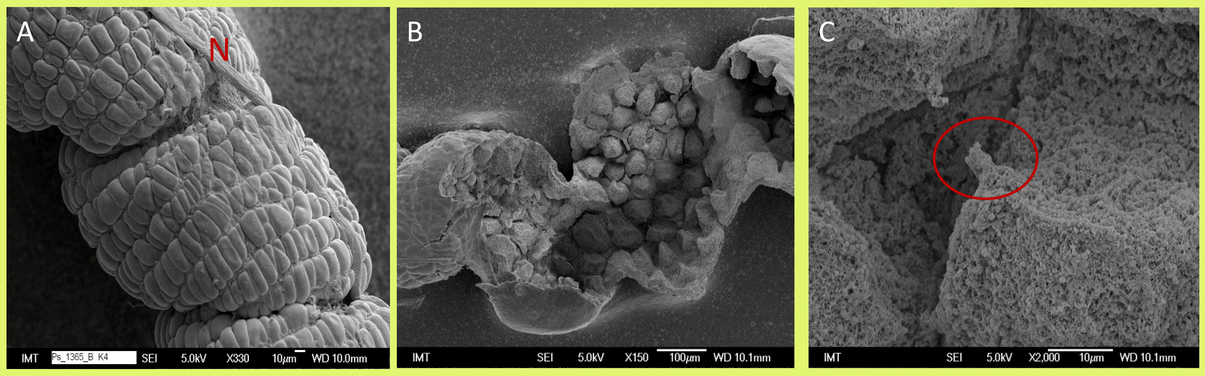


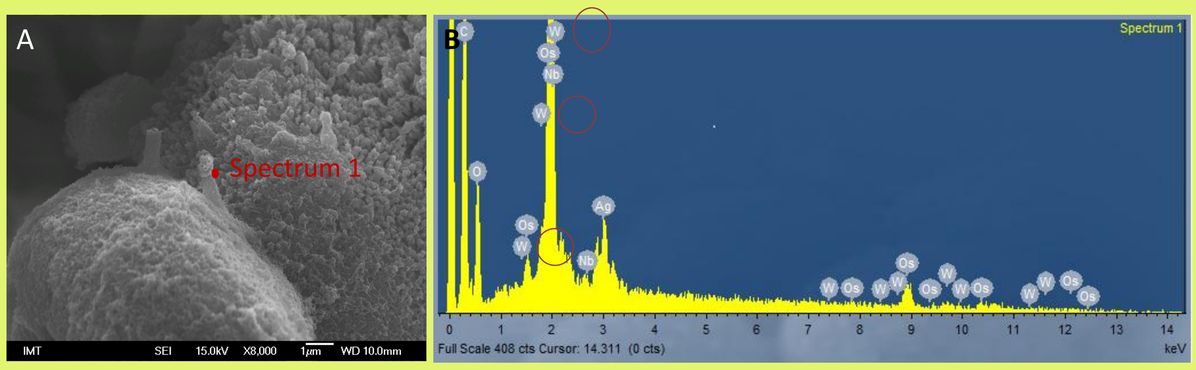
Model organism *P. scaber* with shematic presentation of digestive gland tubes-hepatopancreas (H)

MECHANICAL INTERACTION

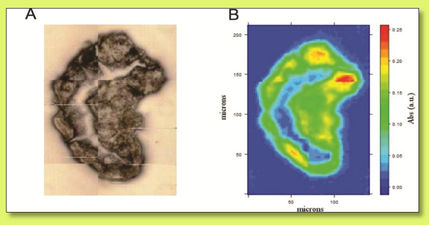
MOLECULAR DEVIATIONS



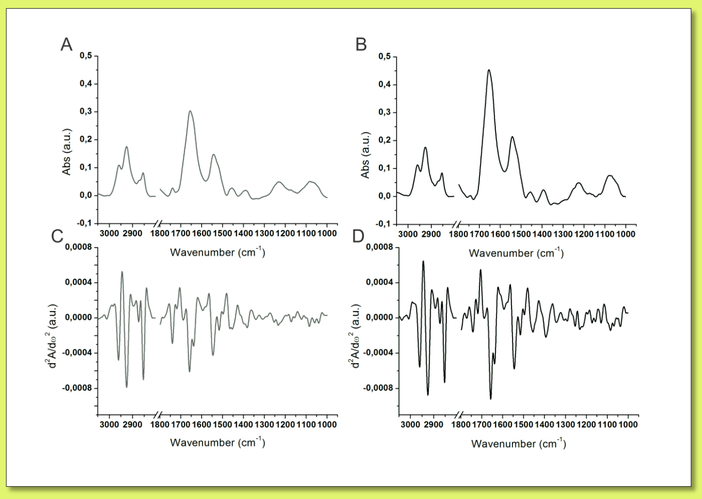
Digestive glands of *Porcellio scaber* investigated with scanning electron microscopy.
(A) Digestive gland tube with a nerve (N) on the surface. **(B)** Inner part of digestive gland exposing the cells where fibre-like structures were observed. **(C)** Part of nanofibres embedded into the cell membrane (encircled).



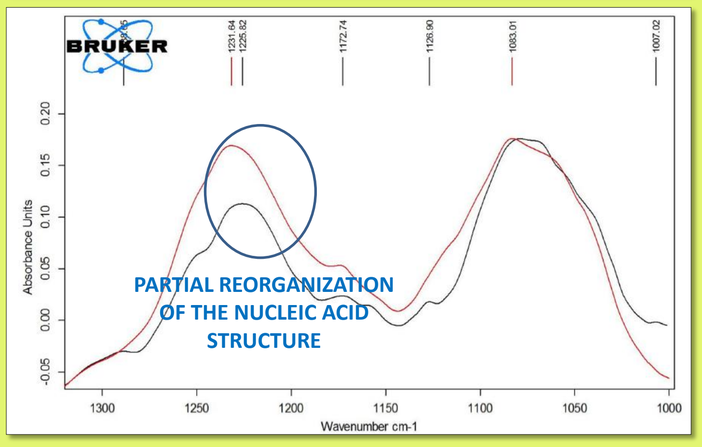
Scanning electron microscopy (SEM) and Energy dispersive X-Ray (EDX) composition of fibre-like structures in digestive gland.
(A) Surface of digestive gland epithelium with fibre-like structures found in one of the cells. The structures where EDX spectrum was taken is marked (spectrum 1). **(B)** EDX spectra of observed area where presence of tungsten is evident (encircled).



Optical and Fourier transform infrared (FTIR) image of control sample. (A) Optical image of the control sample K2 of a section of the digestive gland, defining the imaged area (340X510 microns). **(B)** FTIR image of the control sample K2 obtained by integration of the protein spectral region between 1720-1480 cm^{-1}.



Average spectra of the central regions of digestive gland tissue. (A,B) Average spectra of the central regions of WO_x treated (grey) and control (black) samples. **(C,D)** Second derivative of the average spectra of treated (grey) and control (black) samples, respectively; Savitzky-Golay algorithm (17 smoothing points).



FTIR measurements in the spectral range between 1300 and 1000 cm^{-1}, characteristic for nucleic acids and carbohydrates. The asymmetric stretching band of the PO_2^- in the backbone of nucleic acids shifts to higher wave numbers upon nano-WO_x ingestion (from 1226 to 1232 cm^{-1}, red line). Black line represents the control.

CONCLUSIONS

- Ingestion of nano-WO_x does not affect severely the cell membrane stability and feeding behaviour.
- FTIR results showed changes in protein to lipid ratio, structural changes of nucleic acids and indicate lipid peroxidation.
- We interpreted FTIR results as responses indicating non-homeostatic state before oxidative stress and toxic responses are evidenced.
- Nano-WO_x can cause injuries on epithelial cells of digestive gland tube.
- Physical forces of peristaltic may have important role in nanofibre digestive/intestinal cell interactions what could not be predicted *in vitro*.

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