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Comparative eco-toxicity of nanoscale TiO_2, SiO_2, and ZnO water suspensions

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ABSTRACT

The potential eco-toxicity of nanosized titanium dioxide (TiO_2), silicon dioxide (SiO_2), and zinc oxide (ZnO) water suspensions was investigated using Gram-positive *Bacillus subtilis* and Gram-negative *Escherichia coli* as test organisms. These three photosensitive nanomaterials were harmful to varying degrees, with antibacterial activity increasing with particle concentration. Antibacterial activity generally increased from SiO_2 to TiO_2 to ZnO, and *B. subtilis* was most susceptible to their effects. Advertised nanoparticle size did not correspond to true particle size. Apparently, aggregation produced similarly sized particles that had similar antibacterial activity at a given concentration. The presence of light was a significant factor under most conditions tested, presumably due to its role in promoting generation of reactive oxygen species (ROS). However, bacterial growth inhibition was also observed under dark conditions, indicating that undetermined mechanisms additional to photocatalytic ROS production were responsible for toxicity. These results highlight the need for caution during the use and disposal of such manufactured nanomaterials to prevent unintended environmental impacts, as well as the importance of further research on the mechanisms and factors that increase toxicity to enhance risk management.

1. Introduction

Titanium dioxide (TiO_2), silicon dioxide (SiO_2), and zinc oxide (ZnO) are common additives with a variety of applications. TiO_2 is a good opacifier and is used as a pigment in paints, paper, inks, and plastics. Crystalline SiO_2 is employed in electronics manufacturing as both semiconductor and electrical insulator. The ceramic nature of ZnO permits its function as both pigment and semiconductor. Nanoscale TiO_2, SiO_2, and ZnO offer greater surface area than their bulk counterparts, allowing for improved performance in established applications.

Accompanying the well-established use of TiO_2, SiO_2, and ZnO, research has been conducted on their potential toxicity (Rincon and Pulgarin, 2004; Lonnen et al., 2005). A wealth of information exists on the toxicity of TiO_2 towards bacteria (e.g. Wei et al., 1994; Block et al., 1997; Kwak et al., 2001). TiO_2 is reputed to be toxic to both Gram-negative and Gram-positive bacteria. In a mixed culture experiment, an unidentified Gram-positive *Bacillus subtilis* was less sensitive than a pure culture of Gram-negative *Escherichia coli* to the effects of TiO_2, possibly due to the ability of *B. subtilis* to form spores (Rincon and Pulgarin, 2005). However, other studies have found Gram-positive bacteria to be more sensitive than Gram-negative bacteria to the antibacterial effects of TiO_2 (Fu et al., 2005). The antibacterial properties of TiO_2 have been exploited in water treatment reactors. A concentration of TiO_2 ranging from 100 to 1000 ppm has been reported to completely disinfect water containing 10^5–10^6 *E. coli* cells per ml in 30 min under illuminated conditions (Wei et al., 1994; Maness et al., 1999).

Fewer studies have been initiated on the antibacterial activities of either SiO_2 or ZnO. Bulk SiO_2 has been used as a

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control particle in several studies due to its postulated lack of toxicity towards bacteria (e.g. Liang et al., 2004). ZnO has been reported to exhibit antibacterial activity with Gram-positive *B. subtilis* being more sensitive to its effects than the Gram-negative *E. coli* (Sawai et al., 1995). The minimal inhibitory concentrations ranged from 2000 to 12,500 ppm for *B. subtilis* and 50,000 to 100,000 ppm for *E. coli* depending on particle size (Sawai et al., 1996). While these data suggest that ZnO is much less toxic to *E. coli* than TiO_2, it is not possible to directly compare these studies due to differences in experimental design (e.g., particle size, concentration of bacteria, application of light).

The differential toxicity of TiO_2, SiO_2, and ZnO may be related to the mechanisms by which the particles act on cells. It is documented that these three compounds are photosensitive and produce reactive oxygen species (ROS) in the presence of light (Yeber et al., 2000; Fubini and Hubbard, 2003; Kubo et al., 2005). However, a positive correlation between photocatalytic ROS production and antibacterial activity has been determined only for TiO_2. Light in these reactions is usually provided by specific wavelength high-intensity lamps; however, one study showed that TiO_2 exhibited antibacterial properties when sunlight was the source of illumination (Wei et al., 1994).

In previous studies, TiO_2 particles that were toxic to bacteria ranged in size from tens of nanometers to hundreds of micrometers. It is not currently clear whether particle size is a key determinant of toxicity or whether surface chemistry and morphology are more important. With the rapid emergence of nanoparticles, it is important to identify the factors that accentuate toxicity. Currently, legislation of nanomaterials is limited, mainly due to the lack of toxicological information and the novelty of the field (Hogue, 2005). However, it is crucial that we understand the fate and impact of potential "contaminants" to permit the development of appropriate disposal mechanisms that mitigate the contamination of surface and groundwater resources.

Little published research has focused on the antibacterial effects related to disposal or accidental spillage of TiO_2, SiO_2, and ZnO. Many studies using nanoscale TiO_2 have incorporated solublising agents (e.g., hydroxyl groups) into the suspension (Kwak et al., 2001) or have immobilised the TiO_2 onto glass (Rincon and Pulgarin, 2004), stainless steel (Yu et al., 2003) or acetate sheets (Lonnen et al., 2005) or have utilized artificial (relatively intense) light sources. While these studies focused on parameters of their particular application, they might not be representative of the effect of raw nanoscale TiO_2 release into the aqueous environment. Therefore, we used nanoparticle water suspensions and natural sunlight to better model natural nanoparticle exposure.

This paper compares and contrasts the toxic effects associated with TiO_2, SiO_2, and ZnO water suspensions using two model bacterial species, Gram-negative *E. coli* and Gram-positive *B. subtilis*. The objectives of this study were to (a) determine the concentrations at which the three suspensions are toxic to our test organisms, (b) determine whether the size of the released nanoparticle affects antibacterial activity, and (c) determine whether natural light stimulates toxicity of the nanoparticles to bacteria.

2. Methods

2.1. Organism cultivation

E. coli DH5α and *B. subtilis* CB310 (courtesy of Dr. Charles Stewart, Rice University, Houston, TX) were maintained on Luria–Bertani (LB) plates. For all experiments, the bacteria were cultivated in a minimal Davis medium (MD). MD is a variation of Davis medium in which the potassium phosphate concentration was reduced by 90% (Atlas, 1993). This medium consisted of 0.7 g K_2HPO_4, 0.2 g KH_2PO_4, 1 g $(NH_4)_2SO_4$, 0.5 g Na-citrate, 0.1 g $MgSO_4 \cdot 7H_2O$, and 1 g glucose in 1 l of Milli-Q® at pH 7.0. MD medium was chosen as the antibacterial test medium as previous research has shown that other nano-sized aggregates precipitate out of suspension in media containing high phosphate concentrations (Lyon et al., 2005).

2.2. Preparation of nanoparticle suspensions

TiO_2 (66 nm, 950 nm, and 44 μm advertised particle size), SiO_2 (14 nm, 930 nm, and 60 μm advertised particle size), and ZnO (67 and 820 nm advertised particle size) powders were obtained from Sigma-Aldrich (St. Louis, MO, USA). ZnO powder at 44 μm particle size was obtained from Alfa Aesar (Ward Hill, MA, USA). The 66 and 950 nm TiO_2 are mixtures of anatase and rutile and the 44 μm TiO_2 is almost pure anatase. The advertised particle size was compared to the measured particle size in suspension. Each of the powders was added to 100 ml of Milli-Q® water to obtain a final concentration of 10 g/l and shaken vigorously. The actual size of the particles in suspension in water and in MD was determined using a dynamic light scattering device (Brookhaven Instrument Corporation, Holtsville, NY, USA) for particles below 1 μm diameter, and optical microscopy (Nikon Optiphot, Japan) for those above this limit. All sizes were confirmed using TEM. To facilitate comparative discussion, the three differently sized suspensions obtained for each compound will be termed small, medium, and large, respectively after the relative advertised sizes of the starting materials.

2.3. Assessment of toxicity to bacteria

Petri plates containing liquid MD media were supplemented with appropriate concentrations (10–5000 ppm) of nanoparticle suspensions to achieve a final volume of 5 ml prior to inoculation with an overnight culture of *B. subtilis* or *E. coli* ($OD_{600} = 0.002$). Antibacterial activity assays were conducted in the presence of sunlight with the small-sized particle suspensions. To obtain data on the effect of size and light on toxicity, suspensions were added at pre-determined toxic concentrations. Control plates were prepared containing only MD medium and bacteria. Plates were sealed with Parafilm (American National Can, Chicago, IL, USA) and wrapped in aluminium foil to simulate dark conditions where required. All plates were placed on a rocker platform (Bell Company Biotechnology, Vineland, NJ, USA) to maintain the nanoparticles in suspension and left in direct sunlight for 6 h (9 AM to 3 PM). The experiments were conducted in the window of a southeast facing laboratory on bright days (23 °C average