Journal of Hazardous Materials 248–249 (2013) 478–486

Contents lists available at SciVerse ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat

# Comparative study of respiratory tract immune toxicity induced by three sterilisation nanoparticles: Silver, zinc oxide and titanium dioxide

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## HIGHLIGHTS

- Three typical nanoparticles can cause an increase in oxidative injury to the lungs.
- Nanoparticles can cause disorders in regulation of the cytokine network to the lungs.
- The phagocytic function of alveolar macrophages was reduced by nanoparticles.
- These nanoparticles can destroy cell membrane and cause cytotoxicity.
- The toxicity of nanomaterials with various properties was significant differences.

## ARTICLE INFO

*Article history:*
Received 10 August 2012
Received in revised form 3 November 2012
Accepted 21 January 2013
Available online 28 January 2013

*Keywords:*
Silver nanoparticles
Zinc oxide nanoparticles
Titanium dioxide nanoparticles
Respiratory tract immune toxicity
Comparative study

## ABSTRACT

Silver, zinc oxide, and titanium dioxide nanoparticles are used as sterilisation materials to enhance the performance of disinfectants. We investigated the respiratory tract immune toxicity ("immunotoxicity") of these nanoparticles *in vivo* and *in vitro*, and we explored the relationships between particle size, particle shape, chemical composition, chemical stability and the toxicological effects of these typical nanoparticles in rats. *In vivo*, the rats were exposed to nanoparticles by intratracheal instillation. Exposure to nanoparticles caused an increase in oxidative injury to the lungs and disorders in regulating the cytokine network, which were detected in the bronchoalveolar lavage fluid, suggesting that oxidative stress might be important for inducing the respiratory immunotoxicity of nanoparticles. *In vitro*, the phagocytic function of alveolar macrophages (AMs) was dose-dependently reduced by nanoparticles, and ZnO nanoparticles induced greater cytotoxicity than the silver and titanium-dioxide nanoparticles, which were coincident with the results of multiple measurements, such as a cell viability assay by WST-8 and LDH measurements. Comparative analyses demonstrated that particle composition and chemical stability most likely had a primary role in the biological effects of different nanoparticles.



## 1. Introduction

Because of increasing interest in their potential toxicity, the adverse effects of manufactured nanoparticles *in vivo* and *in vitro* have been investigated intensively. Nanoparticles entering the body through the respiratory tract can penetrate the pulmonary epithelium and reach the interstitium to be deposited in peripheral lung tissue [1,2]. They can cross the pulmonary blood barrier and gain access to the blood circulation. Once within the circulatory system, they can be transferred to the liver and other tissues/organs to induce toxic effects [3].

Moreover, most authors have focused on the effects of one type of particle or several types of particles of the same substance but with a different size or shape. Few studies have compared the toxicological effects of different types of nanoparticles, especially silver nanoparticles (nano-Ag), zinc oxide nanoparticles (nano-ZnO) and titanium dioxide nanoparticles (nano-$TiO_2$). For one type of nanomaterial, the bioactivity increases as the particle size decreases [4–6]. However, for different types of nanomaterials, bioactivity is most likely related to the size, shape, solubility of the particle, and other physicochemical properties [7]. Meanwhile, the relationship between the bioactivity and the properties of nanoparticles is not understood.

The respiratory tract is one of the main conduits of access to the body for foreign particles. Therefore, it is the main site at which

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http://dx.doi.org/10.1016/j.jhazmat.2013.01.046

**Table 1**
Characterisation of the three nanomaterials.

| Particle | Supplier | Size (nm) | Density (g/cm$^3$) | Specific Surface area (m$^2$/g) | Shape | Composition |
|---|---|---|---|---|---|---|
| Nano-Ag | Sigma–Aldrich | 52.25 ± 23.64 | 10.49 | 5 | Sphere | Ag >99.5% |
| Nano-ZnO | Shenzhen Nanguo | 19.61 ± 5.83 | 5.78 | 45 | Hexagonal | ZnO >99.9% |
| Nano-$TiO_2$ | Sigma–Aldrich | 22.82 ± 5.30 | 4.26 | 35–65 | Sphere | $TiO_2$ >99.7% |

particles affects the organism. The immunity of the respiratory tract is an appreciable barrier against the biological effects of nanoparticles. Once nanoparticles gain entry into the organism through the respiratory tract, the organism will inevitably produce a series of defensive responses. The objectives of the study were to explore (1) immunotoxicity within the respiratory tract induced by these three nanomaterials (nano-Ag, nano-ZnO and nano-$TiO_2$) and the potential mechanisms of action and (2) the relationship between the properties of the nanomaterials and the immunotoxicity.

The balance of the cytokine network plays an important part in maintaining the immune and inflammatory responses of organisms. Hence, the levels of interleukin (IL)-1, IL-6, tumour necrosis factor-alpha (TNF-α) and macrophage inflammatory protein (MIP-2) in bronchoalveolar lavage fluid (BALF) of rats exposed to nanomaterials were measured using an enzyme-linked immunosorbent assay (ELISA) to illustrate the effects of nanoparticles on the immune function of the respiratory tract. The present study also focused on the oxidative effects induced by nanoparticles. Therefore, the levels of malondialdehyde (MDA), superoxide dismutase (SOD), reduced glutathione (GSH) and nitrogen oxide (NO) in BALF were measured using biochemical methods. In addition, cytotoxicity was measured using the water-soluble tetrazolium (WST) and lactate dehydrogenase (LDH) assays as well as by observing cellular morphology. The effects of nanoparticles on the phagocytic function of alveolar macrophages (AMs) were measured by the neutral red phagocytosis assay.

## 2. Materials and methods

### 2.1. Ethical approval of the study protocol

The study protocol was approved by the Chinese Association for Laboratory Animal Science.

### 2.2. Particle preparation

Manufactured nanoparticles of ZnO, Ag and $TiO_2$ were purchased from the commercial suppliers detailed in Table 1. The particles were prepared in foetal bovine serum (FBS; Gibco, Billings, MT, USA) by vortexing the suspension 10 times for 10 s followed by sonication (ten times, 30 s every 2 min) at 4 °C to break down agglomerates and ensure a uniform suspension. The samples were then characterised using transmission electron microscopy (TEM) (JEM-100CX, Japan) (Fig. 1). The properties of the nanoparticles are summarised in Table 1.

### 2.3. In vivo study

#### 2.3.1. Animals and intratracheal instillation

Forty-two Wistar rats (body weight, 160–180 g) were divided randomly into seven groups: control group, and 3.5 mg/kg (body weight) or 17.5 mg/kg (body weight) dosage groups for the three nanomaterials. One week prior to the beginning of the experiment, the rats were housed in pairs under controlled environmental conditions (temperature 24 ± 1 °C, humidity 50 ± 5%, lights on 07:00–19:00 h). The treatment was performed in a Grade II animal room, and there were no other air pollutants in the environment. Rodent diet and water were provided *ad libitum*. The rats were exposed to nanomaterials by intratracheal instillation once every 2 days for 5 weeks.

#### 2.3.2. Detection of oxidative damage in BALF

The levels of GSH, SOD, MDA and NO in BALF were detected to determine the oxidative damage to the lung tissue of rats by nanoparticles. The rats were killed by bloodletting from the abdominal aorta under anaesthesia with ether 24 h after the last exposure to nanoparticles. The right lung was then lavaged with normal (physiological) saline at 37 °C to collect BALF. BALF was centrifuged at 2500 rpm for 15 min, and the supernatants were collected. The levels of GSH, SOD, MDA and NO were measured using reagent kits purchased from Jiancheng Bioengineering (Nanjing, China).

#### 2.3.3. Detection of cytokines in BALF

The levels of IL-1, IL-6, MIP-2 and TNF-α in BALF were detected using the double-antibody sandwich ELISA method to determine the immune effects within the respiratory tract of rats after exposure to nanoparticles. The levels of these cytokines were measured using reagent kits purchased from R&D Systems (Minneapolis, MN, USA). Optical density was measured using an Automatic Multifunction Microplate Reader (Multiskan MK3; Thermo Scientific, Waltham, MA, USA) at 450 nm.

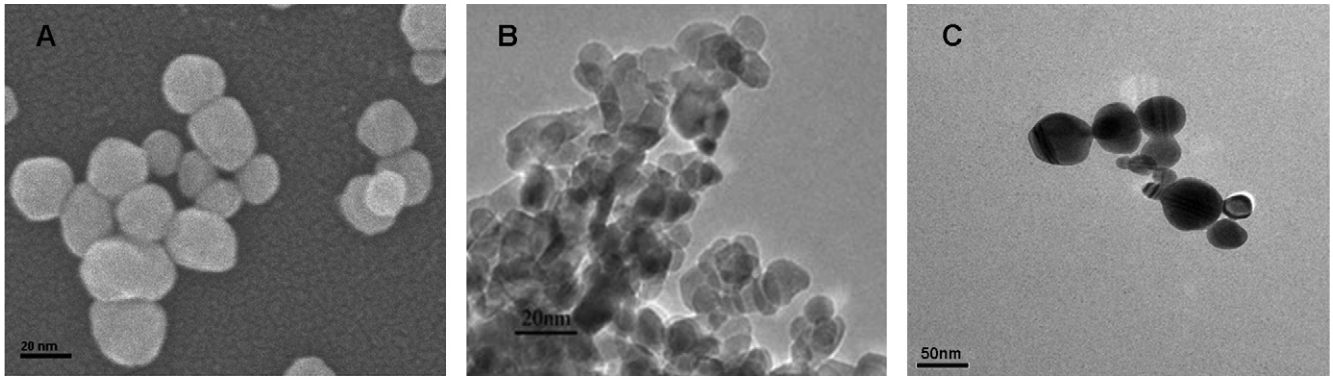


**Fig. 1.** Images of typical particles of (A) nano-$TiO_2$; (B) nano-ZnO; and (C) nano-Ag by TEM.