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Effect of relative humidity and variation of particle number size distribution on the inactivation effectiveness of airborne silver nanoparticles against bacteria bioaerosols deposited on a filter

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ABSTRACT

Airborne silver nanoparticles were found to be effective in controlling bacteria bioaerosols deposited on filters. However, the applicability of those findings is still unclear because the findings were obtained under limited environmental and experimental conditions. To increase the applicability of the findings, this study examines how airborne silver nanoparticles affect airborne bacteria on filters under various experimental conditions, especially with regard to relative humidity and the particle number size distribution of airborne silver nanoparticles. In this study, bacteria bioaerosols and airborne silver nanoparticles are quantitatively generated, and bioaerosols deposited on filters are exposed to airborne silver particles under various experimental conditions. The properties of the airborne bacteria and airborne silver nanoparticles are measured with aerosol measurement devices. The study tests three bacteria species: gram-positive *Staphylococcus epidermidis*, *Bacillus subtilis*, and gram-negative *Escherichia coli* bacteria bioaerosols. The experimental results demonstrate that a particle number concentration threshold is required for the airborne silver nanoparticles effect, and that the effect of airborne silver nanoparticles is stronger when the relative humidity is low.

1. Introduction

Airborne microorganisms, termed bioaerosols, cause public health problems in relation to airborne respiratory diseases. Moreover, nosocomial infection, which is a hospital-acquired infection this is common among immune-deficient patients such as infants and the elderly, and outbreaks of respiratory diseases through public facilities have received considerable attention and heightened the need for further study and development of a method of controlling and inactivating bioaerosols. In a previous paper, we reviewed the following bioaerosol control methods (Lee & Lee, 2006): ultraviolet (UV) irradiation (Nicas & Miller, 1999; Noakes, Fletcher, Beggs, Sleigh, & Kerr, 2004; Riley, Knight, & Middlebrook, 1976), air electric ion emission (Lee, Yermakov, & Grinshpun, 2004a, 2004b, 2005), and thermal energy (Lee & Lee, 2006; Mullican, Buchanan, & Hoffman, 1971).

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Currently, most indoor air-conditioning systems have internal filters that capture physically airborne microorganisms. However, the bioaerosols captured by filters remain viable and may be re-suspended in air due to any reverse flow caused by a temporary reversal of the surrounding pressure or a breakdown and maintenance of the filters. Accordingly, there is a high demand for methods of controlling airborne microorganisms on filters. We previously suggested that airborne silver nanoparticles could serve as a new control method against bioaerosols on filters (Lee, Yun, Ji, & Bae, 2008) but, at the time, did not fully consider experimental conditions such as the particle number size distribution of airborne silver nanoparticles and other practical environmental variables.

To increase the applicability of the previous findings, we now test the effect of the particle number size distribution of airborne silver nanoparticles. In particular, we examined how four different particle number size distributions affect three bacteria bioaerosols deposited on filters. We hypothesize that relative humidity is a major environmental variable in terms of the effect of silver nanoparticles; accordingly, we test three different conditions of relative humidity as the experimental variables of the current study.

The effect of silver on microbial activation was reviewed in a previous paper (Lee et al., 2008). Silver has been used as an antimicrobial agent against microorganisms in a liquid phase and a solid phase (Cho, Park, Osaka, & Park, 2005; Li, Li, Wu, Wu, & Li, 2005; Morones et al., 2005), and several studies have attempted to elucidate the mechanisms of silver as an antimicrobial agent in a liquid phase. Those mechanisms of silver are forming pits on the surface of *Escherichia coli* bacterial cells (Sondi & Salopek-Sondi, 2004), removing the proton motive forces between the walls of *Vibrio cholerae* bacterial cell (Dibrov, Dzioba, Gosink, & Hase, 2002), and damaging the DNA of *E. coli* and *S. aureus* (Feng et al., 2000). The inactivation mechanism of silver in an air environment is thought to differ significantly from these mechanisms in the liquid phase and solid phase because of the different surroundings of the microorganisms. In this study on the inactivation effect of variables such as particle number size distribution and relative humidity, we attempt to understand the mechanism of airborne silver nanoparticles in air.

2. Material and methods

For the experiments, we used three microorganisms: *Staphylococcus epidermidis* (*S. epidermidis*, KCTC 1917), *Bacillus subtilis* (*B. subtilis*, KCCM 11316) and *Escherichia coli* (*E. coli*, KCTC 1039). The first two, *S. epidermidis* and *B. subtilis*, which are gram-positive bacteria, are commonly used in bioaerosol research with gram-negative *E. coli* bioaerosols (Lee, Kim, & Kim, 2002; Lee & Kim, 2003; Lee & Lee, 2006). *Staphylococci* are common parasites of humans and animals and occasionally cause serious infections. *S. epidermidis* is common in indoor air environments and is commonly found on the skin or mucous membranes of humans (Madigan, Martinko, & Parker, 2000, Chapters 3, 5, 13). The gram-positive rod-shaped bacterium *B. subtilis* has been used as a test organism (Lee et al., 2002; Mullican et al., 1971) in biological aerosol studies, because it is easy to handle and representative of a resistant gram-positive airborne bacteria. The gram-negative microorganism *E. coli* has been assessed in numerous microbiology and bioaerosol studies (Lee et al., 2002; Lee & Kim, 2003; Lee et al., 2008). Airborne *E. coli* has been found in indoor air environments (Zucker, Trojan, & Muller, 2000), and one study has suggested that *E. coli* O157:H7 is spread in an airborne manner (Varma et al., 2003).

The three species *S. epidermidis*, *B. subtilis*, and *E. coli* were grown in a nutrient agar (beef extract 0.3%, peptone 0.5%, agar 1.5%, Difco). For the experiment, quantities of *S. epidermidis*, *B. subtilis*, and *E. coli* were newly inoculated into a new nutrient broth liquid media (nutrient broth; beef extract 0.3%, peptone 0.5%, Difco) and incubated at 37 °C for 4–6 h. After the incubation, the bacteria suspension was diluted until the concentration of bacteria was between 10^3 and 10^4 colony forming units (CFUs) per milliliter so that it could be used in the bioaerosol generation process.

An 1-Jet Collison nebulizer from the Microbiological Research Establishment (BGI Collison Nebulizer, Porton, UK) (Lee et al., 2008) was used to artificially generate the bacteria bioaerosols from the diluted suspensions.

Fig. 1 shows the experimental setup. The overall experimental setup and methods are similar to those of previous studies (Lee et al., 2008). However, the system was revised so that we could vary the relative humidity and the particle number size distributions of the silver nanoparticles.

The generated bioaerosols, which contained moisture, were mixed with clean, dry air that had passed through a HEPA filter and a diffusion dryer. The bioaerosols were flowed and deposited onto filters (MicroCheck® II Beverage Monitor, PALL Corp., Ann Arbor, MI, USA; GN Metricel membrane of mixed cellulose esters and metricel black membrane of modified polyethersulfone; 61 mm of diameter; 0.45 μm of pore size), which were mounted on two identical bioaerosol filter samplers (MP-sigma 500, Sibata).

We designed the experimental process to simulate airborne bacteria captured by the filter of an air-conditioning system (Lee et al., 2008). In the experimental process, two samplers were used to sample the bioaerosols at 3 L/min for 3 min. As shown in Fig. 1, mass flow controllers (MFC) were used to control the aerosol flow rates.

After the sampling of bioaerosols on the filter, airborne silver nanoparticles were carried in a stream of air through one filter sampler, thus, the bioaerosols in that filter were exposed to the airborne silver nanoparticles. Before going to the sampler, the airflow of airborne silver nanoparticles was mixed with humid air that passed through humidifiers; this method was used to control the relative humidity conditions. The relative humidity of the airflow that contained the silver nanoparticles was measured after it was mixed with humid air. The experiments were conducted under three relative humidity conditions of 17%, 40%, and 70%.