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Cerium dioxide (CeO_2) nanoparticles decrease arsenite (As(III)) cytotoxicity to 16HBE14o- human bronchial epithelial cells

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ABSTRACT

The production and application of engineered nanoparticles (NPs) are increasing in demand with the rapid development of nanotechnology. However, there are concerns that some of these novel materials could lead to emerging environmental and health problems. Some NPs are able to facilitate the transport of contaminants into cells/organisms via a "Trojan Horse" effect which enhances the toxicity of the adsorbed materials. In this work, we evaluated the toxicity of arsenite (As(III)) adsorbed onto cerium dioxide (CeO_2) NPs to human bronchial epithelial cells (16HBE14o-) using the xCELLigence real time cell analyzing system (RTCA). Application of 0.5 mg/L As(III) resulted in 81.3% reduction of cell index (CI, an RTCA measure of cell toxicity) over 48 h when compared to control cells exposed to medium lacking As(III). However, when the cells were exposed to 0.5 mg/L As(III) in the presence of CeO_2 NPs (250 mg/L), the CI was only reduced by 12.9% compared to the control. The CeO_2 NPs had a high capacity for As(III) adsorption (20.2 mg/g CeO_2) in the bioassay medium, effectively reducing dissolved As(III) in the aqueous solution and resulting in reduced toxicity. Transmission electron microscopy was used to study the transport of CeO_2 NPs into 16HBE14o- cells. NP uptake via engulfment was observed and the internalized NPs accumulated in vesicles. The results demonstrate that dissolved As(III) in the aqueous solution was the decisive factor controlling As(III) toxicity of 16HBE14o- cells, and that CeO_2 NPs effectively reduced available As(III) through adsorption. These data emphasize the evaluation of mixtures when assaying toxicity.

1. Introduction

Nanoparticles (NPs) are defined as materials with at least one dimension between 1 and 100 nm in size. Their extremely small size gives NPs unique electronic, optical and chemical properties compared to their bulk counterparts (Klaine et al., 2008; Navarro et al., 2008). With the rapid development of nanotechnology, NP production and subsequent applications are growing continuously. The global production of engineered NPs is estimated to be higher than 10 million tons per year (Holden et al., 2014). The first Nanotechnology Consumer Product inventory was created in 2005, listing 54 products containing nanomaterials; in 2015, the inventory listed 1814 products from 622 companies located in 32 countries (Vance et al., 2015). With the growing production and application of engineered NPs, environmental and unintentional human exposure is also increasing (Gottschalk and Nowack, 2011; Gottschalk et al., 2013; Keller and Lazareva, 2014; Kuhlbusch et al., 2011; Sun et al., 2014). Therefore, there are concerns that release of these novel materials may lead to emerging health problems.

So far most research has focused on the potential ecological impacts and human health effects resulted from pristine NPs (Buzea et al., 2007; Lowry et al., 2012; Nowack and Bucheli, 2007; Seaton et al., 2010), however, in the environment, NPs are present with different types of contaminants including toxic materials such as metals and metalloids. Due to the large specific surface area and surface reactivity characteristic of NPs, different contaminants could accumulate on the surface of NPs and subsequently affect the fate and toxicity of these materials. It has been reported that some NPs could act as "Trojan Horses", enhancing the toxicity of adsorbed materials by facilitating their transport into cells or organisms. For example, lead (Pb) loaded on cerium dioxide (CeO_2) or titanium dioxide (TiO_2) NPs in the gastrointestinal tract of the water flea, *Ceriodaphnia dubia*, enhanced the bioavailability of Pb, resulting in a higher toxicity (Hu et al., 2012b). Enhanced toxic effects have been observed in experiments using arsenate (As(V)) in the presence of ferric oxide (Fe_2O_3), aluminum oxide (Al_2O_3) or TiO_2 NPs (Hu et al., 2012a; Wang et al., 2011). In a different study, nano-

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diamonds were shown to facilitate the transport of adsorbed heavy metals (*e.g.* Cu^{2+}) into living cells, causing subsequent release of ions in the interior of cells and leading to oxidative stress (*i.e.*, generation of reactive oxygen species (ROS)) and cytotoxicity (Zhu et al., 2015). Similarly, Limbach et al. (2007) observed enhanced ROS production in lung epithelial cells exposed to cobalt and manganese in the presence of nano-SiO_2 compared to the silica-free controls free of SiO_2 NPs. While information about the toxicity of NPs is valuable, it is also important to understand the synergistic effect of these materials and contaminants occurring together in the environment.

Arsenic is a well-known contaminant with high toxicity and carcinogenicity (ATSDR, 2007). Acute exposure to As can cause effects range from gastrointestinal distress to death; chronic As exposure could affect several major organ systems based on the dose (Hughes et al., 2011). The World Health Organization guideline of As in drinking water is set to 10 μg/L, while a number of large aquifers with As concentrations significantly higher than 50 μg/L have been identified in different parts of the world (Smedley and Kinniburgh, 2002). In these regions, a significant relationship between consumption of As contaminated water and increased risks of lung diseases/cancer was found (Ferreccio et al., 2000; Smith et al., 2000; Xie et al., 2014). In natural water, inorganic trivalent arsenite (As(III)) and pentavalent As(V) in the forms of oxyanions are the most predominant As species.

Different NPs have been reported to have outstanding capacity for As(III) and As(V) adsorption (Cui et al., 2012; Feng et al., 2012; Hristovski et al., 2007; Jegadeesan et al., 2010), among them, cerium dioxide (CeO_2) is an important industrial material with the annual global production about 7500 to 10,000 t (Holden et al., 2014; Keller and Lazareva, 2014). CeO_2 is unique with the ease of switching the valence state (between +3 and +4 state) in favorable environment. Due to this high oxygen mobility, CeO_2 NPs are widely use in catalyst, fuel additives and medical applications (Kumar et al., 2014). In addition, a primary application of CeO_2 NPs is chemical and mechanical planarization (CMP), a key process applied to polish wafers when fabricating integrated circuits (Krishnan et al., 2010). In CMP, NPs such as CeO_2 are used in the slurry as an abrasive to remove unwanted materials on the wafer and create a flat surface. The semiconductor industry requires large amounts of water, consequently generating high volumes of wastewater. The waste stream from CMP contains high concentrations of inorganic oxide NPs and other chemicals present in the original slurry (*e.g.*, oxidizers, surfactants, dispersants, corrosion inhibitors) as well as soluble species removed from the wafer. Arsenic containing semiconductor materials such as indium arsenide (InAs) and gallium arsenide (GaAs) are increasingly used in light emitting diodes (LEDs), liquid crystal displays (LCDs), and photovoltaics biosensors and microcircuits due to their high electron mobility, attractive optoelectric properties, and low power requirements (Dayeh et al., 2009; Dick et al., 2010; Yamaguchi et al., 2008). The introduction of arsenic-containing materials in semiconductor manufacturing is expected to result in CMP wastewaters that also contain toxic soluble arsenic species. The potential that inorganic oxide NPs in CMP effluents (*e.g.* CeO_2) may act as carriers of toxic arsenic species is a concern. However, information about the effect of CeO_2 NPs on arsenic transport and toxicity is still lacking.

In vitro cytotoxicity assays are common alternatives to animal tests in toxicity assessment (Xing et al., 2005). Conventional cytotoxicity assays (*e.g.* MTT assay) depend on absorbance, fluorescence or luminescence measurements. These methods have a defect as the test results can be greatly obscured when measuring materials (*e.g.* mesoporous SiO_2 NPs) that tend to interfere with optical measurements (Fisichella et al., 2009; Ke et al., 2011). Also, single end-point assays provide only limited information about the interaction between testing materials and the target cells. The xCELLigence real time cell analysis (RTCA) system is a novel label-free, dynamic and high throughput technique for cytotoxicity and cell viability assessment. In this system, the biological status of adherent cells is monitored through impedance measurements (Atienza et al., 2006). Since impedance determination is not invasive, the cells remain in their normal physiological state during the assay. This system has been applied in different studies investigating the toxicity of arsenic, mercury, sodium dichromate (Xing et al., 2005) and inorganic nanoparticles (Otero-Gonzalez et al., 2012), among many other toxicants. Although RTCA measurements are reliable and highly sensitivity in cytotoxicity assessment (Limame et al., 2012), a disadvantage of this toxicity bioassay is its inability to provide mechanistic information. Nonetheless, several studies have demonstrated a strong correlation between RTCA measurements for different toxicants and results obtained in conventional cytotoxicity bioassays (*e.g.* MTT assay) (Otero-Gonzalez et al., 2012; Xing et al., 2005).

The objective of this study was to investigate the synergistic toxic effect of CeO_2 NPs and As(III). To this end, RTCA system was used to assess the cytotoxicity of As(III) on human bronchial epithelial cells (16HBE14o-) in the presence of CeO_2 NPs. In addition, CeO_2 uptake by 16HBE14o- cells was investigated using transmission electron microscopy (TEM). This study intends to better understand potential risks from NPs with the presence of other contaminants in the environment.

2. Materials and methods

2.1. Materials

CeO_2 NP powder (20 nm) was obtained from MTI Corporation (Richmond, CA, USA). Minimum essential medium with Earle's salts (MEM) were purchased from Invitrogen (Carlsbad, CA, USA). Fetal bovine serum (FBS) and sodium meta-arsenite ($NaAsO_2$, ≥ 90%) were from Sigma-Aldrich (St Louis, MO, USA). In the experiments, all the solutions were prepared using ultrapure water (Milli-Q Water System, Millipore, Billerica, MA, USA).

2.2. Cell culture

In this work, 16HBE14o- cells were obtained from California Pacific Medical Center Research Institute (San Francisco, CA, USA). The cells were initially grown as described elsewhere (Flynn et al., 2011). In brief, cells were grown in tissue culture flasks coated with a collagen/fibronectin/bovine serum albumin (CFB) matrix in a controlled growth medium (CGM) that contains MEM supplemented with 10% (v/v) FBS, 2 mM glutamax, penicillin and streptomycin at 37 °C in a 5% CO_2 atmosphere. Subsequently, the cells were transferred to RTCA assay plates coated with CFB and maintained with a reduced serum (5% FBS) medium.

2.3. Characterization of CeO_2 NPs

The primary particle size of CeO_2 NPs was determined by TEM as describe below in Section 2.8. In addition, the particle size and zeta potential (ζ potential) of the NPs (500 mg/L) in MEM were determined. The hydrodynamic particle size was measured by dynamic light scattering (DLS) using a Zetasizer Nano ZS (Malvern Instruments, Sirouthborough, MA, USA) with a laser wavelength of 633 nm and a scattering angle of 173°. ζ potential was determined by electrophoresis using the same equipment. The Smoluchowski equation was applied to correlate particle electrophoretic mobility to ζ potential value.

2.4. As(III) adsorption on CeO_2 NPs

Firstly, an As(III) stock solution (160 mg/L) was prepared, and the pH of the solution was adjusted to near 7.0 using diluted HCl. Then the solution was diluted to 16.0, 8.0, 1.6, 0.8 and 0.16 mg/L using serum-free MEM supplemented with 2 mM glutamax, penicillin and streptomycin in 50 mL centrifuge tubes with total liquid volume of 10 mL. Finally, 250 mg/L CeO_2 NPs were added into each tube. The dispersions were mixed for 48 h using an orbital shaker at 150 rpm at room