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Exposure to metal oxide nanoparticles in physiological fluid induced synergistic biological effects in a keratinocyte model

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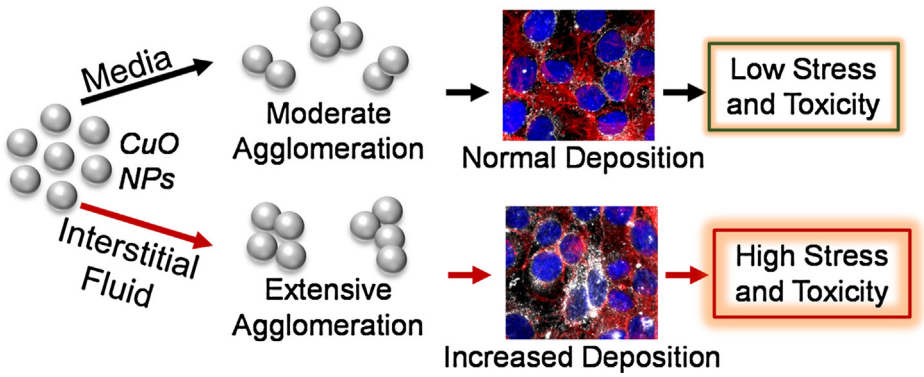
HIGHLIGHTS

- Synergistic toxicity occurred during joint CuO NP and interstitial fluid exposure.
- This observed toxicity correlated with increased NP agglomeration and deposition.
- Physiological fluids provide a means to characterize NPs in relevant environments.

GRAPHICAL ABSTRACT



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ABSTRACT

Nanoparticles (NPs) possess distinctive physicochemical properties that in addition to differentiating them from their bulk counterparts can induce negative cellular consequences. Standard in vitro systems have served as the primary model for NP safety evaluations, but suffer from a lack physiological relevance. One way to overcome this limitation and evaluate NP characteristics under more accurate conditions is through the use of artificial physiological fluids, which mimic the composition of in vivo environments. In this study, we identified that copper oxide (CuO) and titanium dioxide (TiO_2) NPs displayed modified behavior when dispersed in artificial interstitial fluid (IF) versus traditional media, including extensive agglomeration and increased particle deposition. When keratinocyte cells underwent CuO NP exposure, synergistic stress and toxicity responses occurred within an IF environment, correlating with augmented particle deposition. However, following IF incubation alone or concurrently with TiO_2 NPs, which are not innately toxic, no combinatorial responses were identified. These results indicate that synergistic outcomes arise when toxic NPs undergo fluid-induced alterations to key physicochemical properties and behaviors. This study highlights the necessity of carrying out NP characterization and safety assessments in physiologically-representative environments; as altered behavior patterns have the potential to induce bioresponses not identified within traditional models.

1. Introduction

Recently, nanoparticle (NP) usage has dramatically grown in a number of markets, including consumer, medical, energy, and industrial (Gupta et al., 2015). NP physicochemical properties differ

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from their bulk counterparts, resulting in NP-specific behaviors such as increased transport potential and catalytic activity. These traits, along with the ability to specify NP composition, size, and surface chemistry, make nano-sized materials advantageous for product development and applications. For example, copper oxide (CuO) NPs act as a catalyst in numerous materials, including propellants, ceramics, and semiconductors (Li et al., 2013; Molteni et al., 2006). Nano-sized titanium dioxide (TiO_2) is frequently used as a pigment, making it one of the most predominantly employed NPs (Shi et al., 2013). The majority of the TiO_2 produced for pigment is used in paint, but also includes cosmetic, pharmaceutical, and food applications (Weir et al., 2012).

This exponential growth of NPs in everyday life increases the likelihood of potentially harmful exposure to these materials, with consequences yet to be fully elucidated or characterized. This uncertainty has produced the need for extensive toxicological investigations in order to determine health and safety procedures as well as to establish recommended exposure limits (Hussain et al., 2015). Further complications arise from the extreme variability of NPs themselves, including tunable parameters such as size, shape, surface modification, and composition. Previous studies have identified that modification to any of these NP characteristics directly impacts observed biological consequences (Kim et al., 2015; Podila and Brown, 2013). NPs are able to induce toxicological, stress, inflammatory, signal transduction, and genetic modifications; highlighting the far-reaching health consequences following NP exposure (Cho et al., 2011a; Podila and Brown, 2013). To further complicate the matter, NP characterization and behavior, including extent of agglomeration and ionic dissolution rates, are a function of environmental factors, meaning exposure conditions are able to directly influence cellular outcomes (Comfort et al., 2014a; Hakami et al., 2015).

One current limitation with regards to NP safety evaluations is that the majority of these studies have been carried out in traditional in vitro environments. While in vitro models provide a fast and cost effective option for NP screenings (Arora et al., 2012), these basic methods are not physiologically representative and have resulted in poor correlations to animals models (Frohlich and Salar-Behzadi, 2014; Voight et al., 2014). Recent investigations have begun utilizing artificial physiological fluids, which mimic an in vivo environment, to characterize NP behavior and nanotoxicological concerns under more relevant exposure conditions (Braydich-Stolle et al., 2014; Breitner et al., 2015; Comfort et al., 2013b, 2014b). These studies have uncovered significant fluid-induced modifications to NP physicochemical properties within alveolar, cerebrospinal, and lysosomal compositions. For example, one study identified that gold NPs experienced extensive agglomeration, increased ionic dissolution, and augmented NP deposition in alveolar fluid (Breitner et al., 2015). These preliminary efforts suggest that while a NP or nano-based applications may appear safe during an in vitro investigation, previously unidentified consequences may appear within a true physiological system.

As such, the goal of this study was to characterize the behavior of CuO and TiO_2 NPs and biological responses following exposure in both a traditional media environment and within artificial interstitial fluid (IF). The keratinocyte, HaCaT, cell line was employed as dermal is the most likely route of exposure for CuO and TiO_2 NPs, with known applications of surface treatments, antibacterial agents, sunscreen, and cosmetics (Gabbay et al., 2006; Monteiro-Riviere et al., 2011). Additionally, HaCaTs have become a model cell line for NP safety assessments with well documented toxicological profiles (Carrola et al., 2016; Crosera et al., 2015; Comfort 2013a). As IF is the fluid surrounding tissues, including the skin, it was the most physiologically appropriate for this study. When dispersed in IF, CuO and TiO_2 NPs both agglomerated to a much higher degree than in media. Additionally, CuO NP incubation in IF produced a synergistic increase in both HaCaT stress and cytotoxicity levels, directly correlating with an augmented rate of NP deposition. Concurrent IF and TiO_2 NP exposure resulted in no significant changes versus a media environment, demonstrating that combinatorial results were not a function of fluid exposure alone. These results suggest that when an innately toxic NP enters a physiological system, environmental-dependent modifications may generate previously unidentified cellular consequences; an area that is in need of further examination.

2. Materials and methods

2.1. Cell culture

The human keratinocyte, HaCaT, cell line was a kind from the Air Force Research Laboratories. The HaCaT cells were cultured on treated petri dishes in RPMI 1640 media supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin. The cells were stored in a humidified incubator maintained at 5% CO_2 and 37 °C.

2.2. Preparation of artificial interstitial fluid

Artificial interstitial fluid (IF) was selected for this study in order to mimic the fluid surrounding cells within skin tissue. Artificial IF was synthesized using the previously published recipe by Stopford et al. (2003). IF contains numerous salt solutions and has a biological pH of 7.4. As artificial IF contains no innate proteins, FBS was added to a final concentration of 1% (v:v) to help maintain basic cellular function and increase physiological relevance. For experimentation, cells were grown and seeded using traditional cellular media, but were washed and replenished with IF for experimentation.

2.3. NP preparation and characterization

The experimental NPs in this study were purchased from Sigma Aldrich. The NPs were characterized in order to identify their physicochemical properties and behaviors. Primary size and morphology were analyzed through transmission electron microscopy (TEM) on a Hitachi H-7600. NP agglomerate size was determined using dynamic light scattering (DLS) on a Malvern Zetasizer Nano-ZS. NP surface charge in solution was acquired through zeta potential analysis, also carried out on the Malvern Zetasizser. For both DLS and zeta potential analyses, the NPs were resuspended in either cell culture media or IF at a concentration of 25 μg/mL.

2.4. Cell viability assessment

Cytotoxicity was evaluated through measurement of lactate dehydrogenase (LDH) release, using the CytoTox 96 NonRadioactive Cytotoxicity Assay (Promega). HaCaT cells were plated in a 96-well plate at a density of 2×10^4 cells per well and exposed to the denoted conditions for 24 h. The level of LDH release was quantified following the manufacturer's instructions. In order to identify fluid-specific effects and normalize experimental conditions, fluid controls were included for LDH viability assessments.

2.5. ROS activation

Reactive oxygen species (ROS) levels were used to assess cellular stress. HaCaTs were seeded in black 96-well plates at a concentration of 2×10^4 per well in media and incubated for 24 h.