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# Mitochondrial injury induced by nanosized titanium dioxide in A549 cells and rats

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ABSTRACT

The nanosized titanium dioxide (nano-$TiO_2$) is an important nanoscale compound applied in many different fields because of its superior performance. Here, an anatase nano-$TiO_2$ showed cytotoxicity in a dosage-dependent manner, which was in accordance with changes of A549 cell ultrastructure, A549 cell viability and intracellular ATP level. The lungs of rats treated with single intratracheal instillation of nano-$TiO_2$ were injured, which was demonstrated by changes of alveolar epithelial cell ultrastructure, lung tissue pathology and lung tissue MDA level. The results of this study indicated that nano-$TiO_2$ should be related to the generation of intracellular reactive oxygen species (ROS), which injured mitochondria and prevented the synthesis of ATP. The cells were approaching to apoptosis eventually. In macroscopic view, the lungs inevitably suffered.



## 1. Introduction

In recent years, a large number of nanomaterials with special physical and chemical properties have been synthesized and applied in many different fields, such as nanocatalysis (Vinod, 2010), nanoceramics (Hoda et al., 2012) and biomedicine (Jung and Jong, 2011). Among the various nanomaterials, nanosized titanium dioxide (nano-$TiO_2$) was chemically inert, noncorrosive and photocatalytic, thus nano-$TiO_2$ was widely applied in consumer products, such as pigments (Taavitsainen and Jalava, 1995), photocatalysts (Sun et al., 2004) and cosmetic products (Marta et al., 2010). However, the fact that nano-$TiO_2$ could enter the human body through different routes such as inhalation (respiratory tract), ingestion (gastrointestinal tract), dermal penetration (skin) and injection (blood circulation) (Liu et al., 2010) was demonstrated by many experts. Lots of investigations had shown that nano-$TiO_2$ was able to cause injuries in various cells such as human hepatocellular carcinoma cell (SMMC-7721), human liver cell (HL-7702), rat hepatocarcinoma cell (CBRH-7919) and rat liver cell (BRL-3A) (Sha et al., 2011). Nano-$TiO_2$ showed toxicity in many animal organ types, including lung (Freyre-Fonseca et al., 2011; Liu et al., 2009; Rehn et al., 2003), kidney (Gui et al., 2011), liver (Cui et al., 2011; Palaniappan and Pramod, 2011) and brain (Shin et al., 2010). The toxicity of nano-$TiO_2$ was much attributed to the generation and accumulation of reactive oxygen species (ROS) which resulted in an inflammatory response (Gonzalez

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et al., 2008; Jaeger et al., 2012; Park et al., 2008). The role of cellular ROS induced by nano-$TiO_2$ had been gained much attention, but the exact mechanism was not fully described. Studies of mitochondrial injury induced by nano-$TiO_2$ which might be related to the mechanism were rarely reported yet.

The present study was designed to investigate the effect of nano-$TiO_2$ in A549 cells and rats. Cell ultrastructure, cell viability and intracellular ATP level were detected to access the cytotoxicity of nano-$TiO_2$. Three methods including alveolar epithelial cell ultrastructure, lung tissue pathology and lung tissue MDA level were taken to check the effect of nano-$TiO_2$ in rats. The effects of nano-$TiO_2$ were comprehensively understood by experiments which were carried out both in A549 cells and rats. ATP assay and lipid peroxidation MDA assay helped us to reveal the possible mechanism of the toxicity induced by nano-$TiO_2$.

## 2. Materials and methods

### 2.1. Chemicals and reagents

All chemicals and reagents were obtained from Sinopharm Chemical Reagent Beijing Co., Ltd. (Shanghai, China) unless otherwise stated. Modified Eagle Medium (MEM), fetal bovine serum (FBS) were purchased from Thermo Fisher Scientific (Shanghai, China). Nano-$TiO_2$ particles were supplied by Shanghai Institute of Ceramics, Chinese Academy of Sciences (Shanghai, China). The cell counting kit-8, ATP assay kit and lipid peroxidation MDA assay kit were purchased from Beyotime Institute of Biotechnology (Jiangsu, China).

### 2.2. Preparation and characterization of nano-$TiO_2$ suspension

Nano-$TiO_2$ particles were sterilized by heated at 120 °C for 2 h, then suspended in MEM (for A549 cells) or normal saline (for rats) to a stock solution and kept at 4 °C until used. Before application, the suspension was ultrasonicated for 30 min in a break every 10 min for vortex. A stable suspension of nano-$TiO_2$ was obtained in this way and used immediately. Morphology and structure of nano-$TiO_2$ were analyzed by TEM (H-7500, Hitachi, Japan) imaging.

### 2.3. Cell culture

A549 cells were obtained from China Center for Type Culture Collection (Beijing, China). Cells were grown in MEM supplemented with 10% (v/v) FBS. Cells were maintained at 37 °C in a humidified atmosphere of 5% $CO_2$ and passed at 80% of confluence.

### 2.4. Animals and treatment

Twenty healthy male rats (200 ± 2 g) were purchased from Experimental Animal Center of Chinese Academy of Sciences (Shanghai, China). The rats were kept in a positive pressure air-conditioned unit for animal housing. Distilled water and sterilized food were available. The rats were allowed to acclimate 5 days prior to experimental use.

The rats were randomly divided into four groups, including a control group treated with normal saline and three experimental groups treated with 0.1, 1.0 and 10.0 mg/mL nano-$TiO_2$ suspension, respectively. Rats were anesthetized with chloral hydrate, and then nano-$TiO_2$ suspension was instilled into the rat's trachea using an indwelling needle. Three days later, all rats were dissected for the following study.

### 2.5. Cell viability assay

Cell viability was detected by cell counting kit-8 according to the manufacturer's instruction. Briefly, A549 cells were seeded in 96-well culture plates with $5 \times 10^3$ cells in 100 μL MEM per well, after 24 h of cell attachment, A549 cells were treated with nano-$TiO_2$ for 4 h. Six replicate wells were used for each control and tested concentrations. After incubated for 4 h, 10 μL 2)-2H-tetrazolium, monosodium salt (WST-8) solution was added to each well and the cells further incubated at 37 °C for 1 h in a 5% $CO_2$ humidified incubator. Absorbance was quantified at 450 nm using a microplate reader (Synergy2, Bio-tek, USA). The results of the treated groups were compared to the control group and represented as the percentage of the control value.

### 2.6. Mitochondria state

Intracellular ATP level was a sensitive readout of mitochondrial state and determined using a firefly luciferase-based ATP assay kit. Briefly, cells treated with nano-$TiO_2$ for 4 h were lysed and centrifuged at 12,000 × *g* for 5 min in 24-well plates, 100 μL supernatant was mixed with 10 μL ATP detection working dilution. Luminance was measured by a luminometer (Synergy2, Bio-tek, USA). ATP content was calculated according to a standard curve (Chen et al., 2009).

### 2.7. Measurement of MDA

Malondialdehyde (MDA), a product of lipid peroxidation, was analyzed by using an assay kit (Beyotime Institute of Biotechnology). All procedures were completely complied with the manufacturer's protocol. The concentration of MDA could be measured at a wavelength of 532 nm by reacting with thiobarbituric acid (TBA) to form a stable chromophoric production. MDA contents were calculated according to a standard curve.

### 2.8. Histopathological observation

Lungs obtained from rats were washed with phosphate buffer solution (PBS) and fixed with 2.5% glutaraldehyde. All fixed lung tissues were routinely processed, embedded in paraffin, sectioned at 3 μm, and stained with hematoxylin and eosin (H&E) for light microscopic examination.

### 2.9. Ultrastructure observation

Samples obtained from experiments were fixed with 2.5% glutaraldehyde and post-fixed with 1% $OsO_4$. Then all samples were routinely washed with PBS, dehydrated in a graded series of acetone, and embedded in epoxy resins. Ultrathin