Colloids and Surfaces B: Biointerfaces 156 (2017) 157–164

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

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

Nanocubes of indium oxide induce cytotoxicity and apoptosis through oxidative stress in human lung epithelial cells

Maqusood Ahamed[a,*], Mohd Javed Akhtar[a], M.A. Majeed Khan[a], Hisham A. Alhadlaq[a,b], Ali Aldalbahi[a,c]

[a] King Abdullah Institute for Nanotechnology, King Saud University, Riyadh, Saudi Arabia
[b] Department of Physics and Astronomy, College of Science, King Saud University, Riyadh, Saudi Arabia
[c] Department of Chemistry, College of Science, King Saud University, Riyadh, Saudi Arabia

ARTICLE INFO

Article history:
Received 2 March 2017
Received in revised form 27 April 2017
Accepted 8 May 2017
Available online 9 May 2017

Keywords:
Indium oxide nanocubes
Biointeraction
A549 cells
Potential mechanisms
Oxidative stress

ABSTRACT

The demand for semiconductor indium oxide (In_2O_3) nanocrystals is increasing because of their diverse applications, including in biomedicine. However, there is a scarcity of studies on the biological interaction of indium oxide nanocrystals. Here, we explored the underlying mechanisms of toxicity induced by indium oxide nanocubes in human lung epithelial (A549) cells. Prepared indium oxide nanocubes were crystalline with an average size of 21 nm. Biointeraction studies have shown that indium oxide nanocubes induce cell viability reduction and cell membrane damage in a dose- and time-dependent manner. Indium oxide nanocubes were also found to induce reactive oxygen species (ROS) generation, glutathione depletion and lower activity of superoxide oxide dismutase. Further, indium oxide nanocubes induced a mitochondrial membrane potential loss and altered the mRNA expression levels of apoptotic genes (p53, bax, bcl-2, CASP3 & CASP9). The activities of apoptotic enzymes (caspase-3 and −9) were also higher in indium oxide nanocube-treated cells. Finally, we observed that the cytotoxicity and apoptosis induction of indium oxide nanocubes were efficiently prevented by N-acetyl-cysteine. We believe that this is the first report suggesting that indium oxide nanocubes induce toxicity in lung cells *via* ROS generation and oxidative stress. This study warrants future research on the toxicity mechanisms of indium oxide nanoparticles in animal models.

© 2017 Elsevier B.V. All rights reserved.

1. Introduction

Since the last decade, the demand for indium compounds, including indium oxide (In_2O_3) nanoparticles, is growing rapidly because of their application in various fields. Indium oxide nanoparticles are being used in sensing devices, liquid crystal displays and solar cells due to their high electronic conductivity, transparency and mechanical resistance [1,2]. Several forms of indium oxide are being produced and include nanofilms, nanowires, nanopowders and indium-metal complexes [3–5]. Indium oxide is an n-type semiconductor with a wide band gap (~3.6 eV) that has potential to be applied in the biomedical field, including biosensor and tissue imaging [6,7]. Therefore, it is necessary to explore the interaction of indium oxide nanoparticles with biological systems.

Several clinical cases of lung disease caused by the exposure of indium compounds have been reported around the world [8–10]. Jiang et al. [11] recently reported that indium-containing semiconductor nanomaterials induce pro-inflammatory and pro-fibrotic responses in the murine lung. Some investigators have shown that indium-tin-oxide (ITO) nanoparticles induce inflammation, cytotoxicity and genetic damage in lung cells [12–15]. ITO is a sintered material consisting of indium oxide (In_2O_3) and tin oxide (SnO_2) in a ratio of 90:10 (wt: wt). These data are not able to differentiate between the toxicity of ITO nanoparticles and that of tin oxide or indium oxide nanoparticles. Specific studies on the biological response of indium oxide nanoparticles are largely lacking. Only one recent investigation showed that indium oxide nanoparticles cause lung injury to female Wistar rats [16]. This study also suggested that indium oxide nanoparticles should be grouped with highly toxic nanoparticles such as CuO and NiO.

Programmed cell death (apoptosis) might be induced by various extracellular stimuli, including nanoparticles [17,18]. Several genes are known to be involved in the signaling pathway of apoptosis. The tumor suppressor gene p53 acts as a master guardian of

* Corresponding author at: King Abdullah Institute for Nanotechnology, King Saud University, Riyadh 11451, Saudi Arabia.
E-mail addresses: maqusood@gmal.com, mahamed@ksu.edu.sa (M. Ahamed).

http://dx.doi.org/10.1016/j.colsurfb.2017.05.020
0927-7765/© 2017 Elsevier B.V. All rights reserved.

the genome [19]. The bax/bcl-2 gene expression ratio determines the fate (death or life) of cells in response to apoptotic stimuli [20]. Mitochondrial disintegration also leads to the activation of signaling cascades of caspases [21]. The underlying mechanisms of toxicity caused by nanoparticles are still underway. To date, one mechanism frequently discussed is the generation of reactive oxygen species (ROS) and oxidative stress [22]. Nanoparticle-induced ROS generation may lead to oxidative damage of cellular lipids, protein, DNA and ultimately apoptosis [17,23]. There are several semiconductor nanoparticles that can induce cytotoxicity and apoptosis *via* ROS [24,25]. Due to the semiconducting nature of indium oxide nanoparticles, we hypothesized that this material might also induce toxicity in lung cells *via* ROS generation. Hence, this study was designed to explore the potential mechanisms of toxicity caused by indium oxide nanocrystals in human lung epithelial (A549) cells. The A549 cell line has been widely used as an *in vitro* model to examine the pulmonary effects of nanoparticles [26–28].

2. Materials and methods

2.1. Preparation and characterization of indium oxide nanocubes

Indium oxide (In_2O_3) nanocubes were prepared by the thermal treatment of indium oleate as reported in our previous paper [4]. The crystal texture of indium oxide nanocrystals was assessed by X-ray diffraction (XRD) (PANalytical X'Pert) equipped with Cu Kα radiation (λ-1.5406 Å). The surface morphology of indium oxide nanocrystals was observed using a scanning electron microscope (FESEM, JSM-7600F, JEOL, Inc., Japan). The shape and size of the nanocrystals were determined by field emission transmission electron microscopy (FETEM, JEM-2100F, JEOL, Inc., Japan). The hydrodynamic size and zeta potential of indium oxide nanocrystals in distilled water and culture medium were measured by ZetaSizer Nano-HT (Malvern Instruments, UK) as described by Murdock et al. [29].

2.2. Cell culture and treatment of indium oxide nanocubes

The human lung epithelial (A549) cell line was bought from American Type Culture Collection (ATCC) (Manassas, VA). Cells were cultured in DMEM medium supplemented with 10% fetal bovine serum (FBS) and 100 U/ml penicillin-streptomycin at 5% CO_2 and 37 °C. At 80–90% confluence, cells were harvested using 0.25% trypsin and were sub-cultured in 96-well plates or 6-well plates according to the selection of experiments.

Indium oxide nanocubes were suspended in DMEM medium and were prepared at different dilutions (5–100 μg/ml). Next, nanocrystal suspensions were sonicated for 10 min at 40 W to avoid agglomeration before treatment to cells. In a few experiments, the antioxidant N-acetyl-cysteine (NAC) (2 mM) was co-exposed with or without indium oxide nanocubes. For some parameters, ZnO nanoparticles (50 μg/ml) were used as a positive control [30,31].

2.3. Assay of cytotoxicity markers

Briefly, 1×10^4 cells were seeded in 96-well plates and were allowed to attach to the surface for 24 h. Next, cells were treated with various concentrations (5–100 μg/ml) of indium oxide nanocubes for different time intervals (24–72 h). At the end of the exposure time, cells were harvested to carry out the MTT and LDH assays. Cell viability against indium oxide nanocubes exposure was measured by the MTT assay [30] with some modifications to avoid the interference of nanocrystals [27]. This method assesses the function of mitochondria by measuring the potential of live cells to reduce MTT into blue formazan product. Lactate dehydrogenase (LDH) enzyme leakage due to rupture of the cell membrane was measured using a commercial kit (Bio-Vision, Inc., Milpitas, California, USA) [31].

2.4. Assay of oxidative stress markers

In brief, 1×10^4 cells were seeded in 96-well plates and were allowed to attach to the surface for 24 h. Next, cells were treated with indium oxide nanocubes at the concentrations of 25 and 50 μg/ml for 24 h. After the completion of the exposure time, the oxidative stress parameters were determined. The intracellular reactive oxygen species (ROS) level was measured using 2,7-dichlorofluorescin diacetate (DCFH-DA) fluorescent probe as described by Wang and Joseph [32] with some specific changes [33]. The ROS level was assessed by two different methods. First, the quantitative level of ROS was measured using a microplate reader (Synergy-HT, BioTek). Second, fluorescence images of the DCF-DA probe in control and treated cells were captured using a fluorescence microscope (OLYMPUS CKX 41). The intracellular GSH level was quantified by Ellman's reagent [34]. Superoxide dismutase (SOD) enzyme activity was measured using a commercially available kit (Cayman Chemical Company, Michigan, OH, USA) [31].

2.5. Cell morphology

The A549 cells were exposed to indium oxide nanocubes (100 μg/ml for 24 h) in the presence or absence of N-acetyl-cysteine (NAC) (2 mM). After exposure, the morphology of the control and treated cells was examined by a phase-contrast microscope (Leica DMIL, Germany).

2.6. Assay of apoptotic markers

For the measurement of the mitochondrial membrane potential (MMP), cells were cultured in 96-well plates (1×10^4 cells/well) and treated with indium oxide nanocubes (25 & 50 μg/ml for 24 h) in the presence or absence of N-acetyl-cysteine (NAC) (2 mM). The MMP level was assessed using Rhodamine-123 (Rh-123) fluorescent dye as reported by Siddiqui et al. [33]. The MMP level was measured by two different techniques: first, by quantitative analysis of MMP using a microplate reader (Synergy-HT, BioTek) and second, by fluorescence imaging using an Rh-123 probe within cells and a fluorescence microscope (OLYMPUS CKX 41).

For the analysis of the mRNA level of apoptotic genes (p53, bax, bcl-2, CASP3 & CASP9), cells were cultured in 6-well plates (5×10^4 cells/well) and were treated with indium oxide nanocubes at a concentration of 25 μg/ml for 24 h in the presence or absence of NAC (2 mM). After the completion of the exposure time, total RNA was extracted from cells using the Qiagen RNeasy mini Kit (Valencia, CA). cDNA was synthesized from total RNA by reverse transcriptase using M-MLV (Promega, Madison, WI) and oligo (dT) primers (Promega). Quantitative real-time PCR was performed using the QuantiTect SYBR Green PCR kit (Qiagen) and the ABI PRISM 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA). The specific sets of primers for p53, bax, bcl-2, CASP3, CASP9 and β-actin were mentioned in our previous work [27]. The expression levels of genes were normalized to those of the β-actin gene (control).

For the assay of caspase-3 and caspase-9 enzymes, cells were cultured in 96-well plates (1×10^4 cells/well) and were treated with indium oxide nanocubes (25 μg/ml for 24 h). The activity of these enzymes was assessed using a colorimetric kit (Bio-Vision, Inc., Milpitas, California, USA) [27].

Download English Version:

https://daneshyari.com/en/article/4983269

Download Persian Version:

https://daneshyari.com/article/4983269

Daneshyari.com