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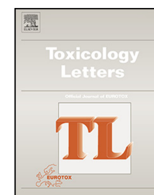

Toxicity of nanoparticles embedded in paints compared to pristine nanoparticles, *in vitro* study

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HIGHLIGHTS

- Toxic effects of pristine ENPs were compared to those embedded in a paint matrix.
- The paint matrix was aged, whether or not containing ENPs (TiO_2, Ag and SiO_2).
- Toxicity was assessed in a tri-culture model.
- Pristine ENPs show some toxic effects in our *in vitro* model.
- No additional significant toxic effects were observed in paints containing ENPs.

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ABSTRACT

The unique physicochemical properties of nanomaterials has led to an increased use in the paint and coating industry. In this study, the *in vitro* toxicity of three pristine ENPs (TiO_2, Ag and SiO_2), three aged paints containing ENPs (TiO_2, Ag and SiO_2) and control paints without ENPs were compared. In a first experiment, cytotoxicity was assessed using a biculture consisting of human bronchial epithelial (16HBE14o-) cells and human monocytic cells (THP-1) to determine subtoxic concentrations. In a second experiment, a new coculture model of the lung–blood barrier consisting of 16HBE14o- cells, THP-1 and human lung microvascular endothelial cells (HLMVEC) was used to study pulmonary and extrapulmonary toxicity. The results show that the pristine TiO_2 and Ag ENPs have some cytotoxic effects at relative high dose, while pristine SiO_2 ENPs and all aged paints with ENPs and control paints do not. In the complex triculture model of the lung–blood barrier, no considerable changes were observed after exposure to subtoxic concentration of the different pristine ENPs and paint particles. In conclusion, we demonstrated that although pristine ENPs show some toxic effects, no significant toxicological effects were observed when they were embedded in a complex paint matrix.

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1. Introduction

Nanomaterials are increasingly being used in a broad range of applications in the construction industry (Hanus and Harris, 2013). Addition of metal oxide engineered nanoparticles (ENPs) and carbon nanotubes to concrete can improve structural efficiency, durability and strength, while incorporation of nanomaterials in solar cells reduces costs and increases the energy conversion efficiency (Guo, 2011). Lately, extensive research has been done on the development of new coating and paint systems for wood, metals, ceramics, natural stone, concrete, composites and plastics

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(Hanus and Harris, 2013). Incorporation of ENPs give rise to paints and coatings with for example anti-UV (TiO_2, ZnO), self-cleaning (TiO_2), antimicrobial (Ag, TiO_2), fire-resistant (SiO_2, CNT) and scratch-resistant (SiO_2, CNT) effects (Hanus and Harris, 2013; Lee et al., 2010; Pacheco-Torgal and Jalali, 2011). In order to reduce the prevalence of illness and discomfort, antimicrobial coatings already have applications in hospital environments, childcare centers and nursing homes (Hanus and Harris, 2013). Currently, coatings, paints and pigments are the most important applications of ENPs in terms of overall use (Keller et al., 2013). A study of Mueller and Nowack in 2008 showed that 35% of the nano-Ag production and 25% of the nano-TiO_2 production in Europe are used by the paint and coating industry, making this sector the first end-user of nano-Ag and the second end-user of nano-TiO_2 (Mueller and Nowack, 2008).

Exposure to ENPs used in paints and coatings can occur during production, handling and application of the paint on a surface or after aging. Different inhalation and instillation studies already have shown the infiltration of inflammatory cells, release of pro-inflammatory cytokines and oxidative stress in the lung after exposure to ENPs (Napierska et al., 2010; Shi et al., 2013). Moreover, ENPs have pro-trombotic effects and are able to induce endothelial dysfunction (Vesterdal et al., 2010).

Both monoculture and complex coculture *in vitro* models of the lung are being used or are in development to study respiratory toxicity. An overview on the state-of-the-art of relevant lung cel6l-based *in vitro* assays, which are currently under development for the evalutation of particles and chemicals can be found in the review paper of Klein et al. (2011). Recently, in our lab, a new *in vitro* coculture model of the lung–blood barrier was developed and validated by Luyts et al. (2014). The triculture model consists of human bronchial epithelial cells (16HBE14o-) and human monocytic THP-1 cells representing the pulmonary epithelium and macrophages respectively. Human lung microvascular endothelial cells (HLMVEC) were used as a model for the cells of the pulmonary circulation. Epithelial and endothelial cells were grown on opposite sides of a Transwell insert membrane until confluence while the monocytes were added before the experiment. A schematic overview of the model setup can be found in Fig. 1. The combination of the different cell types in this model form a good representation of the lung–blood barrier as it is *in vivo*. The model can be used to assess the toxicity of ENPs; different parameters can be measured to assess pulmonary and extrapulmonary toxicity including barrier functionality, inflammation, oxidative stress and endothelium activation.

Although a lot of research has been performed regarding the toxic effects of pristine ENPs, little is known about their toxicity when they are embedded in a complex paint or coating matrix. In this study, we compared the *in vitro* toxicity of three pristine ENPs

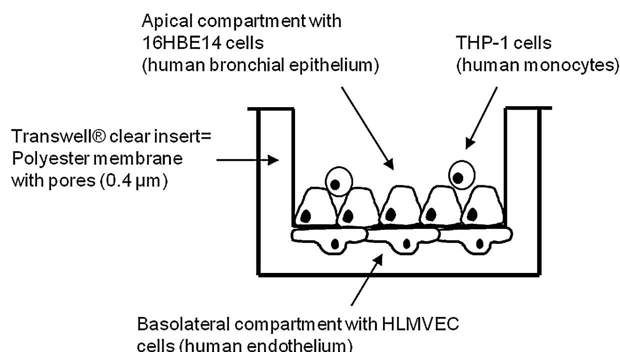

Fig. 1. *In vitro* triculture model setup.
Triculture model of the lung–blood barrier consisting of human bronchial epithelial (16HBE14o-) cells, human monocytic cells (THP-1) and endothelial cells (HLMVEC).

(TiO_2, Ag and SiO_2), three aged paints containing ENPs (TiO_2, Ag and SiO_2) and corresponding control paints without ENPs.

In a first experiment, the cytotoxicity of the different particles was assessed using a biculture consisting of 16HBE14o- cells and THP-1 cells. In a second experiment, a coculture model of the lung–blood barrier consisting of 16HBE14o- cells, THP-1 and HLMVEC, developed and validated by Luyts et al. (2014), was used to study pulmonary and extrapulmonary toxicity.

2. Materials and methods

2.1. Materials

Pristine ENPs (TiO_2, Ag and SiO_2), paints containing these ENPs and control paints without ENPs were provided by industrial project partners. Dulbecco's Modified Eagle Medium:Nutrient Mixture F-12 (DMEM/F12), Roswell Park Memorial Institute 1640 (RPMI) medium, Hank's balanced salt solution (HBSS), phosphate buffered saline (PBS), penicillin–streptomycin, fungizone, L-glutamine, fetal calf serum (FCS), and Transwell® membrane inserts were purchased from Sigma–Aldrich (Bornem, Belgium). Human low density lipoprotein was purchased from Stemcell Technologies (Grenoble, France).

2.2. Manufacturing aged powder paint particles

Paints were applied on a plastic panel using a film applicator creating a uniform film of 200 μm thickness. After drying for 24 h at room temperature (20 °C), paints were removed manually using a metallic spatula to obtain a powder paint. Then, powders were milled using a planetary ball mill PM 100 (Retsch, Haan, Germany) and finally exposed to UV-A (Philips TL20W/09N) as an aging process.

2.3. Experimental strategy

The *in vitro* cytotoxicity was assessed using a biculture consisting of human bronchial epithelial (16HBE14o-) cells and human monocytic cells (THP-1). The cells were exposed to particles (3-fold serial dilution: 0, 1, 3, 9, 27, 81 and 243 μg/ml) during 24 h. Cytotoxicity was assessed with two different assays: lactate dehydrogenase (LDH) release (Napierska et al., 2009) and WST-1 assay.

Subsequently, based on the previous experiment, 2 subtoxic concentrations were determined for each particle that were used in a triculture model of the lung–blood barrier consisting of human bronchial epithelial (16HBE14o-) cells, human monocytic cells (THP-1) and endothelial cells (HLMVEC) (Fig. 1). Different parameters including barrier integrity, inflammation and oxidative stress were investigated.

2.4. Particle preparation

All particles were dispersed in Baxter water (stock concentration: 4.86 mg/ml) and sonicated using a Microson™ ultrasonic cell disruptor (Misonix, Newtown, USA) during 16 min at 400 W. Subsequently, the particles were diluted 10-fold in the culture medium to obtain final concentrations.

2.5. Cell culture

16HBE14o- cells (16HBE), kindly provided by Dr. Gruenert (University of California, San Fransisco, USA) were cultured in DMEM/F12 medium supplemented with 5% FBS, 100 U/ml penicillin, 100 μg/ml streptomycin, 2 mM L-glutamine and 2.5 μg/ml fungizone. Human lung microvascular endothelial cells (HLMVEC)

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