Hindawi
Indoor Air
Volume 2023, Article ID 8484714, 12 pages
https://doi.org/10.1155/2023/8484714

Research Article

Spreading a Durable Protective Layer of Quaternary Ammonium Agents on an N95 Respirator for Predecontamination of Airborne *Mycobacterium tuberculosis* and Viruses Using *Mycobacterium smegmatis* and Bacteriophage MS2 as Models

Bakhytbol Khumyrzakh,[1] **Yung-Chuan Cheng**,[1] **Chane-Yu Lai**,[2] **Kai-Chih Chang**,[3] **and Chun-Chieh Tseng**[1]

[1]*Department and Graduate Institute of Public Health, Tzu Chi University, Hualien, Taiwan*
[2]*Department of Occupational Safety and Health, Chung Shan Medical University, Taichung, Taiwan*
[3]*Department of Laboratory Medicine and Biotechnology, Tzu Chi University, Hualien, Taiwan*

Correspondence should be addressed to Chun-Chieh Tseng; tsengcc@mail.tcu.edu.tw

Received 17 February 2023; Revised 13 April 2023; Accepted 28 October 2023; Published 22 November 2023

Academic Editor: Shah Fahad

Tuberculosis (TB) and coronavirus disease 2019 (COVID-19), caused by *Mycobacterium tuberculosis* (MTB) and severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), respectively, are serious public health issues. N95 respirators are commonly used to protect people from infections in high-risk environments. Consequently, we used *Mycobacterium smegmatis* and bacteriophage MS2 as MTB and SARS-CoV-2 surrogates to evaluate the ability of a quaternary ammonium agent (QAA) coating on the surface of new N95 respirators to reduce the microbial burden upon aerosol exposure. Regarding the burden (10^5 CFU (or PFU)/m^3) of *M. smegmatis* and MS2 phage that settled onto the respirator surface, the QAA yielded average reduction efficiencies (***R***%) of 92.4% and 99.8%, respectively. In addition, the antimicrobial activity of the coated respirator was maintained for one week. For bioaerosols that contacted the respirator (10^5 CFU (or PFU)/m^3), the ***R***% of the QAA was 90.7% for *M. smegmatis* and 94.4% for MS2 phage on the outermost layer of the respirator. Moreover, filtration efficiencies between a QAA-coated respirator and an untreated respirator were not significantly altered ($\boldsymbol{p = 0.332}$). These results demonstrate that this QAA product has a durable antimicrobial activity and could reduce the MTB and SARS-CoV-2 concentrations on the N95 respirator surface. However, it is recommended that such a coating respirator not be worn for more than 4 hours based on hemolysis assay results.

1. Introduction

Tuberculosis (TB) has been a major global public health issue and is one of the top 10 leading causes of death from infectious diseases worldwide [1, 2]. TB can be transmitted by contact with airborne *Mycobacterium tuberculosis* (MTB) through coughing, sneezing, or spitting by infected persons. According to air sampling and real-time PCR quantification, TB patients may generate MTB concentrations from 1.9 to 5.2×10^4 copies/m^3 [1]; however, people are at high risk if they are exposed to even low numbers of MTB (<10 bacilli) [2, 3]. In addition, severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) is a novel coronavirus that is the cause of a serious disease known as coronavirus disease 2019 (COVID-19) [4]. SARS-CoV-2 can be transmitted via respiratory droplet and contact routes, but aerosol transmission is also possible [5]. To date (April 2023), 6.84 million people worldwide have died from COVID-19.

The use of engineering controls such as ultraviolet germicidal irradiation (UVGI) to prevent the spread of infectious droplet nuclei should reduce the concentration of airborne microbes [6]. Nevertheless, during the pandemic,

filtering facepiece respirators (FFRs) have been widely used to protect individuals from respiratory infections [7, 8]. Among FFRs, the N95 respirator exhibits a filtration efficiency of at least 95% or higher when it is used to capture bacteria or viruses [9]. However, U.S. CDC guidance reported that FFRs worn one time could potentially be contaminated and recommended against reuse by the same person or coworkers [10].

Since FFRs are recommended for single use, many medical staff members have experienced widespread N95 respirator shortages during the current SARS-CoV-2 pandemic [11–13]. Consequently, different methods for decontamination of FFRs have been investigated, such as bleach, ethylene oxide, microwave irradiation, UVGI, heat incubation, microwave steam, autoclave, and hydrogen peroxide exposure [12, 14–16]. Guidance provided by OSHA indicates that FFRs can be reused only if the device has not been obviously damaged and retains its ability to function properly. However, decontamination methods may damage the filter material structure and result in aerosol penetration values lower than the National Institute for Occupational Safety and Health (NIOSH) certification criteria [17]. Many technologies are unsuitable for decontaminating FFRs due to the devices' fragility and operational use.

Among the decontamination methods, the use of quaternary ammonium compounds on the surface of filter material may be another option. Our previous study described the use of a commercial quaternary ammonium agent (QAA), Goldshield 5 (GS5; 1% active ingredient), coated onto mask surfaces before use to evaluate the bactericidal effects [18]. The primary ingredient in GS5 is a quaternary ammonium salt known as 3-trihydroxysilyl propyldimethyl-octadecyl ammonium chloride, abbreviated as Si-QAC. In general, such a QAA product attached to the surface of the mask provided more than a 99.3% bactericidal effect for all three tested bacterial species [18]. Additionally, the mask's filtration performance remained largely unchanged, which is of utmost importance. The major difference between using the QAA in our previous study and other methods is that the QAA is coated onto the clean filter material before use, not after the filter material has been contaminated. Secondly, QAAs can provide bactericidal properties for at least one week after application. Another study also demonstrated that QAAs can protect fabric materials for 14 days [19]. However, whether QAAs can produce the same bactericidal efficiency on the N95 respirator, especially when the respirator is exposed to major health-threatening microbial species, is worth further study.

This study is aimed at evaluating the predecontamination effects of commercial Goldshield 75 (GS75; 0.75% active ingredient) on N95 respirators in a chamber. For safety reasons, the rapidly growing *Mycobacterium smegmatis* and bacteriophage (phage) MS2 were applied as surrogates of MTB [20] and SARS-CoV-2, respectively. *Escherichia coli* was used as the control for comparison. This study examined two aspects of GS75 predecontamination effects. In the first phase, we investigated the survival of microbes deposited on the surface of four distinct GS75-coated layers of the N95 respirator. Durability tests were conducted to assess the persistence of GS75's effect after coating. In the second phase, the study evaluated the survival of three types of microbial aerosols when collected by GS75-coated respirator layers. Furthermore, we assessed the influence of the GS75 coating on the filtration efficiency of the N95 respirator. This evaluation is aimed at determining whether the structural integrity of the N95 respirator could be compromised after the application of the coating. Finally, a hemolysis assay was conducted to investigate the drying time required for coating GS75 on the respirator and the suitable wearing duration.

2. Materials and Methods

2.1. Phase One: Predecontamination of Settled Microbial Particles onto Respirator Layers

2.1.1. Test Microorganisms and Culture. M. smegmatis is an aerobic, nonpathogenic species that has many features common with pathogenic mycobacteria. *M. smegmatis* was chosen as a surrogate target of MTB in this study because it grows relatively fast and can be safely tested in a standard biosafety level 2 laboratory. *M. smegmatis* MC^2 (ATCC 70084) cultures were inoculated in Middlebrook 7H9 broth (Difco, Detroit, MI, USA) and incubated for 48 hr at 37°C. In addition, we used *E. coli* (BCRC 10675) as a control. *E. coli* cultures were inoculated in lysogeny broth (LB) and incubated at 37°C for 14 hr. To generate aerosols, the microbial pellets of *M. smegmatis* at stationary phase (48 hr) as well as *E. coli* (14 hr) were collected, aseptically washed with phosphate-buffered saline, and centrifuged ($\mathbf{500 \times g}$, 5 min). Following centrifugation, the supernatants were discarded, and the pellets were resuspended in sterile water to prepare microbial aerosol spray suspensions. In this study, the test virus was MS2 (BCRC 70235), and its host bacteria are *E. coli* (BCRC 50354). A high-titer stock of bacteriophage (10^9 PFU/ml) was prepared via plate lysis and elution. After the supernatant was removed, the resulting phage stock was stored at 4°C. A plaque assay was then used to measure MS2 phage concentrations.

2.1.2. N95 Respirator Selection and GS75 Coating. A single model of N95 respirator (3 M 9210, Maplewood, MN, USA) was evaluated in this study. The N95 respirator was composed of four layers: an outer hydrophobic layer, a second layer of cellulose/polyester, a third filter layer with an electrostatic charge, and an inner hydrophilic layer. In the decontamination test involving deposited microbes, GS75 (from AP Goldshield, LLC, Locust Valley, NY) was utilized at a concentration of 0.75% active ingredient. Afterward, each measuring 11.34 cm^2, all four layers were individually examined and coated with GS75 using a standard spray bottle. The spray bottle was positioned 10 cm away from the N95 respirator, and approximately 1 ml of GS75 was applied to each layer. The volume of GS75 applied was determined by weighing the spray bottle before and after six sprays. As a result, the total amount of GS75 on each respirator layer was approximately 0.088 ml/cm^2.

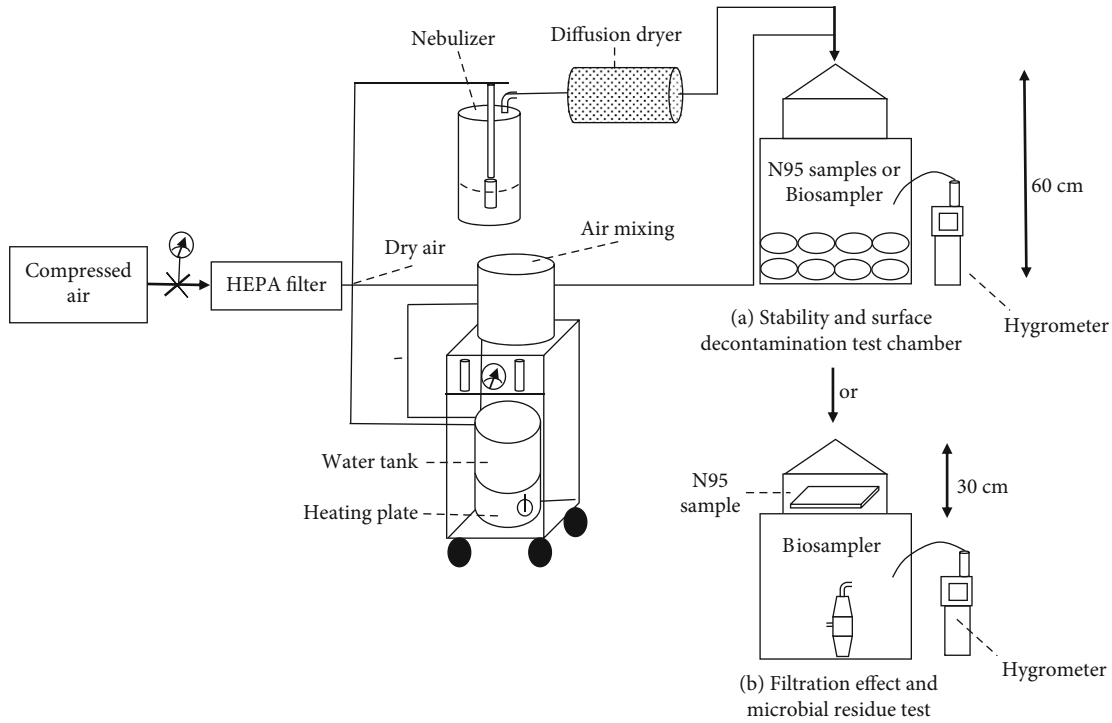


Figure 1: Schematic diagrams of the aerosol test system used in the phase one study for decontamination of settled microbial particles (a) and the phase two study for decontamination of bioaerosols remaining on the respirator surface, along with the filtration performance test (b). The distance from the surface of the test respirator layer to the airflow inlet of the chamber was 60 cm in the phase one study and 30 cm in the phase two study.

Following the GS75 coating, each treated layer was subjected to scanning electron microscopy (SEM) observation. The SEM images provided in the supporting information (Figures S1–S4) illustrated that GS75 effectively adhered to all layers of the N95 respirator, forming a white opaque film. Subsequently, durability tests were carried out, involving a decontamination test conducted at two different intervals (2 hours and seven days) after the GS75 coating process.

2.1.3. Aerosol Generation System. The aerosol test system was adapted from the American Society for Testing and Materials (ASTM) method 2721-10 and our previous study [18] to replicate the deposition of bioaerosols onto surfaces (refer to Figure 1(a)). The acrylic plastic chamber had a volume of 24.3 l, and a Collison three-jet nebulizer (BGI Collison Nebulizer, BGI Inc., Waltham, MA, USA) was applied to generate the *M. smegmatis*, *E. coli*, and MS2 phage aerosols at 3 l/min with compressed laboratory air. Two bacterial suspensions in the nebulizer were 10^5 and 10^6 colony-forming units (CFU)/ml, and the MS2 phage suspensions were 10^5 and 10^6 plaque-forming units (PFU)/ml for each experiment. Consequently, the bioaerosol concentrations in the suspension resulted in aerosol concentrations of 10^4 and 10^5 CFU (or PFU)/m^3. To monitor the bacterial aerosol concentration inside the chamber, a BioSampler at a sampling flow rate of 12.5 l/min was used. The concentrations of all test bioaerosols in the chamber remained stable for a minimum of 90 minutes, with a coefficient of variation (CV%) less than 15%.

The predecontamination test was carried out at a relative humidity (RH) of 55%. To maintain a stable RH in the chamber, the water vapor content was adjusted by modifying the flow rate ratio between the wet and dry gas stream. Real-time RH levels in the chamber were continuously monitored using a hygrometer (Rotronic AG, Bassersdorf, Switzerland). Additionally, a 6-STG impactor (Andersen Samplers, Inc., Atlanta, GA, USA) was employed to measure the size distributions of bioaerosols within various size ranges of 0.65–1.1, 1.1–2.1, 2.1–3.3, 3.3–4.7, 4.7–7.0, and >7.0 μm, operating at a flowrate of 28.3 l/min.

2.1.4. Predecontamination Tests for Settled Microbes. Bioaerosols produced by aerosol systems could settle directly onto the surfaces of both GS75-coated and control respirators inside the chamber. Both the GS75-coated and control respirators were exposed to bioaerosols for 60 minutes. After deposition, the contact plates (23.7 cm^2), which contained 7H11 agar (for *M. smegmatis*) or tryptone soy agar (TSA; for *E. coli*) (Difco, Detroit, MI, USA) with 0.14% (wt/vol) lecithin (Sigma, St. Louis, MO, USA), were used to sample each side of the four layers of the respirator that was facing the airflow. For the MS2 phage deposition test, samples taken from respirator layers were eluted by rinsing with 5 ml of sterile deionized water with 0.14% (wt/vol) lecithin, and the eluate was vortexed with a rotator (Vortex-Genie 2, Scientific Industries Inc., Bohemia, NY, USA) for 60 sec. Lecithin was used to neutralize QAAs to prevent quaternary ammonia compounds from continuously reacting with

bacteria and phages after exposure. Subsequently, the MS2 phages in the eluates were serially diluted and then quantified by the plaque assay. Ultimately, the culturable microbial counts from the respirator layers were calculated. The log reduction in colony (or plaque) counts was determined using the following formula: $\log_{10}(Nt/N_0)$, where N_0 represents the number of bacterial colonies or phage plaques recovered from the control respirator, and $\boldsymbol{Nt}$ is the number of colonies or plaques on the GS75-coated respirator. The colony or plaque reduction efficiency ($\boldsymbol{R}$%) of bacteria and MS2 phage deposited on the respirator surface was calculated as follows:

$$\text{Colony or Plaque reduction efficiency} = \left[1 - \left(\frac{N_t}{N_0}\right)\right] \times 100. \quad (1)$$

2.2. Phase Two: Predecontamination of Microbial Aerosols When They Were Captured by the Respirator Layers. In the phase two study, the targeted microorganisms, types of respirators, and the GS75 coating method remained consistent with those in the phase one study. However, the assessed area of each individual N95 respirator layer was adjusted to 25 cm^2. Notably, there was no durability test conducted in the phase two study.

2.2.1. Aerosol Generation System. The aerosol test system used in the phase two study closely resembled the phase one study (refer to Figure 1(b)). Microbial aerosol concentrations were maintained at 10^4 and 10^5 CFU (or PFU)/m^3 to simulate high-risk environments, similar to the conditions in the previous phase. Following the NIOSH testing standards for aerosol penetration, the particle filtration certification was carried out at a flow rate of 85 l/min to replicate conditions of high physical workload [21]. Considering the surface area (150 cm^2) of the filter material (3 M 9210) utilized in the test regulations, the flow rate of 85 l/min corresponds to a filtration velocity of 9.47 cm/s. In this study, we subjected the respirators to challenge with the same facial velocity, which was based on the airflow rate of 14.2 l/min and a test area of 25 cm^2. Such an experimental condition also allows us to compare the filtration results with those in our previous study [18]. Different from the phase one study, this part of the study was conducted with respirators that were not separated. In both the decontamination and filtration tests, the four individual respirator layers were combined, mimicking real-life respirator usage. These combined layers were then exposed to bioaerosols at a RH of 55%.

2.2.2. Predecontamination Test for Bioaerosols When They Were Captured by Respirator Layers. In the phase two test, the GS75-coated and control respirators were exposed to the test bioaerosols for 60 minutes. Consequently, the bacterial and viral aerosols captured by the respirator layer could be exposed to the remaining GS75, potentially leading to inactivation. It is important to note that in practical application, GS75 was sprayed only on the outer layer of the respirator. Following exposure to bacterial aerosols, contact plates were employed to sample each side of the four respirator layers facing the airflow direction. For MS2 phage, the phage particles remaining on each layer of the respirator were eluted and then quantified by plaque assay as described in a phase one study. Finally, the culturable microbial counts from each respirator layer were cultured and calculated. The survival rates of bacteria and MS2 phage were determined by calculating the colony (or plaque) counts on the GS75-coated respirator ($\boldsymbol{Nt}$) in comparison to the colony (or plaque) counts on the control respirator (N_0). The colony reduction efficiency ($\boldsymbol{R}$%) for bacteria and MS2 phage remaining on the respirator surface was calculated following the same method described in the phase one study.

2.2.3. Determination of Filtration Performance for Bioaerosols. Our study did not perform a fit test because the QAA applied to the outer surface of the respirator was only 1 ml, which had minimal impact on the respirator's shape and structure. However, we recognize that applying QAA may potentially affect the filtration efficiency of the respirator. Hence, we performed tests on filtration efficiency, encompassing bioaerosol penetration testing and NIOSH certification methods. The penetration rate of the three bioaerosols was calculated as the ratio of C_f/C_{in}, where C_f (CFU or PFU/m^3) represents the concentration with a respirator, and C_{in} (CFU or PFU/m^3) represents the concentration without a respirator. Finally, the respirator's filtration efficiency was determined as follows:

$$\text{Filtration efficiency} = \left[1 - \left(\frac{C_f}{C_{\text{in}}}\right)\right] \times 100. \quad (2)$$

The test respirator was treated with GS75, while an untreated respirator was used as a control for efficiency comparison. Culturable concentration measurements were collected downstream of the respirator using an Andersen 1-STG impactor (Andersen Samplers, Inc., Atlanta, GA, USA) for bacteria and a BioSampler for MS2 phage.

2.2.4. Determination of Filtration Performance for NaCl Aerosol. In this assessment, we examined the filtration efficiency of the entire N95 respirator, with GS75 applied solely to its outermost surface. The GS75 coating method remained consistent with the previously mentioned process, and the respirator was allowed to dry for seven days. Following the drying period, an automated filter tester (Model 8130 AFT) from TSI Inc., St. Paul, MN, USA, was utilized to measure the initial percentage of aerosol filter penetration for both GS75-coated and control respirators. The experimental system for the mask penetration test was the same as that in our previous study [18]. Neutralized NaCl aerosol was generated within a mixing chamber and then directed through the filter holder in compliance with NIOSH certification standards [22]. The AFT delivered a polydispersed NaCl aerosol, and all tests were conducted at room temperature with a consistent airflow of 85 l/min. Due to GS75 being diluted with sterile water, a water-coated respirator was employed as the control instead of an untreated mask. This approach is aimed at eliminating the potential effects of water coating in the comparison.

2.2.5. Scanning Electron Microscopic Imaging of M. smegmatis. SEM (scanning electron microscopy) was employed to compare the changes in the morphology of *M. smegmatis* recovered from the respirator layer. After deposition onto the respirator layer's surface, *M. smegmatis* was eluted with sterile deionized water to form pellets. For SEM analysis, the samples were transferred to cover glasses and fixed with 2.5% glutaraldehyde in 0.1 M cacodylate and 1% osmium tetroxide in 0.1 M cacodylate with a final pH of 7.3. The samples underwent an alcohol dehydration series, critical point drying procedures, and gold coating. The cells were examined using SEM at 15 kV (Hitachi S-4700, Hitachi, Japan).

2.2.6. Transmission Electron Microscopy (TEM) of MS2 Phage. The morphology of MS2 phage recovered from the respirator layer was examined by TEM of negatively stained preparations. The phage particles deposited onto the surface of the respirator layer were eluted with sterile deionized water and then applied to the surface of a formvar-coated grid (200 mesh copper grids), followed by negative staining with 2% uranyl acetate (pH 3). Subsequently, phage morphology was observed by using a Hitachi H-7500 transmission electron microscope operated at 80 kV (Hitachi Company, Japan).

2.2.7. Hemolysis Assay. To determine whether the GS75 coated on the N95 respirator would be desorbed upon breathing, we divided the GS75-coated respirators into two groups according to their drying time: drying for 2 hours and drying for 7 days. In this test, we still coated GS75 only on the most exterior side of the respirator. The dried respirator was placed in the system of the phase two study, the compressed air was passed through the respirator, and air samples were collected with a BioSampler (12.5 l/min) for 30 minutes downstream of the respirator at 60, 240, and 480 minutes. The RH of this system was 55%, and the liquid collected by the BioSampler was subjected to a hemolysis assay. The hemolysis assay was determined by hemoglobin release from a blood erythrocyte suspension [23]. Briefly, erythrocytes were washed with phosphate-buffered saline (PBS, pH 7.3) and centrifuged at $\mathbf{1{,}300 \times g}$ at 4°C for 10 min. Next, the erythrocyte suspension was mixed with a suspension collected from the air. The mixtures were incubated at 37°C for 1 h and then centrifuged at $\mathbf{1{,}300 \times g}$ for 5 min. The resulting supernatants were transferred into 96-well plates, and the optical density was measured by a Multiskan Spectrum (Thermo Fisher Scientific, USA) at 540 nm. Hemolysis values for 0% and 100% were determined by incubating the erythrocytes with 10 mM PBS and 0.1% (***v*/*v***) Triton X-100, respectively.

2.2.8. Statistical Analysis. The Kolmogorov–Smirnov test and Shapiro–Wilk test were employed to assess the normality of the data distribution. Following the statistical analysis, nonparametric tests were utilized for data analysis due to a probability of less than 0.05 in the normality test. In the phase one study, differences in colony reduction efficiency among the three different microbes were determined using the Kruskal–Wallis test. The Mann–Whitney–Wilcoxon test was applied for the durability test. The differences in colony (plaque) reduction efficiency among the four layers of the N95 respirator were compared using the Kruskal–Wallis test, followed by Dunn's post hoc test. In the phase two study, the comparison between the filtration efficiency of GS75-coated and untreated N95 respirators was conducted using the Wilcoxon test.

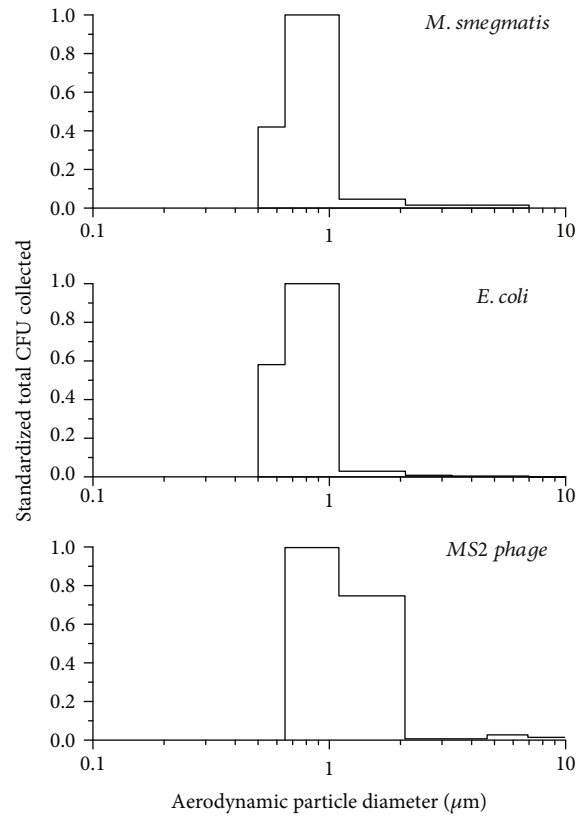


Figure 2: The size distributions of *M. smegmatis, E. coli*, and MS2 phage bioaerosols in the test chamber were measured using an Andersen 6-STG impactor. Each size distribution represents the average of at least three trials.

3. Results

3.1. Phase One: Predecontamination of M. smegmatis Particles Settled onto Respirator Layers

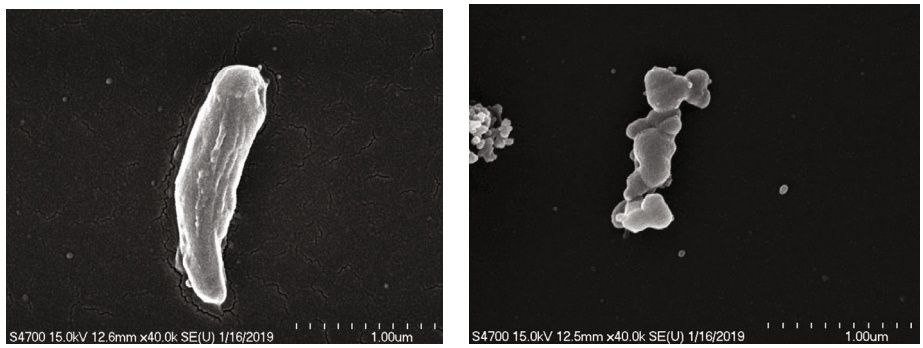
3.1.1. Characteristics of the Aerosolized Bacteria. The particle size distributions of the culturable *M. smegmatis*, *E. coli*, and MS2 aerosols generated by our aerosol system were analyzed using an Andersen six-stage viable particle sizing sampler (6-STG) and are illustrated in Figure 2. Almost all of the test microbes in aerosols were predominantly collected in stage 5 and stage 6 of the 6-STG impactor sampler. As a result, more than 95% of the recovered CFU or PFU of these three bioaerosols had a diameter of less than 2.1 μm.

3.1.2. Predecontamination Test for Microbes Settled on the Surface of Different Respirator Layers. Table 1 illustrates the predecontamination effectiveness of GS75 in inactivating

Table 1: Phase one testing of the colony/plaque reduction efficiency against deposited *M. smegmatis*, *E. coli*, and MS2 phage aerosols of 10^4 CFU (or PFU)/m^3.

Layer	*M. smegmatis*		*E. coli*		MS2 phage	
	2 hours	7 days	2 hours	7 days	2 hours	7 days
1	99.6 (0.5)	96 (3.4)	99.9 (0.05)	99.9 (0)	99.0 (0)	99.0 (0)
2	95.3 (8)	87.5 (12)	99.9 (0.05)	94.4 (5.4)	95.2 (0.03)	99.0 (0)
3	99.9 (0)	99.9 (0)	99.9 (0)	99.9 (0)	97.5 (0.03)	99.0 (0)
4	96 (6.9)	91.9 (5.8)	96.6 (5.74)	89.5 (2.7)	97.5 (0.03)	99.0 (0)
Average	97.7 (2.4)	93.9 (5.3)	99.1 (1.6)	95.9 (5.0)	97.3 (0.03)	99 (0)

The sample size is 72. Values are provided as percentages (%). The numbers in parentheses represent standard deviations.



(a) (b)

Figure 3: *M. smegmatis* on the surface of an untreated N95 respirator (a) and a GS75-coated N95 respirator (b). These two images were observed by SEM and acquired under a magnification of ×40.0 k.

three microbial aerosols with a concentration of 10^4 CFU or PFU/m^3. The table reveals no significant difference in colony or plaque reduction efficiency (***R***%) between drying times of 2 hours and seven days for *M. smegmatis* (***p*** = **0.073**), *E. coli* (***p*** = **0.067**), and MS2 phage (***p*** = **0.06**). Additionally, there was no significant disparity in ***R***% among *M. smegmatis*, *E. coli*, and MS2 phage (***p*** = **0.1**). SEM observations indicated varying degrees of crumpled and dissolved appearances on the surfaces of *M. smegmatis* (Figure 3), suggesting that the death of *M. smegmatis* could be attributed to the destruction of the outer membrane by QAA. In Figure 4, the images display the three-dimensional structure of the MS2 phage in the untreated group, exhibiting a well-defined boundary under staining. However, the boundary of the phage structure in the GS75-coated group appeared blurred and irregular. Nevertheless, due to the limitations of TEM, clear changes in the appearance of phages were not discernible.

Table 2 presents data for different GS75-coated N95 respirator layers that were challenged by microbial aerosols at 10^5 CFU or PFU/m^3. After coating with GS75 for 2 hr, there was no significant difference in *R*% between the two bacterial species ($p = 0.1$). However, the *R*% of *M. smegmatis* after coating with GS75 for 7 days decreased from 92.4% to 87.9%, although no statistical significance was observed ($p = 0.062$). Such an *R*% decrease in the number of *E. coli* was also not obvious, from 93.9% to 93.7% ($p = 0.55$). For MS2 phage, the GS75-coated layer still performed well, with an average *R*% of 99.8%. There was also no significant difference in *R*% among *M. smegmatis*, *E. coli*, and MS2 phage ($p = 0.06$).

3.2. Phase Two: The Predecontamination Process Targeted the Bioaerosols That Persisted on the Respirator Surface. Figure 5(a) shows that only the outermost layer was coated with GS75 and used to capture three microbial aerosols with 10^4 CFU/m^3 or PFU/m^3. The *R*% was 94.4%, 95.2%, and 95.8% for *M. smegmatis*, *E. coli*, and MS2 phage, respectively. There was no significant difference in the *R*% among the three microbes ($p = 0.15$). Figure 5(b) demonstrates that when respirators were coated with GS75 and challenged with a higher bioaerosol concentration of 10^5 CFU (or PFU)/m^3, the *R*% was 90.7% for *M. smegmatis*, 90.9% for *E. coli*, and 94.4% for MS2 phage. Similarly, the *R*% values for all three microbes were not significantly different ($p = 0.52$).

3.2.1. Determination of the Filtration Performance of the N95 Respirator Exposed to Bioaerosols. This part of the study evaluated the filtration efficiency of the N95 respirator with and without coating with the GS75 agent. Here, we still sprayed GS75 onto only the outermost layer of a respirator to mimic the practical application situation. Table 3 demonstrates that the untreated N95 respirator has high filtration efficiencies ranging from 96% to 99.9%, and there was no significant difference in the efficiency among the three microbes ($p = 0.88$) or the two test concentrations ($p = 0.54$). On the other hand, when the outermost layer of a respirator was coated with GS75, the filtration efficiencies ranged from 98.8% to >99.9%. There was still no significant difference among the three microbes ($p = 0.81$) or the two bioaerosol concentrations ($p = 0.93$). Overall, the filtration efficiencies

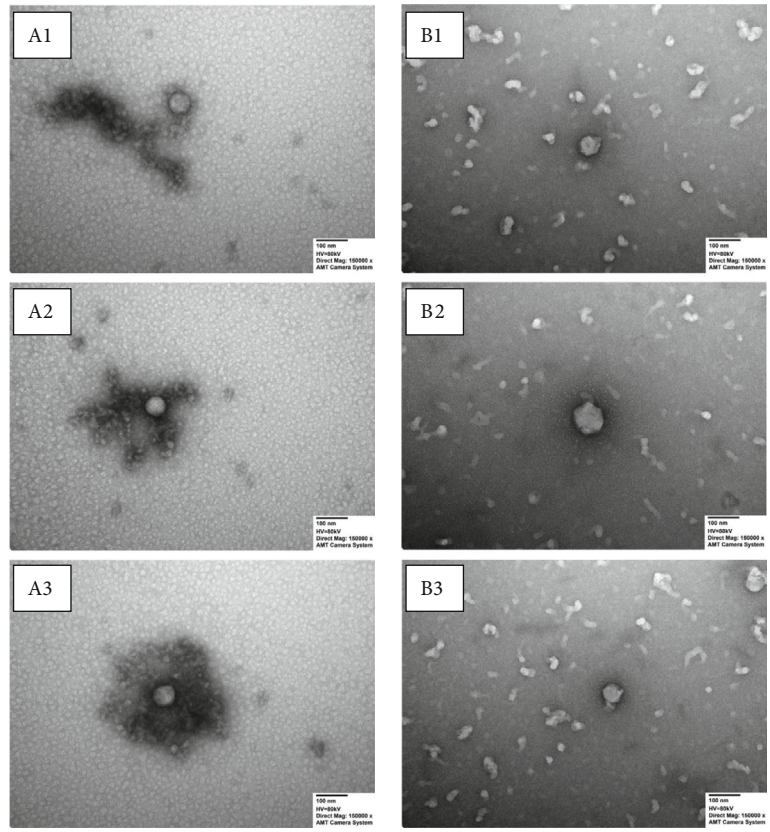


Figure 4: MS2 phages on the surface of untreated N95 respirators (A1–A3) and GS75-coated N95 respirators (B1–B3). These images were observed in three fields by TEM and acquired under a magnification of ×150.0 k.

Table 2: Phase one testing of the colony/plaque reduction efficiency against deposited *M. smegmatis*, *E. coli*, and MS2 phage aerosols of 10^5 CFU (or PFU)/m^3.

Layer	*M. smegmatis*		*E. coli*		MS2 phage	
	2 hours	7 days	2 hours	7 days	2 hours	7 days
1	93.9 (7.4)	90.2 (7)	94 (2.2)	93 (4.5)	99.9 (0)	99.7 (<0.01)
2	86.9 (4)	85.2 (10.7)	87.6 (4.4)	94.4 (5.4)	99.6 (<0.01)	99.8 (<0.01)
3	98.2 (4)	88 (9)	99.9 (0)	98 (3.4)	99.9 (0)	99.7 (<0.01)
4	90.5 (1.9)	88.4 (9.8)	94.4 (1.7)	89.5 (2.7)	99.9 (0)	99.9 (0)
Average	92.4 (4.8)	87.9 (2.06)	93.9 (2.9)	93.7 (3.5)	99.8 (<0.01)	99.8 (<0.01)

The sample size is 72. Values are provided as percentages (%). The numbers in parentheses represent standard deviations.

of the GS75-coated respirator and untreated respirator were not significantly different ($p = 0.79$) when they were exposed to both bioaerosol concentrations.

3.2.2. Filtration Performance Comparison between Sterile Water- and GS75-Coated Whole Respirators for NaCl Aerosol Exposure. Table 4 shows the filtration performance of the whole respirators for NaCl aerosols when only the exterior layer was coated with water or GS75. At an airflow rate of 85 l/min, there was no significant difference in the respirator resistance between water- and GS75-coated respirators ($p = 0.135$). Moreover, there was also no significant difference in the filtration between water- and GS75-coated respirators ($p = 0.332$).

3.3. Desorption Experiment of a GS75-Coated Respirator by Hemolysis Assay. Figure 6 demonstrates that if GS75-coated N95 respirators were dried for 2 hours, the hemolysis rate of the downstream airborne samples was 7.86%, 1.83%, and 2.54% at the 60, 240, and 480 min sampling, respectively. After 7 days of natural drying, the hemolysis rate of downstream airborne samples collected at the first and fourth hours was 0%. However, the hemolysis of the samples at the eighth hour was 1.98%. In addition, the hemolysis rate

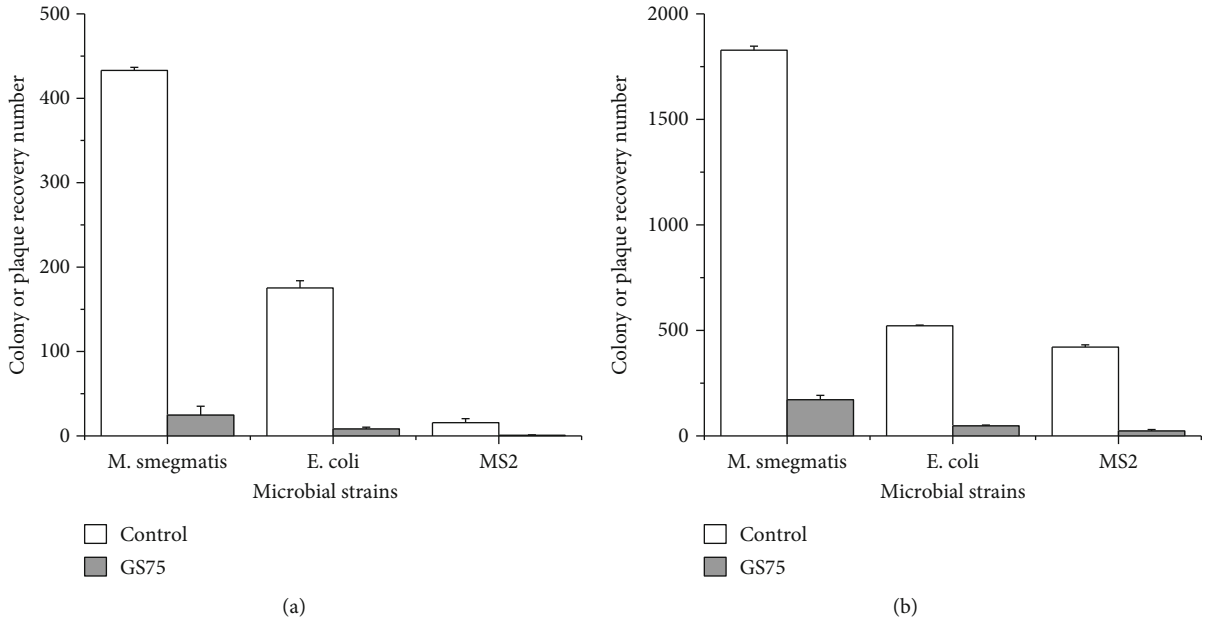


Figure 5: Phase two testing for the predecontamination efficacy of the GS75-coated outer layer of the N95 respirator against *M. smegmatis*, *E. coli*, and MS2 phage, with 10^4 (a) and 10^5 (b) CFU or PFU/m^3.

Table 3: The filtration efficiency of untreated and GS75-coated (only the outer layer) N95 respirator layers.

	M. smegmatis		*E. coli*		MS2 phage	
	10^4 CFU/m^3	10^5 CFU/m^3	10^4 CFU/m^3	10^5 CFU/m^3	10^4 PFU/m^3	10^5 PFU/m^3
Untreated	99.8 (0.34)	99.9 (0.17)	99.8 (0.28)	98.7 (0.20)	96.0 (0.04)	99.2 (0.01)
GS75	>99.9 (0)	99.8 (0.34)	99.6 (0.63)	98.8 (0.37)	99 (0)	99.9 (0)

The sample size is 36. Values are provided as percentages (%). The numbers in parentheses represent standard deviations.

of the original GS75 suspension was 82.85%. The positive control group treated with 1% Triton X-100 had a hemolysis rate of 100%, and the negative control group treated with PBS showed no hemolysis effect.

4. Discussion

The aerosol size distributions of *M. smegmatis*, *E. coli*, and MS2 phage in our chamber closely resembled those of bioaerosols containing *Legionella pneumophila*, *Bacillus subtilis* endospores, and three nosocomial infection-related bacteria, with a geometric mean (GM) ranging from 0.7 to 0.9 μm [18, 24, 25]. All of the tested microbial aerosols in this study had particle size distributions falling within the fifth and sixth stages of the impactor, indicating that these bioaerosols had small particle sizes. Although N95 respirators typically have good filtration efficiency for large particle sizes, their filtration efficiency is more affected by small particles. Therefore, observing the filtration efficiency of N95 respirators for collecting biological aerosols with smaller particle sizes is more representative. The small size distribution indicated that these bioaerosol particles could be suspended in the air for approximately 14 hr. The size distribution of MS2 phage is not the same as that of bacteria, which may be caused by the difference in the composition of the phage culture medium placed in the nebulizer.

Our study utilized high bioaerosol concentrations ranging from 10^4 to 10^5 CFU (or PFU)/m^3. This approach is aimed at representing a worst-case scenario, simulating situations where substantial microbial loads could potentially be deposited on the respirators from the surrounding air. Such a high concentration is equivalent to the concentration that could be measured in the air when there are TB patients in the isolation ward [1]. Even the virus concentration in the air of a SARS-CoV-2-infected patient's ward was only $1.84 - 3.38 \times 10^3$ copies/m^3 [26]. Our phase one study demonstrated that GS75-coated respirators produced a log reduction from 0.91 to 1.64 for settled *M. smegmatis*, and the bactericidal efficiency decreased as the settled aerosol concentration increased. Although there was no difference among the three microbes according to the statistical analysis, the longer the drying time was after applying GS75 onto the respirator, the lower the MTB removal efficiency. Our previous study employed QAA to remove common bacteria after contact with surgical masks. The results demonstrated a colony reduction rate exceeding 99.3% for all tested microbes across all three mask layers [18]. This difference may be related to the active ingredient concentration of QAA applied. The previous study was conducted with an ingredient concentration of 1% (GS5), but this study was evaluated based on the commercially available concentration (0.75%). In addition, the resistance of mycobacteria to QAAs might be higher than that of other general bacteria.

Table 4: The filtration performance of whole sterile water- and GS75-coated masks exposed to a NaCl aerosol.

Model	Resistance (mm H_2O)		Penetration rate (%)	
	Water	GS75	Water	GS75
85 l/min	8.5 (0.73)	9.2 (0.46)	0.17 (0.22)	0.27 (0.34)

The sample size is 12. The numbers in parentheses represent standard deviations.

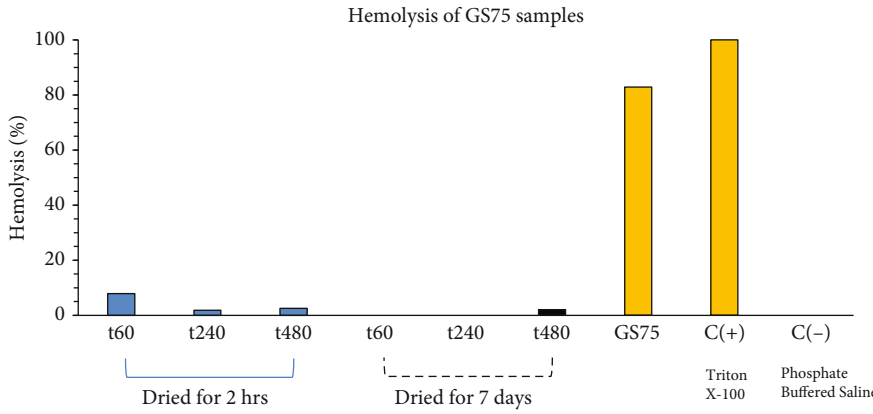


Figure 6: Hemolysis test of GS75-coated samples with different N95 drying and exposure times.

In prior research, a commercial cleaning wipe containing benzalkonium chloride (BAC) was employed for decontamination after the respirator was contaminated by *Staphylococcus aureus*. [27]. The colony reduction rate for 10^4 CFU/m^3 M. *smegmatis* for GS75 was similar to that for wiping with BAC (95.37% to >99.99%) [27]. However, our study suggested the application of QAAs to the respirator layer before wearing rather than after contamination. This proactive approach could reduce the infection risk associated with touching the surface of a contaminated respirator during use. Furthermore, the decontamination effect of GS75 persisted for at least one week after its application onto the respirator. Although GS75 was shown to inactivate general bacteria and viruses on the respirator layer efficiently, the decontamination effect might decrease when highly high concentrations of MTB are present.

In the phase two study, we sprayed GS75 agent onto only the outermost layer of an N95 respirator since this method can be easily applied by the public or medical staff. On average, people may touch their face 23 times per hour [28], and if microbes are deposited on the outermost layer of the respirator, this may cause an extra infection risk for people who touch the surface of their respirator. The results of the phase two study agreed with our hypothesis that regardless of whether the bioaerosols are deposited or penetrated and are retained on the outermost layer of the respirator, GS75 can exert a good inactivation effect. Compared with smaller particles, larger particles, such as *M. smegmatis* (1.08 to 6.27 μm long [29]), tend to deposit on the outer layer of the respirator [30]. Consequently, *M. smegmatis* always accumulated a higher concentration on the surface of the first layer of the respirator than *E. coli* and MS2 phage. Although the aerosol sizes of the three bioaerosols generated by our system are similar, *E. coli* (1 to 2 μm long) and MS2 phage (23-28 nm), with smaller physical sizes, may penetrate deeper following airflow sampling for 60 min. Nevertheless, regardless of the microbial concentration retained on the outermost layer of the respirator, the reduction efficiency can be more than 90%.

A previous study suggested that the filtering efficiency of commercial N95 respirators for MTB can be regarded as 99.5% or even higher [9], and our filtration test yielded similar results despite the use of an MTB surrogate strain [29]. The filtration test conducted in this study also indicated that the physical degradation of the respirator after the GS75 coating seemed negligible. Even when this product was applied to a surgical mask, there was no significant impact on the filtering performance in collecting pathogenic bacterial strains compared to untreated masks [18]. Theoretically, the positive charge of the QAA might interact with the respirator layer, aiding in collecting airborne microbes with a negative charge. However, the QAA may also interfere with the charged layer and reduce the filtration performance. The electret filter used to produce N95 respirator layers may lose the electret charges under high humidity [31] or when exposed to nonionic detergents and sodium chloride [27, 32]. Therefore, the application of QAAs to the N95 respirator may decrease the filtration efficiency, but this effect may be dependent on the composition and amount of QAA used in the commercial product.

When the QAA was applied to inactivate bacteria by the fabric test or carrier test, the reduction in culturable counts of gram-positive bacteria was significantly greater than that for gram-negative bacteria [19]. This difference could be attributed to the higher presence of negatively charged peptidoglycans in gram-positive bacteria compared to gram-negative bacteria. Consequently, more quaternary ammonium salts (Si-QAC) might be trapped by the peptidoglycans

in gram-positive bacteria, damaging their cell membranes [33]. Although *M. smegmatis* is a gram-positive bacterium, the cell wall of *Mycobacterium* spp. consists of not only peptidoglycan but also some unusual glycolipids, including arabinogalactan, lipoarabinomannan, and mycolic acid [34]. These complex components of *Mycobacterium* species are responsible for its resistance to many stresses, including antibiotics, bacteriophages, and disinfectants [35].

From the results of the phase one study, we found that when the respirator was coated with GS75 for one week, the decontamination efficiency for *M. smegmatis* decreased by 4.3%, which is much higher than that for *E. coli* (1.7%) and MS2 phage (0.85%). More than 10% of lipids make up the weight of the mycobacteria and could protect their cell membrane from being penetrated by QAAs. Earlier studies have also demonstrated that some specific QAAs had poor activities for inactivation of *Mycobacterium* species [35, 36], and this effect may be concentration dependent. After the GS75-coated respirator is dried for one week, the concentration of its main ingredients may decline with time, which may also lead to a decrease in its bactericidal effect, although there was no significant difference in the *R*% among the three test microbes. Our study indicates that precleaning before coating surfaces with QAAs is often necessary because the effectiveness of QAAs would be reduced if soap or organic matter was present on the applied surface [35]. Consequently, if a QAA is applied to already used N95 respirators, the decontamination effect should be reduced, especially when the respiratory is challenged with highly stress-resistant *Mycobacterium* spp.

The advantages of the GS75 coating process introduced in this study include convenience, and the problem of physical degradation of the respirator could be limited. However, any modifications made to a respirator after it has been certified by NIOSH will cause it to lose its approval. To avoid this issue, the manufacturer must address it by applying QAA and sending the product for subsequent certification. Our experimental results demonstrate that respirators coated with QAA have a high likelihood of meeting certification standards, as their filtration efficiency remains unaffected. Moreover, there are two advantages to using this method: firstly, the application of QAA is more consistent and uniform, and secondly, healthcare workers and the general public can more easily access these QAA-coated respirators.

On the other hand, safety issues for human health must be addressed. QAAs are considered safer than chemical disinfectants such as chlorine and glutaraldehyde. However, there may be irritant or cytotoxic effects of QAAs on human cells/tissues [37]. The QAA product evaluated in this study has not, to our knowledge, ever had cytotoxicity testing published in a peer-reviewed publication. However it has been tested using the international organization for standardization's agarose overlay method, and unpublished testing demonstrated that there is only slight reactivity involving cytotoxicity [19]. Although GS75 may cause low cytotoxicity, it may still cause hemolysis.

We also tested whether GS75 could be desorbed from the respirator and further enter the respiratory tract due to the influence of airflow. Consequently, our suggestion is that such a GS75-coated respirator should be dried for a week and should not be worn for more than 4 hours, which may reduce the impact of QAAs on human health. Epidemiological studies have indicated that QAAs could be a contributing factor to work-related asthma [38, 39]. Therefore, assuming that GS75-coated respirators are proposed for use in practice, we recommend wearing them in a high-risk environment for short periods (<4 hr) rather than wearing them for a long time.

There are several limitations in our study. Firstly, we examined only one specific type of N95 respirator and one type of QAA compound. To generalize these findings, different respirator models and QAA compounds should be tested before implementing such practices. Secondly, human respiratory secretions, such as proteins or mucin released during breathing, might create a shield, reducing microbial susceptibility to GS75. Lastly, surrogate species were used in this study, and their susceptibility to QAAs may differ from that of target species such as MTB and SARS-CoV-2. Further research with target species is necessary to confirm these findings [35]. Although we simulated the decontamination ability with higher microbial concentrations, caution should be taken when applying the results directly to the strains with high health risks.

5. Conclusions

Wearing N95 respirators is a fundamental personal protective measure to prevent infections from high-risk microorganisms like MTB or SARS-CoV-2. However, N95 respirators can only capture and not inactivate microbes that have settled or become trapped on the layers. While various physical or chemical decontamination methods have been proposed, these techniques might damage the respirator layers or diminish their filtering efficiency. Our study shows that a quaternary ammonium compound (QAA) called GS75 can be applied to the filter layers of N95 respirators, providing a lasting antimicrobial effect for seven days when bioaerosols settle on or penetrate the first layer of the respirator. The convenient QAA coating procedure introduced in this study could potentially be used to reduce the possibility of secondary microbial infections by virulent microbial strains. In addition, the problem caused by GS75 coating resulting in physical degradation of the respirator layer was negligible. However, although a QAA-coated respirator is recommended as a predecontamination method for infection prevention, QAA-related ingredients may still have potential health hazards. Therefore, we recommend that this method should be used when people must be exposed to high-risk environments, and individuals should avoid wearing the coated respirator for more than 4 hours.

Data Availability

The data used to support the findings of this study are included within the article and supporting information.

Additional Points

Practical Implications. (i) Coating N95 respirators with an antimicrobial surfactant prior to use could potentially decrease the concentration of harmful pathogens, such as MTB and SARS-CoV-2, on the respirator surface. (ii) The long durability (at least one week) and easy coating method introduced in this study make this method more feasible than other methods. (iii) The problem caused by the QAA coating resulting in physical degradation of the respirator layer is negligible. (iv) It is recommended that individuals who are exposed to high-risk environments should not wear the coated respirator for more than four hours.

Conflicts of Interest

The authors have declared no competing financial interests.

Authors' Contributions

BK and YCC contributed equally and are co-first authors. CCT conceived and designed the experiments. BK, YCC, and CYL performed the experiments. BK, YCC, and CCT analyzed the data. KCC contributed the reagents, materials, and analysis methods. CCT, BK, and CYL wrote the paper.

Acknowledgments

This work was supported by the grant MOST 105-2628-B-320-001-MY3 from the Ministry of Science and Technology, Taiwan. The authors appreciate the technical assistance received from personnel at the Electron Microscopy Laboratory at Tzu Chi University.

Supplementary Materials

Figure S1: untreated and GS75-coated outer layers of the N95 respirator. Figure S2: untreated and GS75-coated second inner layers of the N95 respirator. Figure S3: untreated and GS75-coated middle layers of the N95 respirator. Figure S4: untreated and GS75-coated inner layers of the N95 respirator. *(Supplementary Materials)*

References

[1] P. S. Chen and C. S. Li, "Concentration profiles of airborneMycobacterium tuberculosisin a hospital," *Aerosol Science Technology*, vol. 42, no. 3, pp. 194–200, 2008.

[2] G. E. Pfyffer, "*Mycobacterium*: general characteristics, laboratory detection, and staining procedures," in *Manual of Clinical Microbiology, Eleventh Edition*, pp. 536–569, American Society of Microbiology, 2015.

[3] M. Gilchrist, "Biosafety precautions for airborne pathogens," in *Laboratory safety principles and practices*, pp. 67–76, American Society for Microbiology, Washington, DC, 2nd edition, 1995.

[4] M. T. Adil, R. Rahman, D. Whitelaw et al., "SARS-CoV-2 and the pandemic of COVID-19," *Postgraduate Medical Journal*, vol. 97, no. 1144, pp. 110–116, 2021.

[5] N. Chams, S. Chams, R. Badran et al., "COVID-19: a multidisciplinary review," *Frontiers in Public Health*, vol. 8, p. 383, 2020.

[6] H. Luo and L. Zhong, "Ultraviolet germicidal irradiation (UVGI) for in-duct airborne bioaerosol disinfection: review and analysis of design factors," *Building and Environment*, vol. 197, article 107852, 2021.

[7] D. Paul, A. Gupta, and A. K. Maurya, "Exploring options for reprocessing of N95 filtering facepiece respirators (N95-FFRs) amidst COVID-19 pandemic: a systematic review," *PloS One*, vol. 15, no. 11, article e0242474, 2020.

[8] J. Alt, R. Eveland, A. Fiorello et al., "Development and validation of technologies suitable for the decontamination and reuse of contaminated N95 filtering facepiece respirators in response to the COVID-19 pandemic," *The Journal of Hospital Infection*, vol. 119, pp. 141–148, 2022.

[9] Y. Qian, K. Willeke, S. A. Grinshpun, J. Donnelly, and C. C. Coffey, "Performance of N95 respirators: filtration efficiency for airborne microbial and inert particles," *American Industrial Hygiene Association Journal*, vol. 59, no. 2, pp. 128–132, 1998.

[10] N. N. Bock, P. A. Jensen, B. Miller, and E. Nardell, "Tuberculosis infection control in resource-limited settings in the era of expanding HIV care and treatment," *The Journal of Infectious Diseases*, vol. 196, Supplement 1, pp. S108–S113, 2007.

[11] K. Al-Hadyan, G. Alsbeih, A. Nobah et al., "In-house filtration efficiency assessment of vapor hydrogen peroxide decontaminated filtering facepiece respirators (FFRs)," *Journal of Environmental Research and Public Health*, vol. 18, no. 13, p. 7169, 2021.

[12] S. I. Prada, Á. Vivas, M. P. Garcia-Garcia et al., "Safe and effective re-use policy for high-efficiency filtering facepiece respirators (FFRS): experience of one hospital during the COVID-19 pandemic in 2020," *IPEM-Translation*, vol. 3-4, article 100011, 2022.

[13] M. L. Ranney, V. Griffeth, and A. K. Jha, "Critical supply shortages–the need for ventilators and personal protective equipment during the COVID-19 pandemic," *The New England Journal of Medicine*, vol. 382, no. 18, p. e41, 2020.

[14] D. J. Viscusi, M. S. Bergman, B. C. Eimer, and R. E. Shaffer, "Evaluation of five decontamination methods for filtering facepiece respirators," *Annals of Occupational Hygiene*, vol. 53, no. 8, pp. 815–827, 2009.

[15] H. Yang, J. Hu, P. Li, and C. Zhang, "Ultraviolet germicidal irradiation for filtering facepiece respirators disinfection to facilitate reuse during COVID-19 pandemic: a review," *Photodiagnosis and Photodynamic Therapy*, vol. 31, article 101943, 2020.

[16] K. Seresirikachorn, V. Phoophiboon, T. Chobarporn et al., "Decontamination and reuse of surgical masks and N95 filtering facepiece respirators during the COVID-19 pandemic: a systematic review," *Infection Control and Hospital Epidemiology*, vol. 42, no. 1, pp. 25–30, 2021.

[17] D. J. Viscusi, M. S. Bergman, D. A. Novak et al., "Impact of three biological decontamination methods on filtering facepiece respirator fit, odor, comfort, and donning ease," *Journal of Occupational and Environmental Hygiene*, vol. 8, no. 7, pp. 426–436, 2011.

[18] C. C. Tseng, Z. M. Pan, and C. H. Chang, "Application of a quaternary ammonium agent on surgical face masks before use for pre-decontamination of nosocomial infection-related

bioaerosols," *Aerosol Science and Technology*, vol. 50, no. 3, pp. 199–210, 2016.

[19] D. Baxa, L. Shetron-Rama, M. Golembieski et al., "*In vitro* evaluation of a novel process for reducing bacterial contamination of environmental surfaces," *American Journal of Infection Control*, vol. 39, no. 6, pp. 483–487, 2011.

[20] M. J. Lai, C. C. Liu, S. J. Jiang et al., "Antimycobacterial activities of endolysins derived from a mycobacteriophage, BTCU-1," *Molecules*, vol. 20, no. 10, pp. 19277–19290, 2015.

[21] L. Janssen, N. Anderson, P. Cassidy, R. Weber, and T. Nelson, "Interpretation of inhalation airflow measurements for respirator design and testing," *Journal of the International Society for Respiratory Protection*, vol. 22, no. 3/4, p. 122, 2005.

[22] NIOSH, *Determination of Particulate Filter Efficiency Level for N95 Series Filters against Solid Particulates for Non-Powered, Air-Purifying Respirators. Procedure TEBAPR-STP-0059*, National Institute for Occupational Safety and Health, National Personal Protective Technology Laboratory, Pittsburgh, PA, 2007.

[23] Z. G. Tian, T. T. Dong, D. Teng, Y. L. Yang, and J. H. Wang, "Design and characterization of novel hybrid peptides from LFB15(W4,10), HP(2-20), and cecropin a based on structure parameters by computer-aided method," *Applied Microbiology and Biotechnology*, vol. 82, no. 6, pp. 1097–1103, 2009.

[24] C. S. Li, "Evaluation of microbial samplers for bacterial microorganisms," *Aerosol Science and Technology*, vol. 30, no. 2, pp. 100–108, 1999.

[25] C. S. Li, C. C. Tseng, H. H. Lai, and C. W. Chang, "Ultraviolet germicidal irradiation and titanium dioxide photocatalyst for controlling Legionella pneumophila," *Aerosol Science and Technology*, vol. 37, no. 12, pp. 961–966, 2003.

[26] P. Y. Chia, K. K. Coleman, Y. K. Tan et al., "Detection of air and surface contamination by SARS-CoV-2 in hospital rooms of infected patients," *Nature Communications*, vol. 11, no. 1, p. 2800, 2020.

[27] B. K. Heimbuch, K. Kinney, A. E. Lumley, D. A. Harnish, M. Bergman, and J. D. Wander, "Cleaning of filtering facepiece respirators contaminated with mucin and *Staphylococcus aureus*," *American Journal of Infection Control*, vol. 42, no. 3, pp. 265–270, 2014.

[28] Y. L. Kwok, J. Gralton, and M. L. McLaws, "Face touching: a frequent habit that has implications for hand hygiene," *American Journal of Infection Control*, vol. 43, no. 2, pp. 112–114, 2015.

[29] H. Yamada, M. Yamaguchi, Y. Igarashi et al., "Mycolicibacterium smegmatis, Basonym mycobacterium smegmatis, expresses morphological phenotypes much more similar to Escherichia coli than mycobacterium tuberculosis in quantitative structome analysis and CryoTEM examination," *Frontiers in Microbiology*, vol. 9, p. 1992, 2018.

[30] W. G. Lindsley, J. D. Noti, F. M. Blachere, J. V. Szalajda, and D. H. Beezhold, "Efficacy of face shields against cough aerosol droplets from a cough simulator," *Journal of Occupational and Environmental Hygiene*, vol. 11, no. 8, pp. 509–518, 2014.

[31] E. Motyl and B. Lowkis, "Effect of air humidity on charge decay and lifetime of PP electret nonwovens," *Fibres & Textiles in Eastern Europe*, vol. 14, pp. 39–42, 2006.

[32] E. S. Moyer and M. Bergman, "Electrostatic N-95 respirator filter media efficiency degradation resulting from intermittent sodium chloride aerosol exposure," *Applied Occupational and Environmental Hygiene*, vol. 15, no. 8, pp. 600–608, 2000.

[33] K. Kawahara, K. Tsuruda, M. Morishita, and M. Uchida, "Antibacterial effect of silver-zeolite on oral bacteria under anaerobic conditions," *Dental materials*, vol. 16, no. 6, pp. 452–455, 2000.

[34] M. Jankute, J. A. Cox, J. Harrison, and G. S. Besra, "Assembly of the mycobacterial cell wall," *Annual Review of Microbiology*, vol. 69, no. 1, pp. 405–423, 2015.

[35] M. Best, S. A. Sattar, V. S. Springthorpe, and M. E. Kennedy, "Efficacies of selected disinfectants against Mycobacterium tuberculosis," *Journal of Clinical Microbiology*, vol. 28, no. 10, pp. 2234–2239, 1990.

[36] A. D. Russell, S. A. Hammond, and J. R. Morgan, "Bacterial resistance to antiseptics and disinfectants," *The Journal of Hospital Infection*, vol. 7, no. 3, pp. 213–225, 1986.

[37] H. Nagamune, T. Maeda, K. Ohkura, K. Yamamoto, M. Nakajima, and H. Kourai, "Evaluation of the cytotoxic effects of bis-quaternary ammonium antimicrobial reagents on human cells," *Toxicology in Vitro*, vol. 14, no. 2, pp. 139–147, 2000.

[38] C. Paris, J. Ngatchou-Wandji, A. Luc et al., "Work-related asthma in France: recent trends for the period 2001–2009," *Occupational and Environmental Medicine*, vol. 69, no. 6, pp. 391–397, 2012.

[39] M. Gonzalez, J. Jégu, M. C. Kopferschmitt et al., "Asthma among workers in healthcare settings: role of disinfection with quaternary ammonium compounds," *Clinical & Experimental Allergy*, vol. 44, no. 3, pp. 393–406, 2014.