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Comparative toxicities of bismuth oxybromide and titanium dioxide exposure on human skin keratinocyte cells

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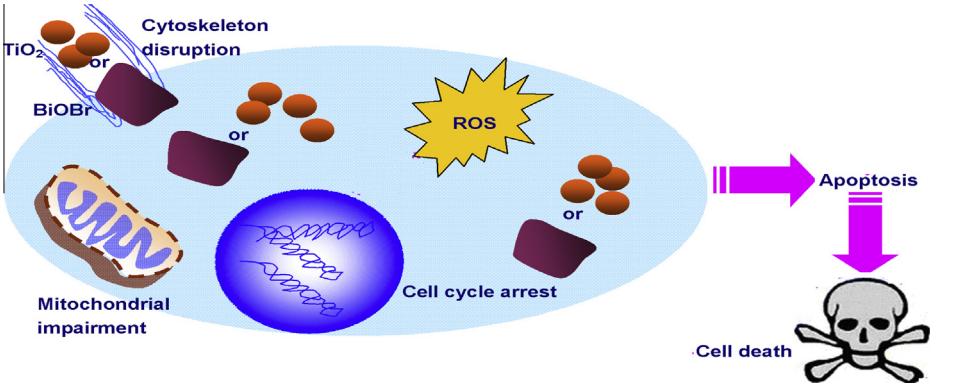
HIGHLIGHTS

- Comparison of toxicities of BiOBr and TiO_2 NPs to HaCaT cells was first designed.
- NPs caused cell death, cellular organelles impairment, apoptosis and cycle arrest.
- TiO_2 NPs showed a more bioavailability than BiOBr.
- A specific "cellular quota" for uptake of TiO_2 NPs in HaCaT cells was found.
- NPs induced elevated level of ROS generation, particular in TiO_2 NPs group.

GRAPHICAL ABSTRACT



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ABSTRACT

Nano-sized bismuth oxybromide (BiOBr) particles are being considered for applications within the semiconductor industry. However, little is known about their potential impact on human health. In this study, we comparatively investigated the cytotoxicity of BiOBr and titanium dioxide (TiO_2) nanoparticles (NPs) using human skin keratinocyte cell line (HaCaT) as a research model. Results indicate that lamellar-shaped BiOBr (length: 200 nm, width: 150 nm, and an average thickness: around 15 nm) has less toxic effects on cell viability and intracellular organelles than TiO_2 (P25) NPs. BiOBr mainly induced late cell apoptosis, while for TiO_2, both early apoptosis and late apoptosis were involved. Cell cycle arrest was found in cells on both NPs exposure, and more prominent in TiO_2-treated cells. More cellular uptake was achieved after TiO_2 exposure, particularly at 10 μg mL^{-1}, presence of TiO_2 resulted in more than 2-fold increase in cellular granularity compared with BiOBr. Furthermore, TiO_2 had a high potential to generate intracellular reactive oxygen species (ROS) in cells, where a 2.7-fold increase in TiO_2 group and 2.0-fold increase in BiOBr group at the same concentration of 25 μg mL^{-1}. Higher cellular uptake and ROS stimulation should contribute to the more hazards of TiO_2 than BiOBr NPs. This knowledge is a crucial component in the environmental and human hazard assessment of BiOBr and TiO_2 NPs.

1. Introduction

Semiconductor (SC) has been expected to be one of the most promising materials to be used for solving energy and pollution problems. Bismuth oxybromide (BiOBr) and titanium dioxide

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(TiO_2) are two important groups of these SC materials. As a traditional SC material, TiO_2 is widely used, however, it is restricted in practical applications by low solar harvest and quantum efficiency (Zhang et al., 2013). Furthermore, TiO_2 has been shown to pose a serious threat to human and environmental health (Jaeger et al., 2012; Ghosh et al., 2010). In this case, many novel and efficient SC materials have been pursued. Among the new emerging SC materials, bismuth oxyhalides (BiOX (X = Cl, Br, I)), particularly BiOBr is important due to its unique properties, such as excellent visible-light response, high photocorrosion stability, and superior photocatalytic performance. To date, BiOBr with various dimensionalities and morphologies has been fabricated successfully, including nanoparticles (Henle et al., 2007), nanosheets (Cheng et al., 2012), eggshells (Deng and Guan, 2013), lamellas (Shang et al., 2009), microflowers (Zhang et al., 2013), and microspheres (Zhang et al., 2012). Additionally, impurity doping and SC heterojunctions have been developed to further improve their photocatalytic performance (Ai et al., 2011; Wei et al., 2013). All these BiOBr materials have been effectively utilized in air cleaning and water purification. The expanded research and application of BiOBr materials enhance the potential for their environmental release and unfavorable human exposures. Therefore, it is rather imperative for us to carry out the research and explore of their health effects.

Recently, the total number of BiOBr-related reports has increased significantly, and most studies focus exclusively on preparation methods, formation mechanisms, and environmental applications. Unfortunately, little information is available on the potential toxic effects of BiOBr on human health and living organisms, especially its behavior in a cellular level is still an enigma. Because of the chemical, sized, and being not biodegradable properties, BiOBr materials will distribute throughout environment with largely unknown consequences on human health (Nel et al., 2006; Wang et al., 2009; Yin et al., 2012). Massive experiments have demonstrated that BiOBr exhibits better performances on the treatment of environmental pollutants than TiO_2 (Shang et al., 2009; Deng and Guan, 2013). For the practical application, however, an environmentally robust SC material is a crucial component. Furthermore, a new technology will only be successful when it has been proven to be safe. Consequently, potentially hazardous effects on human health should be identified for safe use of these BiOBr materials.

Short-term bioassay systems are relevant for preliminary screening on the potential hazardous effects of environmental chemicals (Park et al., 2008; Mater et al., 2014; Pinto et al., 2014). In particular, skin is the largest primary defense organ in our body and directly comes into contact with environment. Here, the study was designed to compare the toxicological effects of BiOBr and TiO_2 (P25) nanoparticles (NPs) using a human skin derived cell line, namely, HaCaT keratinocytes. Although the cytotoxicity of TiO_2 has been reported, current data are often contradictory resulting in hardly comparable outcomes. One of the reasons is the various evaluation protocols. If the identical evaluation protocol and experiment procedure were conducted to compare the cytotoxicity of two different NPs at the same time, one should obtain some valuable results. For this purpose, the cytotoxicity of TiO_2 was also included in this study. Some toxic endpoints, such as cell viability, morphology, intracellular organelles (mitochondria, cell nuclei, and actin filaments), apoptosis, as well as cell cycle distribution were accurately accomplished. To elucidate the observed difference in toxicological effects of BiOBr and TiO_2 NPs, possible mechanisms in terms of NPs internalization and intracellular reactive oxygen species (ROS) accumulation were also explored. To the best of our knowledge, this article comparatively investigated toxicological effects of BiOBr and TiO_2 NPs for the first time. We hope this information may provide helpful guidance on their potential risk assessment.

2. Materials and methods

2.1. Materials

TiO_2 (P25) was obtained from a commercial company (Degussa, Germany). 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), RNase, and propidium iodide (PI) were purchased from Sigma–Aldrich Co. (Madrid, Spain). Mito-Tracker Green, FITC-labeled phalloidin, Hoechst 33342, and 2′,7′-dichlorodihydro-fluorescein (DCFH-DA) were purchased from Beyotime Biotech. (China). Annexin V/PI staining kit was obtained from KeyGEN Biotech. (China).

2.2. BiOBr preparation

Hydrolysis is a very common method in the preparation of BiOBr materials (Xu et al., 2013a,b; Dash et al., 2014; Mao et al., 2014; He et al., 2015). In this paper, BiOBr was synthesized by a simple hydrolysis method modified by our group (Mao and Fan, 2013). Firstly, 0.005 mol of $Bi(NO_3)_3{\cdot}5H_2O$ was dissolved in 30 mL ethanol under stirring at room temperature. Then stoichiometric amount of KBr was added into the above solution, and reacted for 6 h. After that, the precipitate was filtrated and washed thoroughly with absolute alcohol and ultrapure water. Finally, the product was dried at 80 °C for 5 h before further characterization.

2.3. Characterization of BiOBr and TiO_2 NPs

Both BiOBr and TiO_2 NPs were characterized for different physicochemical parameters. The nanocrystalline quality and structure were evaluated by X-ray diffraction (XRD) analysis. An X-ray diffractometer (Shimadu, Japan) was scanned at 40 kV and 30 mA with Cu Kα radiation. The particulate size and shape were observed using transmission electron microscopy (TEM, JEOL-100CX, Japan), high-resolution transmission electron microscopy (HRTEM, JEOL-100CX, Japan), and scanning electron microscopy (SEM, JEOL JSM-7001F, Japan). Average hydrodynamic size, size distribution, and zeta potential were measured by dynamic light scattering (DLS) using a Zetasizer Nano-ZS equipped with a 4.0 mW laser operating at 633 nm (Model ZEN 3600, Malvern Instruments Ltd., Malvern, UK). To assess the media effects on NPs dispersive status, sample solutions at 100 μg mL^{-1} were conducted in ultrapure water, phosphate buffer saline (PBS), and complete cell culture medium.

2.4. Cell culture and exposure to NPs

HaCaT cells, a human skin keratinocyte cell line, were purchased from Cell Resource Center (Chinese Academy of Medical Sciences & Peking Union Medical College, CAMS & PUMC, China). Cells were cultivated in Minimal Essential Medium containing Earle's balanced salt solution (MEM–EBSS, CAMS & PUMC, China) supplemented with 10% fetal bovine serum (FBS, Gibco, USA) and 1% penicillin–streptomycin (Solarbio Science &Technology Co., Ltd., China). Cells were cultivated at 37 °C in a humidified atmosphere of 5% CO_2. To carry out experiments, cells grown to 70–80% confluence were seeded in 6-(3×10^5 cells mL^{-1})/96-well (2.5×10^4 cells mL^{-1}) plates or coverslips (2.5×10^4 cells mL^{-1}) depending on the assay. Then cells were allowed to adhere for 24 h. The confluence was 50–60% at the time of exposure.

To assess the toxicological effects of BiOBr and TiO_2 NPs, freshly dispersed suspensions (in sterilized, ultrapure water) were separately