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# Use of scanning electron microscopy to monitor nanofibre/cell interaction in digestive epithelial cells

Agron Millaku[a,*], Damjana Drobne[b,c,d], Matjaz Torkar[e,f], Sara Novak[b], Maja Remškar[f], Živa Pipan-Tkalec[b]

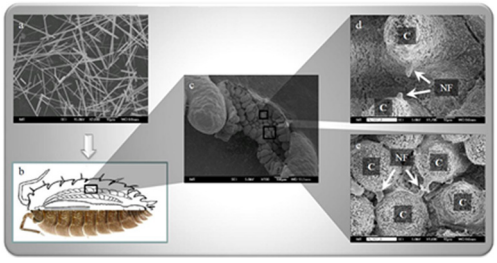
[a] Limnos-Company for Applied Ecology Ltd, Podlimbarskega 31, 1000 Ljubljana, Slovenia
[b] University of Ljubljana, Biotechnical Faculty, Department of Biology, Večna pot 111, 1000 Ljubljana, Slovenia
[c] Centre of Excellence, Advanced Materials and Technologies for the Future (CO NAMASTE), Jamova cesta 39, 1000 Ljubljana, Slovenia
[d] Centre of Excellence, Nanoscience and Nanotechnology (Nanocentre), Jamova cesta 39, 1000 Ljubljana, Slovenia
[e] Institute of Metals and Technology IMT, Lepi pot 11, 1000 Ljubljana, Slovenia
[f] Jožef Stefan Institute, Condensed Matter Physics Department, Jamova cesta 39, 1000 Ljubljana, Slovenia

## HIGHLIGHTS

- Tungsten oxide nanofibres react physically with digestive gland epithelial cells in *Porcellio scaber*.
- Physical peristaltic forces of lead to insertion of nanofibres into the cells.
- No toxic responses as measured by conventional toxicity biomarkers were detected.
- Physical interactions were observed in a majority of the investigated animals.

## GRAPHICAL ABSTRACT

Scanning electron microscopy is particularly well suited to the observation of nanofibre/cell interaction in the endothelial cells lining the hepatopancreas. (a) Tungsten oxide nanofibres, (b) test organism *Porcellio scaber* and schematic appearance of digestive tubes, (c) digestive tube (hepatopancreas) prepared for SEM investigation, (d) digestive gland cells (C) with nanofibres (NF) embedded in the cell membrane and (e) nanofibres inserted deeply in the cells and damaged nanofibres due to peristalsis.

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## ABSTRACT

We provide data obtained by scanning electron microscopy (SEM) and energy dispersive X-ray spectroscopy (EDS) on the interaction of ingested tungsten nanofibers with epithelial cells of the digestive tubes of a test organism *Porcellio scaber*. Conventional toxicity endpoints including feeding behaviour, weight loss and mortality were also measured in each investigated animal. No toxicity was detected in any of exposed animals after 14 days of feeding on tungsten nanofiber dosed food, but when nanofibers enter the digestive system they can react with epithelial cells of the digestive tubes, becoming physically inserted into the cells. In this way, nanofibers can injure the epithelial cells of digestive gland tubes when they are ingested with food. Our SEM data suggest that peristaltic forces may have an important role, not predicted by *in vitro* experiments, in the interactions of nanomaterials with digestive intestinal cells.

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## 1. Introduction

Natural and artificial nanoparticles and nanofibers are present in our environment but data on their interactions with cells of living organisms and consequences of these interactions for the entire organism are sparse [1,2].

* Corresponding author. Tel.: +386 31609707.
*E-mail address:* agron.mi@hotmail.com (A. Millaku).

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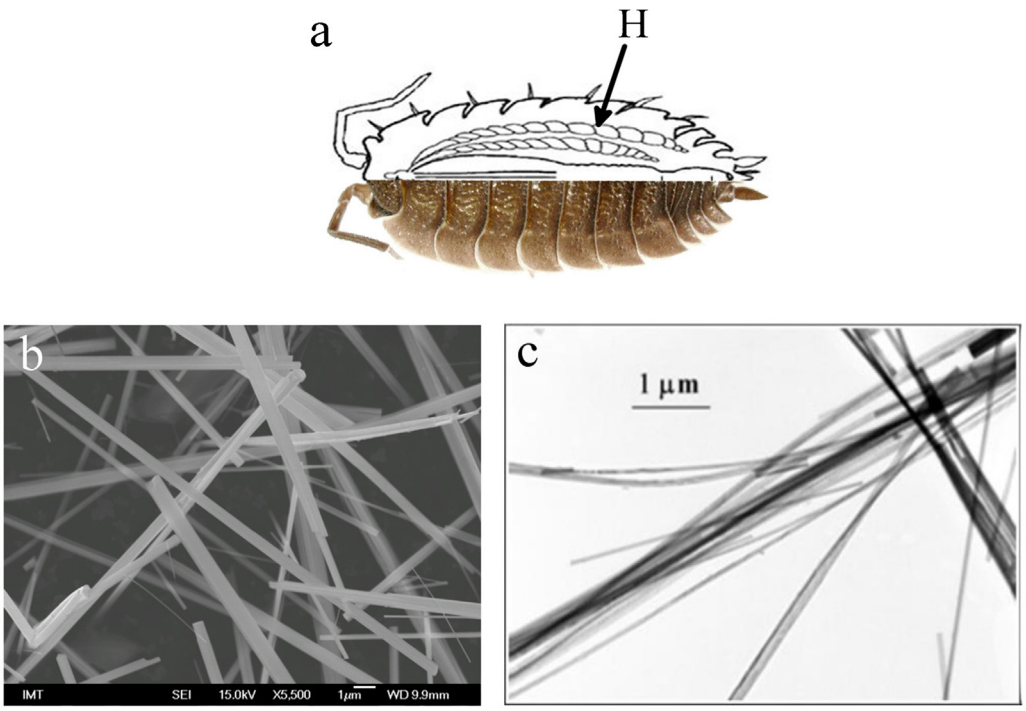


**Fig. 1.** Model organism and nanofibers used in the study. (a) *Porcellio scaber* with digestive glands is shown, (b) scanning electron micrograph of $WO_x$ nanofibers and (c) transmission electron micrograph of $WO_x$ nanofibers.

Nanomaterials (NMs) are defined as materials that have structural features with at least one dimension of <100 nm, and include nanofilms and nanocoatings (<100 nm) in one dimension, nanotubes and nanowires (<100 nm) in two dimensions and NMs (<100 nm) in three dimensions [3]. Tungsten oxides ($WO_3$, $WO_2$, and $WO_x$) have been considered for use in many important applications including optical devices, gas sensors, electrochromic windows, and photocatalysts [4]. Synthesis of tungsten oxides can be accompanied by release of fibre-like nanoparticles and this raises safety concerns reminiscent of those associated with asbestos fibres [5,6]. Large scale production of $WO_x$ nanofibers (nano-$WO_x$) can also result in environmental pollution. Because they can cause free radical damage *in vitro* $WO_x$ nanofibers, either as whiskers or needles, are recognised as being more biologically potent than non-fibrous $WO_x$ [7]. Particles of tungsten carbide (WC) that can cause pneumoconiosis are also well known [8].

Consequently, development of a hazard profile is a critical step in characterising the potential safety of nanofibers, and the associated health and environmental hazards. The most important routes of exposure are *via* respiratory and digestive systems but although there are some *in vitro* studies on appropriate cell models, *in vivo* data are scarce.

In this study we have examined the effects of ingested tungsten nanofibers *in vivo* on a model invertebrate terrestrial isopod *Porcellio scaber* (Isopoda, Crustacea), an organism of choice for testing effects of different ingested substances. Their digestive glands (hepatopancreas) resemble liver and pancreas of vertebrates. Hepatopancreatic cells are directly exposed to substances in partly digested food, and filtered and transported from stomach into the lumen of the hepatopancreas. The digestive gland epithelium is subject to complex physical forces *in vivo* engendered by contact with luminal contents and pressure from peristalsis.

The objective of this work was study by SEM and EDS of the epithelial surfaces of control potential physical interactions between ingested nanofibers and cells *in vivo*. Such morphological studies have been reported on cell cultures [9,10], but in our *in vivo* study some crucial parameters such as peristalsis that govern digestion are included. We have combined data on interactions between nanofibers and digestive gland cells with data on conventional toxic responses of these organisms and we discuss the mechanical interactions between ingested nanofibers and cells and potential toxic outcome.

## 2. Materials and methods

### 2.1. Experimental animals

We have used the woodlouse terrestrial isopod *P. scaber* to test effects of nanofibers on the digestive gland tube *in vivo* (Fig. 1a). Terrestrial isopods, including *P. scaber*, have become organisms of choice in (eco)toxicology, (eco)physiology and recently nanotoxicity studies due to a lot of physiological, stress and toxicological biomarkers that could be controlled under in the laboratory where the exposure dose as well as exposure concentration to selected substance could be assessed [11–17].

Individual *P. scaber* were collected in three different locations near Ljubljana. The animals were kept in a terrarium (20/35/20 cm) for acclimatisation for a period of three months. The terrarium was filled with a 2–5 cm layer of moistened sand and soil and a thick layer of partly decomposed hazelnut (*Corylus avellana*) tree leaves that had been collected in uncontaminated woodland and dried at room temperature. The substratum in the terrarium was heated to 80 °C for several hours to destroy predators (spiders) before the introduction of the isopods. The culture was kept at controlled room temperature (21 ± 1 °C), a 16:8-h light–dark photoperiod, and high humidity.

### 2.2. Preparation and characterisation of nanofibers

$WO_x$ nanofibers that were used in this study were synthesised by a chemical transport reaction [18] from tungsten powder (99.9%) and $WO_3$ powder (99.9%) in the stoichiometric ratio of $WO_{2.86}$.

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