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Gene expression profiles reveal distinct immunological responses of cobalt and cerium dioxide nanoparticles in two *in vitro* lung epithelial cell models

Sandra Verstraelen[a,*], Sylvie Remy[a], Eudald Casals[b], Patrick De Boever[a,d], Hilda Witters[a], Antonietta Gatti[c], Victor Puntes[b], Inge Nelissen[a]

[a] Flemish Institute for Technological Research (VITO NV), Environmental Risk and Health Unit, Mol, Belgium
[b] Institut Català de Nanotecnologia (ICN), Barcelona, Spain
[c] Università di Modena e Reggio Emilia, Laboratorio Biomateriali, Modena, Italy
[d] Hasselt University, Centre for Environmental Sciences, Diepenbeek, Belgium

HIGHLIGHTS

- Gene expression study using A549 and BEAS-2B cells exposed to CoO- and CeO2-NPs.
- Different kinetics of cell responses induced by the NPs between the lung models.
- Mainly downregulation of gene transcription for both CoO- and CeO2-NPs.
- Heterogeneous transcriptional responses among the cell types and NPs.
- Between 1-14% differentially expressed transcripts involved in immune processes.

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ABSTRACT

Fragmentary knowledge exists on cellular signaling responses underlying possible adverse health effects of CoO- and CeO_2-nanoparticles (NP)s after inhalation.

We aimed to perform a time kinetic study of gene expression profiles induced by these NPs in alveolar A549 and bronchial BEAS-2B epithelial cells, and investigated possible immune system modulation. The kinetics of the cell responses induced by the NPs were different between the lung epithelial models. Both CoO- and CeO_2-NP exposure induced mainly downregulation of gene transcription. BEAS-2B cells were found to be more sensitive, as they showed a higher number of differentially expressed transcripts (DET) at a 10-fold lower NP-concentration than A549 cells. Hierarchical clustering of all DET indicated that the transcriptional responses were heterogeneous among the two cell types and two NPs. Between 1% and 14% DET encoding markers involved in immune processes were observed.

The transcriptional impact of the metal oxide NPs appeared to be cell-dependent, both at the general and immune response level, whereas each lung epithelial cell model responded differently to the two NP types. Thus, the study provides gene expression markers and immune processes involved in CoO- and CeO_2-NP-induced toxicity, and demonstrates the usefulness of comprehensive–omics studies to differentiate between NP responses.

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1. Introduction

Nanotechnology is widely considered to be one of the most promising technologies of the 21st century and it has entered our society in many aspects. The increasing use of nanoparticles (NP)s in consumer products, industrial processes, biological, and medical applications has raised concerns with respect to their potential health impact.

Cobalt oxide (CoO)-based NPs reside between the most promising materials for technological applications like information

* Corresponding author. Flemish Institute for Technological Research (VITO NV) Environmental Risk and Health Unit Boeretang 200 2400 Mol Belgium. Tel.: +32 0 14 33 51 07; fax: +32 0 14 58 05 23.
E-mail addresses: sandra.verstraelen@vito.be (S. Verstraelen), sylvie.remy@vito.be (S. Remy), eudald.casals@icn.cat (E. Casals), patrick.deboever@vito.be (P. De Boever), hilda.witters@vito.be (H. Witters), gatti@nanodiagnostics.it (A. Gatti), victor.puntes@icn.cat (V. Puntes), inge.nelissen@vito.be (I. Nelissen).

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storage, magnetic fluids, and catalysts (Puntes et al., 2001), while cerium dioxide (CeO_2) NPs are mainly designed to be used in a dispersive manner as a diesel fuel combustion catalyst (Cassee et al., 2011). In a recent paper, the annual United States production of CeO_2-NPs was estimated at 35 - 700 tons per year (Hendren et al., 2011). Furthermore, the Working Party on Manufactured Nanomaterials (WPMN) of the Organization for Economic Co-operation and Development (OECD) included CeO_2-NPs as one of the thirteen priority listed representative manufactured nanomaterials for safety testing (OECD, 2010). No data on production volume was found for CoO-NPs.

Since nanoscale CoO and CeO_2 are used in automotive exhaust catalysts, the particles may get released into the air, thus may be inhaled by humans. Besides alveolar macrophages, bronchial and alveolar epithelial cells are among the principal cells that get into contact with airborne NPs which penetrate in the lung. As a consequence of this interaction these cells are capable of producing pro-inflammatory mediators that have the ability to elicit both a local and systemic inflammatory response (Lambrecht and Hammad, 2010; Verstraelen et al., 2008). Fragmentary knowledge exists on the adverse health effects of CoO- and CeO_2-NPs following exposure *via* inhalation or other routes, and is generally based on *in vivo* and *in vitro* studies using standard toxicological endpoints.

CoO-NPs induced an increase of tumor necrosis factor-alpha and interferon-gamma release and an inhibition of interleukin (IL)-10 and IL-2 in peripheral blood mononuclear cells after *in vitro* exposure (Petrarca et al., 2006). There are numerous cases of occupational workers who developed adverse lung effects, such as interstitial lung disease or pneumoconiosis, associated with accumulation of cerium in the lungs after prolonged exposure to cerium fumes or dust (Yoon et al., 2005; McDonald et al., 1995). *In vivo* studies using rats showed that CeO_2-NPs induced cytotoxicity *via* oxidative stress, were inflammogenic to the lungs, and may lead to fibrosis (Ma et al., 2011; Srinivas et al., 2011). *In vitro* studies using nanoscale CeO_2 have shown that these metal oxides trigger several reactions that are cell line dependent (A549 and L-132) (Kim et al., 2010). Nano-CeO_2 induced cytotoxicity and oxidative stress in human bronchial (BEAS-2B) and alveolar (A549) epithelial cells (Lin et al., 2006; Park et al., 2008; Eom and Choi, 2009). Other studies using HEK and HT22 cells reported CeO_2-NPs to exhibit anti-oxidant properties (Karakoti et al., 2008; Schubert et al., 2006).

In view of the above described observations of pulmonary effects triggered by metal oxide NPs, we here selected the human alveolar epithelial cell line A549 and the transformed bronchial epithelial cell line BEAS-2B, representing different anatomical regions of the lung, as *in vitro* models to study the molecular response of these cell lines after a short-term exposure to CoO- or CeO_2-NPs. These epithelial cell lines are widely being used for *in vitro* assessment of health impact of nano-sized particles (Eom and Choi, 2009; Veranth et al., 2007; Barillet et al., 2010; Baber et al., 2011; Eom and Choi, 2011; Panas et al., 2012). The cell lines are considered as useful models for integration in a NP hazard assessment approach as part of global harmonization and standardization efforts (OECD).

In this study, we used genome-wide gene expression analysis in combination with comprehensive bioinformatics to identify and compare molecules and cellular pathways that are triggered by CoO- and CeO_2-NP exposure in the two lung cell models. We aimed to investigate whether or not such transcriptional signatures are NP- and/or cell type-dependent.

2. Materials and Methods

2.1. Synthesis of nanoparticles

Two different kinds of colloidal NPs were synthesized by wet chemistry methods and redispersed in the aqueous phase using milli-Q grade water. All reagents were purchased from Sigma–Aldrich (St. Quentin Fallavier, France) and used as received. Glass material was sterilized and depyrogenated prior to use to reduce the levels of lipopolysaccharide (LPS) and other contaminants in the NP preparations. Two different batches of NP suspensions and their pristine solvents were synthesized for the exposure studies in A549 and BEAS-2B cell line, respectively.

CoO-NPs of 7 nm mean diameter were prepared in an organic solvent under controlled Ar-atmosphere, based on the thermal decomposition of cobalt carbonyl ($Co_2(CO)_8$) in o-dichlorobenzene in the presence of oleic acid and trioctylphosphine oxide. This synthesis resulted in size-controlled Co-NPs in organic phase (Puntes et al., 2001), which were transferred to water using aqueous 1 mM tetra-methyl ammonium hydroxide (TMAOH) to exchange the NPs surfactant and thus render the particles water-soluble (Euliss et al., 2003). Under these conditions (exposed to air and wet) the Co-NPs slowly evolve toward Co oxide mirroring natural conditions. Sometimes the oxidation is not complete and a little metallic Co core may remain inside the particle, but the surface is definitively oxidized.

The CeO_2 particles were synthesized by the non-isothermal precipitation procedure with some modifications (Chen and Chang, 2005; Zhou et al., 2002). This method has two stages: precipitation and aging. In the first one (precipitation), 50 ml of cerium(III) nitrate ($Ce(NO_3)_3{\cdot}6H_2O$) solution (0.02 M) were set to 70 °C and 25 ml TMAOH solution (1 M) were then added under constant stirring. As soon as the TMAOH was added, the formation of white precipitates was observed. This stage lasted for 5 minutes to oxidize the Ce(III)–Ce(IV) (Chen and Chang, 2005). Followed by the second stage (aging): the solution was rapidly transferred into another water bath, in which the reaction was continued at 50 °C for 20 hours. Then, the products were centrifuged, washed and resuspended in 50 ml TMAOH 1 mM which confered the colloidal stability to the formed CeO_2-NPs.

2.2. Characterization of nanoparticles

Transmission Electron Microscopy (TEM) images were acquired with a JEOL 1010 electron microscope operating at an accelerating voltage of 80 kV (low voltage beams help to obtain enough contrast to observe these small NPs, even the less dense). Samples for TEM were prepared by drop casting on carbon-coated copper TEM grids. The grids were left to dry at room temperature. Observations were made on different parts of the grid and with different magnifications and more than 400 particles were computer-analyzed and measured for the size distribution.

Z-Potential measurements were made with a Malvern Zeta-Sizer Nano ZS instrument operating at a light source wavelength of 532 nm and a fixed scattering angle of 173° for Z-Potential measure. The colloidal suspension (0.8 ml) of NPs was placed into the specific cuvette, and the software was arranged with the specific parameters of refractive index and absorption coefficient of the material of the NPs, and the viscosity of the solvent, data required to obtain the correct value for each type of NP.

X-Ray Diffraction (XRD) spectra were acquired with a PANalytical XíPert diffractometer that uses a copper and Co Kα radiation source. Samples for XRD consisted of the NPs in powder form. For this purpose, destabilization of the NPs mixing the colloid with a solvent of different polarity was followed by soft centrifugation after which NPs precipitated. The supernatant was discarded, and the pellet of NPs was dried to eliminate all the moisture.

The stability of NPs in complete cell culture media (CCM), determining their interaction with biological entities, was studied. Using Dynamic Light Scattering (DLS) and Z-potential measurements, and TEM analyses CeO_2- and CoO-NPs as synthesized (in pristine solvent) and after 24 hours in complete CCM (RPMI medium + 10% fetal bovine serum) at 37 °C were monitored.

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