Inactivation of *S. epidermidis*, *B. subtilis*, and *E. coli* Bacteria Bioaerosols Deposited on a Filter Utilizing Airborne Silver Nanoparticles

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In the present study, a control methodology utilizing airborne silver nanoparticles is suggested and tested with respect to its potential to control Gram-positive *Staphylococcus epidermidis* and *Bacillus subtilis*, and Gram-negative *Escherichia coli* bacteria bioaerosols deposited on filters. As it is known that the Gram-negative bacteria are sensitive to airflow exposure, the main focus of this study for testing the airborne silver nanoparticles effect was the Gram-positive *Staphylococcus epidermidis* and *Bacillus subtilis* bacteria bioaerosols whereas *Escherichia coli* bioaerosols were utilized for comparison. Airborne bacteria and airborne silver nanoparticles were quantitatively generated in an experimental system. Bioaerosols deposited on the filter were exposed to airborne silver nanoparticles. The physical and biological properties of the airborne bacteria and airborne silver nanoparticles were measured *via* aerosol measurement devices. From the experimental results, it was demonstrated that this method utilizing airborne silver nanoparticles offers potential as a bioaerosol control methodology.

Keywords: Bioaerosols, *Staphylococcus epidermidis*, *Bacillus subtilis*, *Escherichia coli*, silver nanoparticles, filter, airborne bacteria

Airborne microorganisms known as bioaerosols have been receiving attention, owing to their effects on human health. Bioaerosols, such as airborne bacteria, viruses, fungi, and biological fragments, can flow freely with airflow movement and can spread over a wide area in a short period of time. It is thought that bioaerosols could be delivered with Asian dust (Yellow sand), and it is suspected that they may even cross oceans [26]. As such, the skin and respiratory system of humans are highly

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vulnerable to exposure to bioaerosols. Pathogenic airborne microorganisms can cause serious contagious diseases. Notably, this concern was justified by the 2001 bioterror incident in the U.S.A. A recent claim of an abundance of bioaerosols in the atmosphere, meanwhile, increases the scientific importance of bioaerosols [7].

At present, the general public has taken appropriate precautions against pathogens in terms of soil and water pathogens; however, precautions against airborne pathogenic microorganisms remain inadequate [16]. Therefore, recently, governmental actions have been taken to control airborne microorganisms by establishing a standard for bioaerosols in indoor environments (Ministry of Environment. 2006. Law for indoor air quality control in public facilities. http://me.go.kr). Owing to the health effects of bioaerosols and recent governmental policy requiring action, there is now urgent demand for studying control methods regarding airborne microorganisms [12, 16].

Once microorganisms become airborne, in other words when they become bioaerosols, they possess physical characteristics as airborne particles called "aerosols" as well as biological properties as microorganisms. Properties of aerosols are different from those of gas molecules; aerosols have unique characteristics such as thermophoresis, electrophoresis, and diffusion. Furthermore, aerosol movements are subject to fluid flow [5]. Therefore, physical aerosol properties and fluid flow as well as biological properties should be considered in studies on control methodologies against bioaerosols [16].

The control methodologies for bioaerosols were thoroughly reviewed in a previous paper by the author [16]. Ultraviolet (UV) irradiation [21–23], air electric ion emission [13–15], and thermal energy [12, 16, 20] have been tested as control methodologies for bioaerosols. Specifically, attempts have been made to control the viability of bioaerosols, which is a key parameter for the infectivity of bioaerosols and an important parameter to consider when attempting to control bioaerosols.

In the present study, a control methodology that utilizes airborne silver nanoparticles to control bioaerosols on an air filter is newly suggested and tested. Numerous types of filters are employed to capture airborne pollutants, including bioaerosols, in air conditioning devices. After being captured on a filter, the bioaerosols maintain their viability and become resuspended and spread again from the filter *via* filter breakdown or during cleaning and maintenance. Therefore, there is need to develop a control methodology against bioaerosols deposited on air filters.

In this study, a methodology using airborne silver nanoparticles is suggested and tested with *Staphylococcus epidermidis*, *Bacillus subtilis*, and *Escherichia coli* bioaerosols, which were deposited on a filter. As it is known that Gram-negative bacteria is sensitive to airflow exposure [16, 18], the Gram-positive bacteria *S. epidermidis* and *B. subtilis*, which were utilized in the bioaerosol control study as one of the Gram-positive microorganisms [6, 10, 11, 16], were the main focus for testing the airborne silver nanoparticles effect. For comparison, the representative Gram-negative bacteria *E. coli* was also employed in this experiment.

Silver has long been tested as an antimicrobial agent in liquid and solid phases. Several recent studies evaluated the antimicrobial activity of silver nanoparticles in a liquid or solid culture phase [1, 17, 19]. An aqueous solution of silver nanoparticles with various concentrations showed antimicrobial activity on microorganisms on a solid culture [1, 19] and a solution of amoxicillin mixed with silver nanoparticles showed synergetic bactericidal efficiency [17]. In addition, there have been some studies that have attempted to elucidate the mechanisms of silver as an antimicrobial agent in a liquid phase. Silver nanoparticles in a liquid phase were found to form pits on *E. coli* bacterial cell surfaces [24], and silver ions were found to remove proton motive forces between *Vibrio cholerae* bacterial cell walls [2]. After analyzing TEM photographs of *E. coli* and *S. aureus* [4], it was found that DNA lost its replication ability and proteins became inactivated after treatment with silver ions. Although there have been several studies that have sought to explain the antimicrobial mechanisms of silver, thus far there is no widely accepted clear conclusion on how silver functions as an antimicrobial material [1]. Moreover, most previous studies involving silver particles focused on both silver and bacteria in a liquid phase. Silver particles were tested in a solution and bacteria also existed in the solution or on an agar plate. In spite of increasing demand for bioaerosol control study, there have been few studies on the efficacy of airborne silver particles in controlling bioaerosols.

Here, in accordance with the demand for controlling bioaerosols, the effect of airborne silver nanoparticles on bacteria bioaerosols on a filter was studied by experimentation. Airborne silver nanoparticles were manufactured in an experimental system and their particle size distributions were quantitatively measured in the system. Bacteria bioaerosols were artificially generated in the experimental system and their physical and biological properties were measured. The bioaerosols were deposited on a filter for simulation of captured bioaerosols in air pollutant control systems, and they were exposed to airborne silver nanoparticles. From the experimental results and analyses, it is demonstrated that airborne silver nanoparticles have potential in terms of their applicability as antimicrobial agents against bioaerosols on filters.

MATERIAL AND METHODS

Staphylococcus epidermidis (KCTC 1917), *Bacillus subtilis* (KCCM 11316), and *Escherichia coli* (KCTC 1039) were employed as test microorganisms in this study. *S. epidermidis* and *B. subtilis*, Gram-positive bacteria, were previously used in bioaerosol control studies with *E. coli* bioaerosols [6, 10, 11, 16]. 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 [18]. The Gram-positive bacterium *B. subtilis* is rod-shaped. The strain has been used for several decades as a test organism [10, 11, 16, 20], especially in biodefence studies, as it is a feasible representative of a resistant Gram-positive bacteria bioaerosol. *E. coli* is a Gram-negative microorganism and has been assessed in numerous studies [8–12, 16]. In addition, airborne *E. coli* has been found in occupational air environments [27], and a recent study implicated the airborne spread of *E. coli* O157:H7 during an outbreak investigation [25].

S. epidermidis, *B. subtilis*, and *E. coli* were grown in Nutrient Broth media (beef extract 0.3%, peptone 0.5%; Difco). For each experiment, a quantity of *S. epidermidis*, *B. subtilis*, and *E. coli* were newly inoculated into new NB liquid media and incubated at 37°C for 4 to 5 h. During the incubation, the concentration of bacteria in each liquid media was adjusted to between 10^7 to 10^8 colony forming units (CFU)s/ml in order to be used in the bioaerosol generation process.

The bacteria bioaerosols were artificially generated in the experimental system. Details of the aerosolization process of bacteria have been explained in the previous paper by the author [16]. In order to aerosolize bacteria through a nebulization process, a liquid suspension of microorganisms [6] was created. Sterilized water was mixed with the liquid suspension of bacteria. The diluted suspension was then poured into a nebulizer. Utilizing a Microbiological Research Establishment type 1-Jet Collison Nebulizer (BGI Collison Nebulizer, Porton, U.K.), bioaerosols were generated in the experimental system [16].

Fig. 1 shows the experimental setup. The generated bioaerosols, which contained moisture, were mixed with dry and clean air that had passed through a HEPA filter and a diffusion dryer. The dried and diluted bioaerosols were flowed and deposited onto filters (55 plus monitor; Millipore) mounted on two identical bioaerosol filter samplers (MP-sigma 500; Sibata). This experimental process was designed so as to simulate airborne bacteria captured by an air pollutant conditioning system. In the experimental procedures,
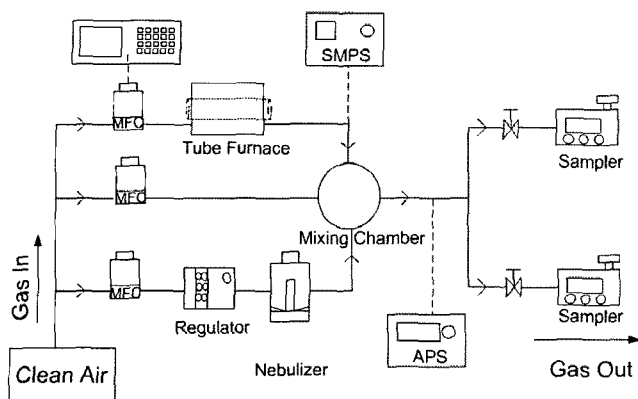
Fig. 1. Schematics of the experimental setup.

bioaerosols were sampled at 3 l/min for 3 min by two filter samplers. The fluid flow rates were controlled by mass flow controllers (MFCs) in the experimental system, as shown in Fig. 1.

After deposition of bioaerosols on the filter, airborne silver nanoparticles were streamed through one filter sampler, carried by air; thus, the bioaerosols in this filter were exposed to the airborne silver nanoparticles. The bioaerosols on the other filter sampler were not exposed to any airflow. Following this step, these filters were attached to agar plates, and bioaerosols on both filters were simultaneously incubated at 37°C for 18 to 40 h, and colonies that formed on the filters were enumerated.

The colony forming units (CFUs) of bioaerosols deposited on the filter were used to calculate the death rates of the bioaerosols. Results under the exposed and nonexposed conditions for the two filters were compared. The death rate due to the airborne silver nanoparticles was calculated by the following equation:

$$\text{Death rate} = 1 - \frac{\text{CFUs(exposure to airborne silver nanoparticles)}/\text{m}^3}{\text{CFUs(nonexposed)}/\text{m}^3}. \quad (1)$$

During exposure to the airborne silver nanoparticles, bioaerosols on the filter are exposed to the airflow simultaneously for the same period of time, because the airborne silver nanoparticles always flow with the air. Therefore, in order to clearly observe the effect of airborne silver nanoparticles, additional experiments involving airflow exposure without silver nanoparticles were conducted. In this experiment, bioaerosols on one filter were exposed to airflow whereas those on the other filter were not exposed to any flow.

The death rate due to the airflow exposure was calculated by the following equation:

$$\text{Death rate} = 1 - \frac{\text{CFUs(exposure to airflow without silver particles)}/\text{m}^3}{\text{CFUs(nonexposed)}/\text{m}^3}. \quad (2)$$

By comparing these two death rates in Eqs. (1) and (2), the effect of the airborne silver nanoparticles on the viability of bioaerosols deposited on the filter could be determined.

The airborne silver nanoparticles that were employed in this study were generated utilizing an electrically heated tube furnace. Airborne silver nanoparticles of two different particle size distributions were artificially generated to investigate the effect of the particle size

distributions on experimental results. Silver powder (ABC Nanotech, Korea) was located in an electrically heated tube furnace, and the temperature of the furnace was increased to 980°C or 950°C depending on the experimental particle size distribution. Dried and filtered air passed through the electrically heated tube furnace. Condensation of silver vapour was then induced and airborne silver nanoparticles were delivered from the furnace to the filter sampler. Particle size distribution of the airborne silver nanoparticles was measured by a Scanning Mobility Particle Sizer (SMPS, DMA model 3085, CPC model 3025A, TSI) system. In order to assess the possibility of a heating effect on the bioaerosol sample arising from the electrically heated tube furnace, the relative humidity and temperature at the inlet of the sampling filters were measured. For the airflow with and without silver nanoparticles to the sampling filters, the relative humidity and temperature at the inlet of the sampling filters remained at approximately 19±1% and 20±2°C, respectively, regardless of operation of the electrically heated tube furnace. Therefore, it was verified that only the effect of the airborne silver nanoparticles could be evaluated in the experimental system whereas any heating effect due to the electrically heated tube furnace would be negligible.

In the experimental procedures, airborne silver nanoparticles were passed through one filter sampler at 3 l/min for 1, 3, 6, and 9 min to investigate the effect of exposure time on the death rate. In addition, for the airflow exposure experiment, the airflow without silver particles was passed through one filter under the same conditions. At least three replications were conducted under the same experimental conditions, and the data were analyzed using a statistical tool (Excel, Microsoft).

RESULTS AND DISCUSSION

Fig. 2 shows one example of the particle size distributions of *S. epidermidis*, *B. subtilis*, and *E. coli* bioaerosols measured in the experiments utilizing an Aerodynamic Particle Sizer (APS model 3321, TSI). The particle size distributions of the generated bioaerosols varied depending on the experimental processes and conditions. In this study, the main focus was on the viability loss of bioaerosols; therefore, a thorough analysis with numerous measurements of bioaerosol size distributions was not conducted. In Fig. 2, the generated *S. epidermidis*, *B. subtilis*, and *E. coli* bioaerosols show peak concentrations at an aerodynamic diameter of 0.8, 1.0, and 0.65 μm, respectively, which were measured after the cleaning process using the centrifuge. The concentration of viable *S. epidermidis*, *B. subtilis*, and *E. coli* bioaerosols measured by the filter samplers ranged from 1×10^4 CFUs/m³ to 9×10^4 CFUs/m³, from 1×10^4 CFUs/ m³ to 4×10^4 CFUs/m³, and from 1×10^4 CFUs/m³ to 3×10^4 CFUs/m³, respectively.

Fig. 3 shows the particle size distributions of the airborne silver nanoparticles. The mode diameter of the airborne silver nanoparticles was 21 nm and the peak concentrations of distributions A and B were 2.6×10^8 and 0.9×10^8 particles/cm³, respectively.

Fig. 3. Particle size distributions of airborne silver nanoparticles measured at the inlet of the filter sampler.

Airborne silver nanoparticles of distributions **A** and **B** were generated with the temperature of the furnace at 980°C and 950°C, respectively. The error bars represent the standard deviations of three measurements.

Fig. 2. Examples of particle size distributions of (**A**) *S. epidermidis*, (**B**) *B. subtilis*, and (**C**) *E. coli* bioaerosols.

The error bars represent the standard deviations of the tests.

Results of *S. epidermidis* Bioaerosols

Fig. 4A shows the death rate of *S. epidermidis* bioaerosols after exposure to the airborne silver nanoparticles and the airflow without silver particles. *S. epidermidis* Gram-positive bacteria bioaerosols showed strong resistance
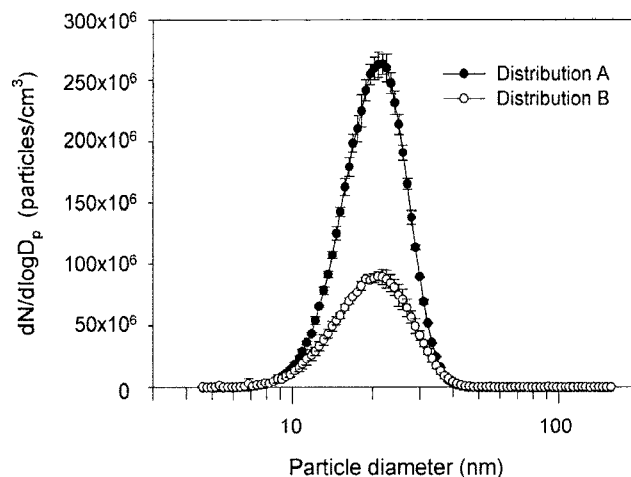
against exposure to the airflow without silver particles. By 9 min of exposure to the airflow on the filter, only approximately 6% of *S. epidermidis* bioaerosols lost their viability on the filter. However, in the case of the airflow containing airborne silver nanoparticles A, approximately 99% of *S. epidermidis* bioaerosols lost their viability because of exposure to the airborne silver nanoparticles A for more than one minute, as shown in Fig. 4A. In order to investigate the effect of the concentration of airborne silver nanoparticles, airborne silver nanoparticles B, which had a smaller concentration than A as shown in Fig. 3, were utilized. The death rate due to silver particles B increased as the exposure time increased. By 1, 3, 6, and 9 min of exposure to airborne silver nanoparticles B, approximately 27, 53, 87, and 99% of *S. epidermidis* bioaerosols lost their viability, respectively. It was found that the concentration of the airborne silver nanoparticles could be a major parameter to control the effect of the silver nanoparticles on the bioaerosols. It could be estimated from the results that some specific threshold concentration existed so that the silver nanoparticles more than the threshold value had effect on the bioaerosols, regardless of the exposure time.

In order to analyze the results, the pure effect of the airborne silver nanoparticles was calculated using the following equation:

Death rate=1

$$-\frac{\text{CFUs}(\text{exposure to airborne silver nanoparticles})/m^3}{\text{CFUs}(\text{exposure to airflow without silver particles})/m^3}. (3)$$

Table 1 shows the effect of airborne silver nanoparticles on bioaerosols on the filter after removal of the airflow exposure effect by employing Eq. (3). As shown in Eq. (3), the death rate due to the airborne silver nanoparticles in
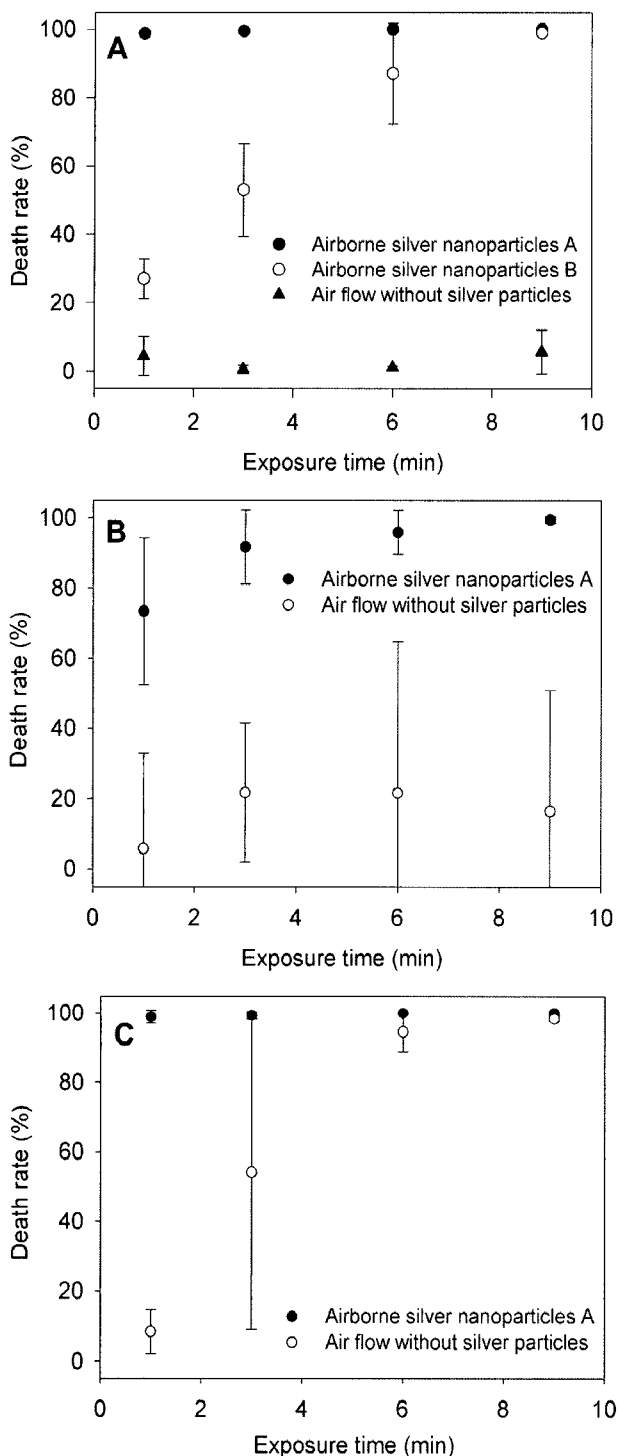
Fig. 4. Death rate of *S. epidermidis* bioaerosols (**A**), *B. subtilis* bioaerosols (**B**), and *E. coli* (**C**) bioaerosols due to exposure to airborne silver nanoparticles or airflow without silver particles.
After being deposited on filters, the bioaerosols were exposed to airborne silver nanoparticles or an airflow without silver nanoparticles.

Table 1. Death rate of *S. epidermidis*, *B. subtilis*, and *E. coli* bioaerosols due to exposure to airborne silver nanoparticles of A and B, after removal of the airflow effect.

	Exposure time	Airflow effect	Airborne silver nanoparticles effect	
S. epidermidis	1 min	4.4%	A	99%
			B	24%
	3 min	0.6%	A	99%
			B	53%
	6 min	1.1%	A	>99%
			B	87%
	9 min	5.7%	A	>99%
			B	99%
B. subtilis	1 min	5.9%	A	72%
	3 min	22%	A	89%
	6 min	22%	A	95%
	9 min	17%	A	99%
E. coli	1 min	8.5%	A	99%

99% viability loss due to exposure to the airborne silver nanoparticles A, thus demonstrating that airborne silver nanoparticles have considerable potential as a control method against *S. epidermidis* bioaerosols deposited on filters.

Results of *B. subtilis* Bioaerosols

Fig. 4B shows the death rate of *B. subtilis* bioaerosols after exposure to airborne silver nanoparticles A and the airflow without silver particles. Although there were deviations from the average values in the experimental data compared with *S. epidermidis* bioaerosols, *B. subtilis* bacteria bioaerosols showed resistance against exposure to the airflow without silver particles. The average death rates of *B. subtilis* bioaerosols on the filter due to the exposure to the airflow ranged from 6% to 22%. In the case of the airflow containing airborne silver nanoparticles, 73, 92, 96, and 99% of *B. subtilis* bioaerosols lost their viability because of exposure to the airborne silver nanoparticles A for 1, 3, 6, and 9 min, respectively. In Table 1, the pure effect of the airborne silver nanoparticles on *B. subtilis* bioaerosols was calculated using Eq. (3). *B. subtilis* bioaerosols showed more than 95% viability loss from 6 min of exposure to the airborne silver nanoparticles A, thus demonstrating that airborne silver nanoparticles have considerable potential as a control method against *B. subtilis* bioaerosols, as well as *S. epidermidis* bioaerosols.

Results of *E. coli* Bioaerosols

Fig. 4C shows the death rate of *E. coli* bioaerosols after exposure to airborne silver nanoparticles A and the airflow without silver particles. As being expected in the introduction, *E. coli* bioaerosols were highly sensitive to the airflow exposure. This sensitivity appeared as large deviations of experimental data under the same exposure conditions.

Table 1 was calculated by dividing the number of colonies of bioaerosols after exposure to the airborne silver nanoparticles by the number of colonies of bioaerosols after exposure to the airflow without silver nanoparticles. In Table 1, *S. epidermidis* bioaerosols showed more than

This sensitivity of *E. coli* is clearly distinguished from the *S. epidermidis* Gram-positive bacteria bioaerosol cases, where the error bars are relatively small. After 1-min exposure to the airflow, an average of 8% of *E. coli* bioaerosols on the filter lost their viability. However, after 3-min exposure to the airflow, a drastic loss of viability was observed, and after 6-min exposure to the airflow, more than 90% of *E. coli* lost their viability because of the airflow effect. At 3 min, the *E. coli* data showed large deviations from the average values, which can be attributed to the significant variation in sensitivity of *E. coli* in this exposure time range. This experimental result of large deviations in a specific range was also observed in other control methodology experiments [16].

The airborne silver nanoparticles A caused 99% loss of viability of the *E. coli* bioaerosols after 1 min. However, owing to the high death rate of *E. coli* after 3-min exposure to the airflow without silver nanoparticles, the silver nanoparticles effect cannot be clearly observed after 3 min. The airborne silver nanoparticles B were not utilized in the *E. coli* experiments, because the airflow effect was too strong to be distinguished from the effect of the higher concentrations of silver particles A.

In order to analyze the meaning of the results shown in Fig. 4C, statistical *t*-tests with *p*-values [3, 13] were conducted using a statistical tool (Excel, Microsoft). A statistical analysis using *t*-test *p*-values was conducted for the case of 1-min exposure to the airflow and the silver nanoparticle airflow. The *p*-values were less than 0.05, indicating that the airborne silver nanoparticles and the airflow exposure effects are statistically different. Therefore, the silver nanoparticles effect on *E. coli* bioaerosols on the filter can be distinguished from the airflow effect. After 3 min, the *p*-values were larger than or close to 0.05 in all cases, indicating that the effect of the airflow exposure and that of the silver nanoparticles cannot be statistically distinguished. Both effects were combined and mixed, and thus, it was not possible to analyze the pure effect of silver nanoparticles after 3 min in the case of *E. coli* bioaerosols. Therefore, the results of pure effects of the airborne silver nanoparticles using Eq. (3) are presented in Table 1 with only the 1-min exposure results. Although the loss of viability of the *E. coli* bioaerosols could not be clearly distinguished from the airflow effect in most conditions, it could be observed that the airborne silver nanoparticles affect *E. coli* bioaerosols on the filter for 1-min exposure.

DISCUSSION

The different results between *S. epidermidis*, *B. subtilis*, and *E. coli* can be attributed to the difference in the cell wall structure of Gram-positive *S. epidermidis*, *B. subtilis*, and Gram-negative *E. coli* bioaerosols. The presence of a strong wall structure in Gram-positive *S. epidermidis* and *B. subtilis* bacteria, which mainly consists of peptidoglycan [18], affords strong resistance against the airflow. The thinner and weak cell wall of Gram-negative *E. coli* bacteria [18] resulted in a sensitive reaction to the airflow. This trend is also in agreement with the results of other control methodologies [16].

Overall, in the present study, the experimental system was designed to simulate control of bioaerosols captured in the filter system, and the methods employing airborne silver nanoparticles were suggested and tested. It was found that airborne silver nanoparticles have strong potential as antimicrobial agents against *S. epidermidis* and *B. subtilis* bioaerosols on filters.

The mechanism of the activity of airborne silver nanoparticles on bioaerosols on filters may be different from the mechanism in a liquid phase, although the mechanism in the liquid phase has yet to be clearly elucidated, because the surrounding environments of bioaerosols are quite different from those of microorganisms in the liquid phase and the effect of this kind of environmental difference has been found to be substantial [16]. Therefore, in future studies, detailed quantifications of the antimicrobial activity of airborne silver nanoparticles through varying experimental conditions, such as the size distributions of airborne silver nanoparticles and exposure conditions such as temperature and relative humidity, can be conducted along with elucidation of the airborne silver nanoparticle activity on bioaerosols. Here, three species were tested, and therefore, the applicability to other microorganisms would be another topic for future study. In addition, the risk of the accidental release of the airborne silver nanoparticles to the surrounding environment can be studied in future studies, to increase the applicability of airborne silver nanoparticles found in this study.

Acknowledgments

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