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Assessment of filtration efficiency, inhalation resistance and antibacterial activity for reusable antibacterial face masks after washing

재사용 항균성 마스크의 세척 후 여과효율,
흡기저항 및 항균성 평가

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Abstract

Assessment of filtration efficiency, inhalation resistance and antibacterial activity for reusable antibacterial face masks after washing

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Introduction: In the early stages of the COVID-19 pandemic, disposable masks were worn several times due to the lack of certified masks. Although reusable masks fabricated using antibacterial fibers are available on the market, scientific data on their performance and antibacterial properties are lacking. Therefore, this study evaluated the filtration efficiency, inhalation resistance, and continuity of the antibacterial activity of reusable antibacterial masks before and after washing with water.

Methods: Reusable silver, copper, and graphene antibacterial masks and cotton masks were used to compare their performance before and after washing. Furthermore, the performance before washing was compared with those of KF94 and N95 masks. Washing of the masks in a washing machine at 40 °C (Method A) and immersion in water at 60 °C for 15 min (Method B) and 90 °C for 15 min (Method C) were the tested washing methods. The masks were washed every day for 10 d, and their performance was evaluated after one, two, five, and ten washing times. The filtration efficiency of the masks was evaluated using NaCl and *Staphylococcus aureus*. Additionally, the antibacterial properties and inhalation resistance of the tested masks were investigated.

Results: The filtration efficiencies of three reusable antibacterial masks were between 10–13% before washing and increased by approximately 6% after washing. Before washing, the bacterial filtration efficiency was 93–98%, and the antibacterial activity was 88–98%. The performance changed irregularly as the washing was repeated, and there was no significant difference in the performance before and after washing. Reusable antibacterial masks showed higher antibacterial activity than the non-antibacterial masks. The inhalation resistance before and after washing was 5–17 mmH_2O, indicating appropriate air permeability.

Conclusion: The filtration efficiency of the three reusable antibacterial masks was inadequate for blocking particles. The performance of the reusable antibacterial masks changed irregularly depending on the washing methods and number of washing cycles. Therefore, it is recommended to wear a certified mask for protecting the respiratory track in daily life.

Keywords: Mask washing, Antibacterial masks, Filtration efficiency, Bacterial filtration efficiency, Antibacterial activity, Inhalation resistance

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1. Introduction

The World Health Organization (WHO) declared the coronavirus disease 2019 (COVID-19) a pandemic in March 2020. By July 2022, the number of confirmed COVID-19 cases worldwide exceeded 6.3 million (WHO, 2022a). With the spread of COVID-19, WHO recommends the use of masks in public places as it reduces the risk of infection by blocking airborne droplets and particles. The National Institute of Occupational Safety and Health (NIOSH) states that appropriate use of masks provides the highest level of protection from particles (Centers for Disease Control and Prevention [CDC], 2022a).

In the United States, masks are defined as N-, R-, and P-series according to NIOSH 42 CFR Part 84 (CDC, 1997). The European standard (EN 149:2001 + A1:2009) divides masks into FFP1, FPP2 and FFP3 according to their level of protection (Wang et al., 2022). In Korea, masks are categorized under filtering respirators (or masks) and KF-anti droplet (KF-AD) or surgical masks and are classified as quasi-drugs under the Pharmaceutical Affairs Act of the Ministry of Food and Drug Safety (MFDS, 2022). In the United States, cloth and surgical masks were mainly used, and less than 6% of the participants in the study about effectiveness of mask use wore N95 or KN95 masks (Andrejko et al., 2021). In Korea, more than 80% of the survey respondents wore KF94 masks (Kwon & Yang., 2022).

In the early days of the COVID-19 pandemic when masks were in short supply, civil society focused on making non-certified masks, and research on how to reuse disposable masks was conducted (Rubio-Romero et al., 2020). Wearing face masks in indoor public places is compulsory in Korea (KDCA, 2020), and more than 50% of the study's participants responded that the number of mask reuse per week was at least one to four times (Shin et al., 2021). CDC suggested that disposable masks should be used for one day and then be discarded (CDC, 2022b), and the WHO has noted that cloth masks need to be washed every day (WHO, 2021). In management plans for fabric masks, washing them at least once a day in hot water at 60 °C using soap or detergent is required; if washing with hot water is not possible, a method of boiling them for 1 min after washing in room temperature water has also been suggested (WHO, 2022b). In Korea, masks are recommended to be worn once and discarded, with no separate requirement for cloth mask management.

Commercial reusable cloth masks are non-certified masks whose filtration efficiency has not been confirmed; such masks do not have to comply with respiratory standards similar to those of filtering masks unless they are used for specific purposes. However, these masks can give a false impression that they have the potential to serve as barriers to particulate matter in the atmosphere. In this regard, the CEN Workshop Agreement (CWA) 17553:2020, which is a recommendation for community face coverings, was published by the European Committee for Standardization (CEN, 2020). However, this document is not designated as an official standard (Nguyên et al., 2021). However, in Korea and the United States, methods for evaluating respiratory-associated microbes and particles for cloth masks have not been established. In the textile field of household goods in Korea, cloth masks are classified into those for cold weather, fashion, and sports (KATS, 2021) The cloth mask test procedure should follow multiple test methods such as dye test, allergy test, lead and cadmium content, and formaldehyde test, and items such as fiber blending ratio should be indicated (KATS, 2021). As the nanofiber market has recently grown, antibacterial masks that can be reused by grafting antibacterial fibers to these fibers are being distributed in the market. Manufacturers of these reusable antibacterial masks advertise that they are microbial resistant; however, there is a lack of scientific evidence on the performance of these masks. It is ambiguous whether they protect the respiratory track of the wearer and for how long they can maintain their antibacterial properties after washing.

Several filtration efficiency and pressure resistance tests are being conducted in cloth masks or handmade masks that do not follow international standards, and research on washing disposable and multi-use masks is underway (Charvet et al., 2022; Sankhyan et al., 2021). Experiments to verify the antibacterial properties by grafting antibacterial substances to fibers are also being conducted (Seidi et al., 2021). However, studies on the change in performance of reusable antibacterial masks by washing under various conditions are lacking. In this study, the following hypotheses are investigated. Masks lacking certification standards related to respirators will exhibit differences in performance compared to masks certified domestically and internationally. Moreover, differences in temperature and washing method will change the mask performance. Finally, the mask performance will change as the number of washes increases.

2. Methods

The entire experimental procedure used in this study is schematically illustrated in Fig. 1.

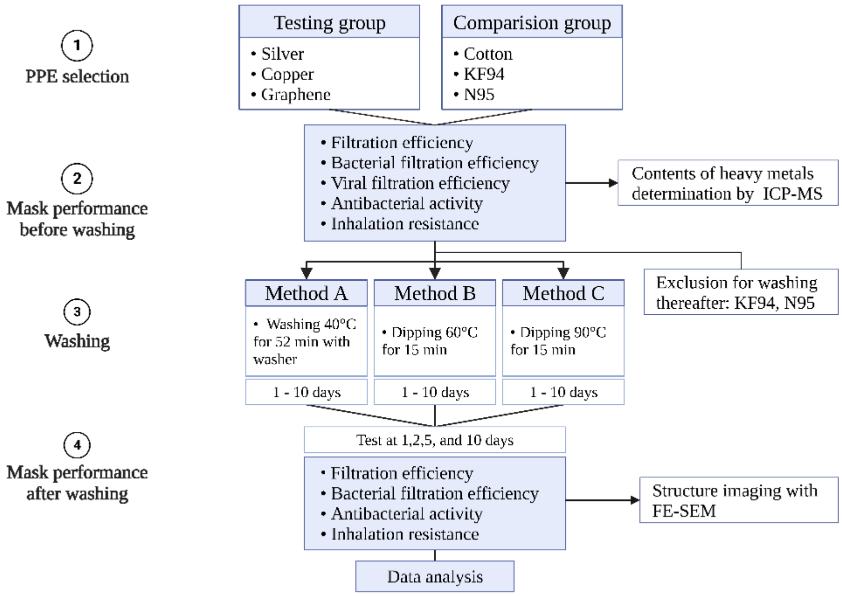


Figure 1. Schematic illustration of the experimental procedure.

2.1 Characteristics of masks under study

Reusable antibacterial silver, copper, and graphene masks were purchased from the market and subjected to experiments. Cotton masks were used as a control for comparing mask performance after washing, and the performance data of unwashed masks were compared with those of KF94 and N95 masks used as controls. The characteristics of the tested face masks are listed in Table 1. According to mask manufacturers, silver and copper masks consist of polyester and spandex, while graphene masks contain polyester fibers. Cotton masks are fabricated from 100% cotton and KF94 and N95 masks are made of polypropylene.

To check the heavy metal content of the masks, 0.2 g of the mask was put into PTFE vessels with 8 mL of 70% nitric acid solution and heated at 160 °C for 5 h in a heating block (OD-98-002P, ODlab, Gwangmyeong-si, Gyeonggi-do, Republic of Korea). After cooling at room temperature, the mixture was transferred to a conical tube and adjusted to 15 g with deionized water. The heavy metal concentrations were obtained using inductively coupled plasma mass spectrometry (ICP-MS).

Table 1. Characteristics of the face masks used in this study.

Group	Mask type	Mask use	Shape	Layers	Surface area (cm^2)[7]	Weight (g) (AM ± SD)[8]
Test group	Silver[1]	Reusable	Flat fold	3	209.5	7.5 ± 0.2
	Copper[2]	Reusable	Flat fold	3	209.4	8.2 ± 0.2
	Graphene[3]	Reusable	Flat fold	3	255.8	8.5 ± 0.2
Comparison group	Cotton[4]	Reusable	Flat fold	2	258.1	15.8 ± 0.3
	KF94[5]	Disposable	Flat fold	3	223.4	4.5 ± 0.1
	N95[6]	Disposable	Flat fold	3	241.5	9.2 ± 0.1

[1]Silver mask (Z Code Antibacterial cooling mask; Wivis, Seoul, Republic of Korea)

[2]Copper mask (IM Mask; International Mold, Paju-si, Gyeonggi-do, Republic of Korea)

[3]Graphene mask (Quantum V1; JD Life Science, Wanju-gun, Jeollabuk-do, Republic of Korea)

[4]Cotton mask (Reset; HM WORKS INC., Gangbuk-gu, Seoul, Republic of Korea)

[5]KF94 mask (201A KF94; Dobu Life Tech, Gwangju-si, Gyeonggi-do, Republic of Korea)

[6]N95 mask (N95 9210+, 3M, St. Paul, MN, USA)

[7]The volume of the area in contact with the face was measured because the ear strap of the reusable antibacterial mask was not separate.

[8]Average mass ± standard deviation

Table 2. Heavy metal contents in masks (*n* = 3).

Heavy metal (μg/g)	Mask type			
	Silver mask	Copper mask	Graphene mask	Cotton mask
Ag	0.4137 ± 0.2645[1]	0.0005 ± 0.0002	0.0010 ± 0.0003	0.0005 ± 0.0009
Co	0.0127 ± 0.0009	0.0006 ± 0.0001	0.0020 ± 0.0004	ND[2]
Cr	0.0013 ± 0.0004	0.0012 ± 0.0002	0.0024 ± 0.0007	0.0007 ± 0.0001
Cu	0.0022 ± 0.0006	8.9050 ± 0.6950	0.0055 ± 0.0027	0.0004 ± 0.0003
Fe	0.0237 ± 0.0253	0.0081 ± 0.0017	0.9638 ± 0.0390	0.0368 ± 0.0100
Mn	0.0038 ± 0.0005	0.0012 ± 0.0001	0.0722 ± 0.0049	0.0016 ± 0.0003
Ni	0.0008 ± 0.0004	0.0003 ± 0.0001	0.0024 ± 0.0004	0.0005 ± 0.0003
Pb	0.0011 ± 0.0003	0.0004 ± 0.0001	0.0018 ± 0.0004	0.0001 ± 0.0002
Sb	1.0865 ± 0.0768	1.3885 ± 0.0524	0.6542 ± 0.1857	ND[2]
Ti	0.0511 ± 0.0062	0.0714 ± 0.0186	0.1040 ± 0.0169	0.0063 ± 0.0014
Zn	0.0028 ± 0.0021	0.0036 ± 0.0012	0.0055 ± 0.0017	0.0021 ± 0.0024

[1]Average mass ± standard deviation

[2]ND: not detected.

Abbreviation: Ag = Silver, Co = Cobalt, Cr = Chromium, Cu = Copper, Fe = Iron, Mn = Manganese, Ni = Nickel, Pb = Lead, Sb = Antimony, Ti = Titanium, Zn = Zinc

Note: Korean Agency for Technology and Standards' standard level of harmful element content: Pb: 100 μg/g.

2.2 Washing methods and cycles

Three washing methods were evaluated. A washing machine (F12WVA; LG Electronics, Seoul, Republic of Korea) was used for washing the masks, and standard washing was performed at 40 °C, which was the default laundry temperature of the machine (Method A). The washing, rinsing, and drying processes were completed in 52 min. Approximately 10 L of water were used for each process without any detergent. Additionally, for Method B (60 °C) and Method C (90 °C), 400 mL of boiled tap water were added according to each temperature in a beaker, and the tested masks were placed in the beaker and left undisturbed for 15 min. The masks were dried at room temperature (20 ± 5 °C, 55 ± 5% RH) and washed once a day. The masks were washed over ten days, and their performance was tested after washing for one, two, five, and ten times.

2.3 Mask performance test

The silver, copper, graphene, and cotton masks were washed once (one cycle) and were then dried at room temperature (20–25 °C, 50–70% RH). Subsequently, the filtration efficiency and inhalation resistance tests were conducted, and the mask washing was performed again on the same mask. Masks tested with bacteria and virus could not be tested on the same mask after washing. Therefore, the masks were prepared for testing their antibacterial activity, bacterial, and viral filtration efficiency by washing one, two, five, and ten times. The masks were conditioned at 21 ± 5 °C and 85 ± 5% RH for 4 h before the antibacterial activity and bacterial and virus filtration efficiency tests were conducted.

(1) ***Filtration efficiency test***

The filtration efficiency test was performed using an automated filter tester (8130A; TSI Incorporated, Shoreview, Mn, USA) with NaCl aerosol, which was generated using an automatic salt aerosol generator. The size distribution of the particles had a count median diameter of 0.075 μm and a geometric standard deviation that did not surpass 1.83 μm. The air flow rate was 85 L/min ± 4 L/min. The initial filtration efficiency of the masks in the first minute was tested with reference to NIOSH 42 CFR 84 (NIOSH, 1995). To calculate the aerosol concentration, the upstream and downstream were measured using a photometer. The filtration efficiency was calculated using the following formula.

$$FE\ (\%)\ = 1 - \frac{C_{down}}{C_{up}} \times 100$$

[Formula]

C_{up} = upstream concentration

C_{down} = downstream concentration

(2) *Bacterial filtration efficiency test*

The bacterial filtration efficiency test was performed using a bacterial filtration efficiency tester (BFET-1853; ART Plus, Icheon-si, Gyeonggi-do, Republic of Korea) with a six-stage Anderson sampler. *Staphylococcus aureus* (*S. aureus*) (ATCC 6538™) was used as a representative bacterial strain. The bacterial filtration efficiency was tested according to the ASTM International (ASTM, 2019) and MFDS (MFDS, 2022) with minor modifications. *S. aureus* was inoculated in Tryptic Soy Broth (TSB) and incubated at 37 ± 2 °C for 24 h. The concentration was calculated using serial dilution with plate counting method. A solution with the concentration maintained at 1,700–3,000 colony-forming units (CFU) was obtained for positive controls and was used as a test solution. The mean particle size (MPS) of the bacterial aerosol was 3.0 ± 0.3 µm. The *S. aureus* suspension was aerosolized and sprayed on a mask sample for 60 s at a constant flow rate of 28.3 L/min and simultaneously allowed to be captured for 120 s onto six Tryptic Soy Agar (TSA) plates. The plates were incubated for 48 ± 4 h at 37 ± 2 °C. The total CFU of the test, positive control, and negative control groups were determined by counting the colonies on all six plates. The bacterial filtration efficiency was determined according to ASTM International (ASTM F2100; ASTM, 2019) and MFDS (MFDS, 2022) as follows.

$$B = \frac{(C - T)}{C} \times 100$$

[Formula]

B = Bacterial filtration efficiency (%)

C = Total CFU measured in positive control group

T = Total CFU measured in test group

(3) *Viral filtration efficiency test*

The viral filtration efficiency was tested using a bacterial filtration efficiency tester (BFET-1853; ART Plus). Murine coronaviruses (mouse hepatitis viruses; ATCC VR-764™) were sprayed into a six-stage Andersen cascade impactor (ACI). Murine coronaviruses were inoculated on a confluent monolayer of L2 cells in a flask and cultured at 37 °C with 5% CO_2 for 2 d. Freezing and thawing were repeated three times to allow the virus to escape the cells. After centrifugation (5,000×*g*, 20 min) at 4 °C, the virus was purified in the culture medium using a Stericup® Filter Unit (0.22 μm; Merck KGaA, Darmstadt, Germany). Thereafter, the supernatant containing the virus was concentrated using an Amicon® Ultra-15 Centrifugal Filter Unit (Merck Millipore Ltd., Cork, Ireland), and a virus stock was obtained and stored at −80 °C until use. The virus stock was diluted with phosphate buffer solution to obtain the positive control group with 1,700–3,000 plaque forming units (PFU). The MPS of the virus aerosol was set to 3.0 ± 0.6 μm. For capturing viruses, a medium (12.5 mL), containing 7% gelatin (from porcine skin; Sigma-Aldrich, St Louis, MO, USA) and 0.5 × Dulbecco's modified Eagle's medium (Gibco, Thermo Fisher Scientific, Waltham, MA, USA), was poured into each Petri dish (100 mm × 15 mm) and allowed to solidify at 4 °C. The aerosol generator sprayed the virus suspension onto the mask sample at a flow rate of 28.3 L/min for 60 s and simultaneously allowed the viruses to be captured onto six Petri dishes for 120 s.

$$V = \frac{(C - T)}{C} \times 100$$

[Formula]

V = Viral filtration efficiency (%)

C = Total PFU measured in positive control group

T = Total PFU measured in the test group

(4) *Antibacterial activity test*

The antibacterial activity of face masks was tested according to the International Organization for Standardization (ISO 20743:2013) and Korean Agency for Technology and Standards (KSK0693:2016) with minor modifications (Fig. 2).

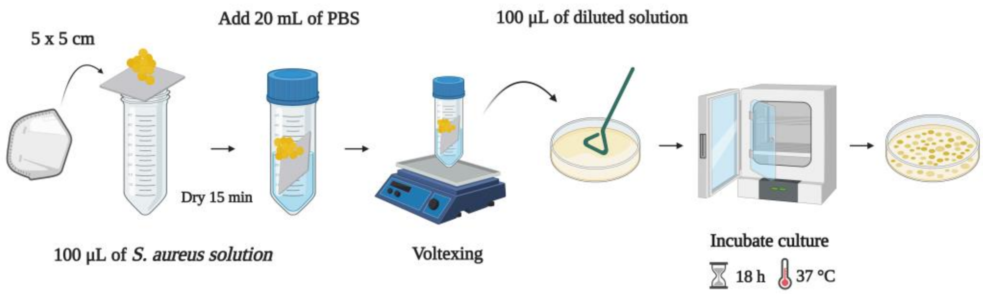


Figure 2. Experimental procedure for antibacterial activity testing.

S. aureus was inoculated in TSB and cultured at 37 °C. Then, the culture was serially diluted with TSA ten times and incubated at 37 ± 2 °C for 18 h. The bacterial concentration was determined using the plate counting method. The experiment was conducted at room temperature (20–25 °C) and at 50–70% RH. Then, 2 g of Tween 80 and 5 g of sodium chloride were added to 1,000 mL of distilled water as a solution for dispensing bacteria on the surface of the sample. The bacterial solution (100 µL) was inoculated onto the surfaces of the mask samples (5 × 5 cm^2). The concentration of the inoculated bacterial solution was 2.0 × 10^6 CFU/mL. For calculating the bacterial concentration at 0 h for each mask sample, the masks were inoculated with the bacterial solution, dried for 15 min, immediately immersed in 20 mL of phosphate buffer solution, and then vortexed to separate the bacteria from the mask sample. The eluate was serially diluted and 100 μL of the diluted solutions were sprayed onto TSA and incubated at 37 ± 2 °C for 24 h. The visible colonies were then counted for calculating the concentration of the eluate. For calculating the bacterial concentration at 18 h from each mask sample, bacteria were inoculated on the surface of the mask sample and dried for 15 min. Then, phosphate buffer solution (20 mL) was added to the conical tube for desorption. The concentration of the eluate was calculated as mentioned above.

(5) *Inhalation resistance test*

The inhalation resistance for each mask type was tested using a mask inhalation resistance tester (ARE-1651; ART Plus) for 1 min at a flow rate of 85 ± 2 L/min. The N95 certification standard of NIOSH (42 CFR 84; NIOSH, 1995) was used for this test.

(6) *FE-SEM*

Field-emission scanning electron microscopy (FE-SEM 800F Prime; JEOL Ltd, Tokyo, Japan) was used to evaluate the changes in surface morphology of the silver, copper, graphene, and cotton masks. A mask sample was attached to a stub using carbon tape. The stub was coated with platinum at 20 mA for 100 s. The FE-SEM images were obtained at an acceleration voltage of 15 kV. The images were magnified by 70 × and 500 ×. *S. aureus* aerosols sprayed on unwashed control masks were observed in images magnified by 10,000 ×.

2.4 Data analysis

Statistical analysis was performed using the R software v. 4.2.2 (R Development Core Team, Vienna, Austria), and graphs were obtained using GraphPad Prism 9.4.1 (GraphPad Software, Inc., La Jolla, CA, USA). The average and standard deviation for the test and comparison groups of the six types of masks (silver, copper, graphene, cotton, KF94, and N95 masks) were obtained (n = 3 for each type). An independent two-sample t-test was used to determine statistical significance between the washing cycles. Statistical significance was set to $P < 0.05$.

3. Results

3.1 Performance of the tested face masks without washing

Table 3 shows the results of five performance tests for each mask type without washing. When washing was not performed, the three reusable antibacterial masks showed a filtration efficiency of 10–13%, while that of the cotton masks was approximately 47%. These filtration efficiency values were significantly lower than those of KF94 and N95 at more than 98% efficiency, which meets the N95 certification standards (NIOSH, 1995).

All five masks except for the silver mask exhibited bacterial filtration efficiency exceeding 95%, which meets the ASTM F2100 and MFDS standards (ASTM, 2019; MFDS, 2022). Overall, the viral filtration efficiency for each mask type was similar to the bacterial filtration efficiency (Table 3). The silver mask exhibited the lowest filtration efficiency for both microorganisms (Table 3). Regarding KF94 and N95 masks, the viral filtration efficiency exceeded 99.9%, which was higher than that of reusable antibacterial masks.

Based on the results of the antibacterial activity test, the antibacterial activity of cotton, KF94, and N95 masks was 84–86%, which was lower than that of reusable antibacterial masks. Additionally, among the reusable antibacterial masks, the graphene mask showed the lowest value (approximately 88%; Table 3).

The inhalation resistance values of the six masks were less than 35 mmH_2O. In contrast to the copper mask, the silver and graphene masks showed inhalation resistance of 5–7 mmH_2O. The mean inhalation resistance values for copper and cotton masks were approximately 16 and 25 mmH_2O, respectively, which were lower than that (approximately 11 mmH_2O) of KF94.

Table 4 shows the mean CFU of *S. aureus* collected on each stage of the ACI with a total of six stages used in this study. The highest number of *S. aureus* was collected at stages 5 and 6, with cut-off diameters of 1.1–2.1 µm and 0.65–1.1 µm, respectively.

The bacterial filtration efficiency of the tested masks for each ACI stage is shown in Fig. 3. Compared to other stages, stages 5 and 6 showed lower efficiency. Among the reusable antibacterial masks, the silver mask showed a bacterial filtration efficiency of 84% at stage 5, and the efficiency sharply decreased to 13% at stage 6. The efficiency of other reusable antibacterial masks also decreased below 90% at stage 6. By contrast, the KF94 and N95 masks showed high efficiency exceeding 99% even at stage 6. The detailed efficiency values at each stage are provided in Appendix 7.

Table 3. Results of the performance test for each mask type without washing.

Test	Mask type						
	n	Silver mask	Copper mask	Graphene mask	Cotton mask	KF94 mask	N95 mask
Filtration efficiency (%)	9[1]	10.07 ± 1.63[2]	13.95 ± 1.13	10.98 ± 1.17	47.88 ± 2.95	98.61 ± 1.44	99.47 ± 0.53
Bacterial filtration efficiency (%)	3	93.49 ± 1.16	98.35 ± 1.04	96.53 ± 1.14	97.89 ± 0.89	99.98 ± 0.04	99.98 ± 0.04
Viral filtration efficiency (%)	3	94.54 ±1.75	95.77 ± 0.21	95.85 ± 0.14	99.16 ± 0.21	99.98 ± 0.03	> 99.99
Antibacterial activity (%)	3	98.84 ± 0.93	98.02 ± 2.75	88.98 ± 3.03	84.54 ± 16.62	84.24 ± 23.80	86.75 ± 6.84
Inhalation resistance (mmH_2O)	3	7.49 ± 0.92	16.97 ± 1.63	5.14 ± 0.49	25.78 ± 1.88	11.38 ± 1.49	7.28 ± 0.54

[1]*n* = 9. The filtration efficiency of silver, copper, and graphene masks was too low; the tests were therefore conducted in two independent laboratories.

[2]Mean ± standard deviation

Table 4. Mean CFU of *S. aureus* deposited at each ACI stage of each mask type without washing (*n* =3).

Stage (cut-off diameter)	Mask type					
	Silver mask	Copper mask	Graphene mask	Cotton mask	KF94 mask	N95 mask
1 (7 μm)	1.00 ± 1.00[1]	0.33 ± 0.58	0.33 ± 0.58	ND[2]	ND[2]	ND[2]
2 (4.7 μm)	0.33 ± 0.58	ND[2]	0.33 ± 0.58	0.33 ± 0.58	ND[2]	ND[2]
3 (3.3 μm)	3.67 ± 2.89	ND[2]	1.00 ± 1.73	0.67 ± 0.58	ND[2]	ND[2]
4 (2.1 μm)	14.67 ± 7.77	0.33 ± 0.58	2.00 ± 2.00	2.00 ± 1.00	ND[2]	ND[2]
5 (1.1 μm)	61.67 ± 9.02	6.00 ± 4.00	12.33 ± 4.93	6.33 ± 1.15	0.33 ± 0.58	ND[2]
6 (0.65 μm)	50.67 ± 9.29	3.00 ± 1.73	4.33 ± 1.53	2.33 ± 2.08	0.33 ± 0.58	0.33 ± 0.58

[1]Mean ± standard deviation

[2]ND: not detected.

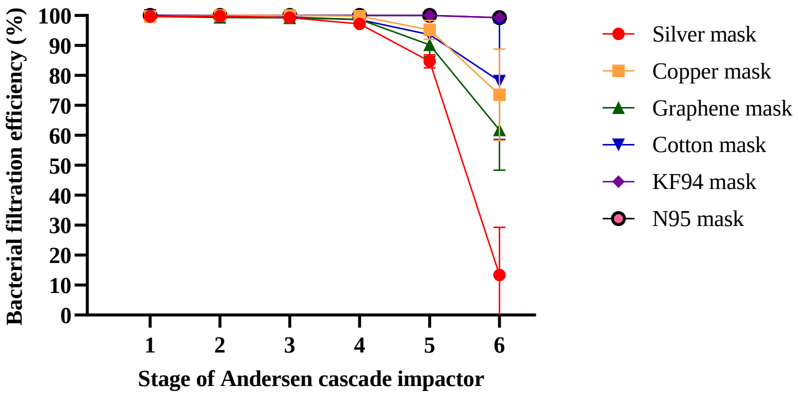


Figure 3. Bacterial filtration efficiency from each stage of ACI for each mask type.

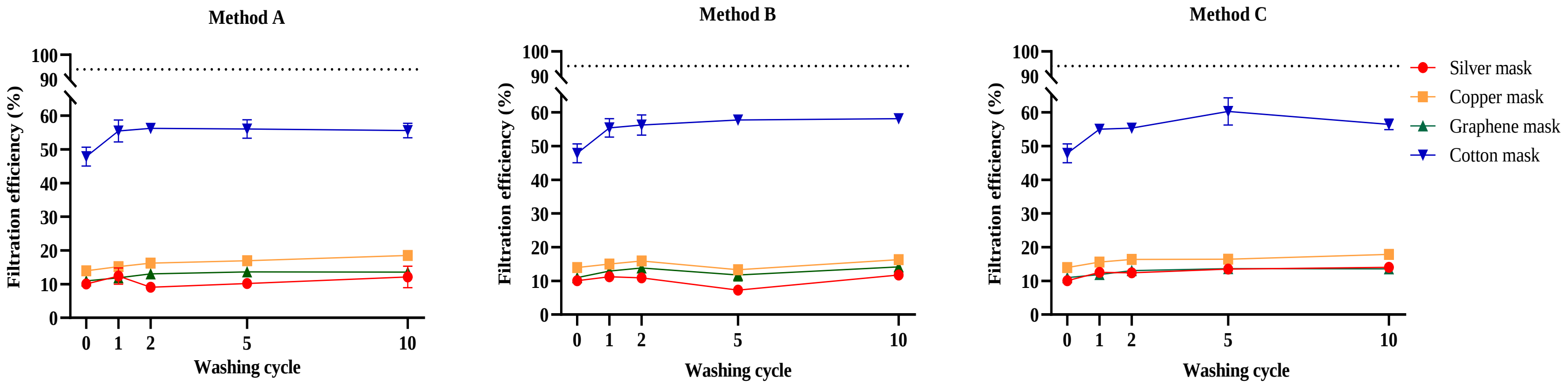
3.2 Performance of the tested face masks after washing

Fig. 4 illustrates the results corresponding to 0, 1, 2, 5, and 10 washing cycles for the four types of washed reusable masks (silver, copper, graphene, and cotton masks) subjected to Methods A, B, and C. The three reusable antibacterial masks and the cotton masks failed to comply with the NIOSH-certified N95 mask standard of 95% (NIOSH, 1995) before washing, and no increase was found to exceed the mask standard after washing (Fig. 4[a]).

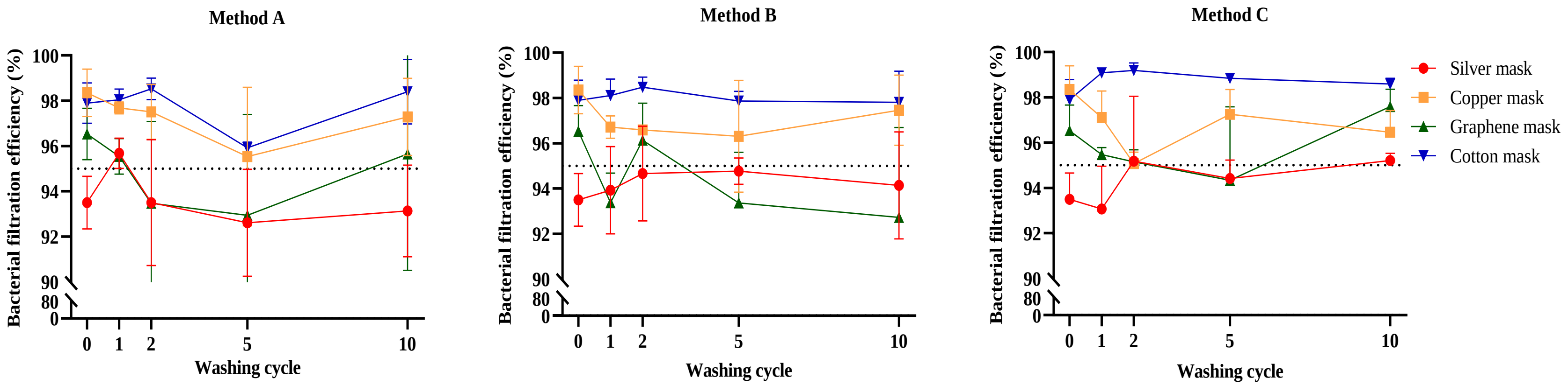
The bacterial filtration efficiency of the silver and graphene masks changed irregularly when washing was repeated. All three washing methods produced bacterial filtration efficiency values below 95%, which did not meet the ASTM and MFDS standards (ASTM, 2019; MFDS, 2022) (Fig. 4[b]). The bacterial filtration efficiency of the unwashed graphene mask exceeded the 95% criterion but fell short of the criterion as the washing was repeated (Fig. 4[b]).

In the antibacterial activity test, the antibacterial activity of the cotton mask was at a minimum value of 76%, which was lower than that of other masks (Fig. 4[c]). The three reusable antibacterial masks and the cotton masks showed irregular changes in antibacterial activity when subjected to 10 washing cycles using the 3 washing methods (Fig. 4[c]).

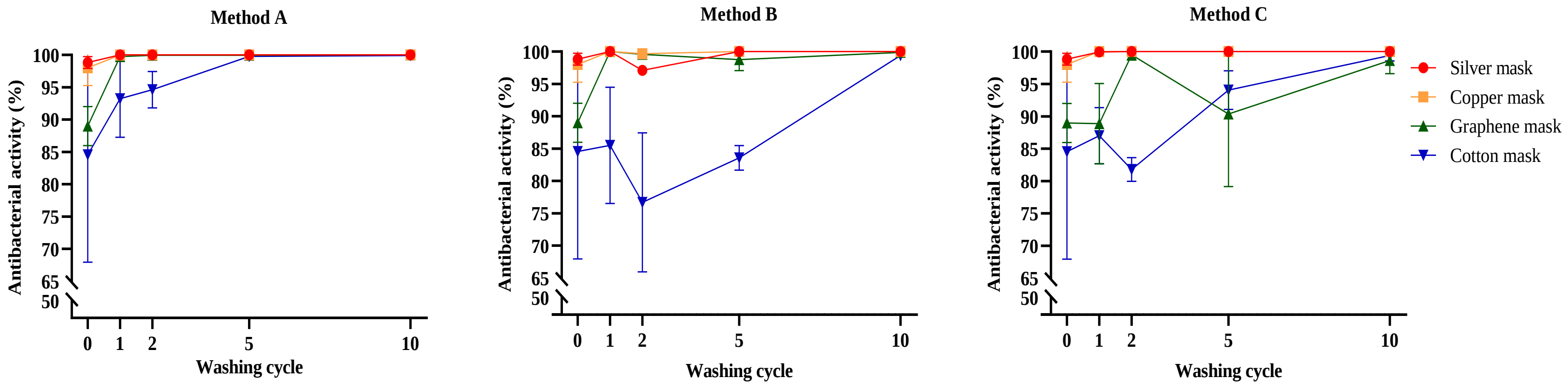
As the washing cycle increased, the inhalation resistance of the cotton mask increased and decreased irregularly, exceeding the N95 certification standard of NIOSH of 35 mmH_2O (NIOSH, 1995). The three reusable antibacterial masks satisfied the N95 certification standards of NIOSH for inhalation resistance even after 10 washing cycles. The silver mask exhibited the smallest variation upon repeated washes, ranging from a minimum of 5 mmH_2O to a maximum of 8 mmH_2O with increasing number of wash cycles (Fig. 4[d]). The detailed results for these four types of masks are provided in Appendices 3, 4, 5, and 6.



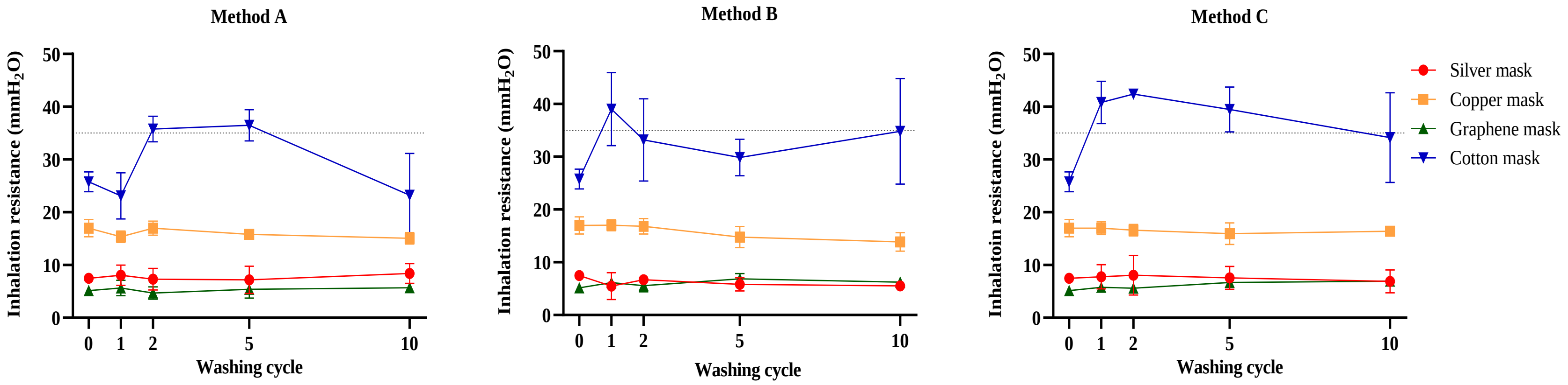
(a) Filtration efficiency according to the three washing methods and number of washing cycles.



(b) Bacterial filtration efficiency according to the three washing methods and number of washing cycles.



(c) Antibacterial activity according to the three washing methods and number of washing cycles



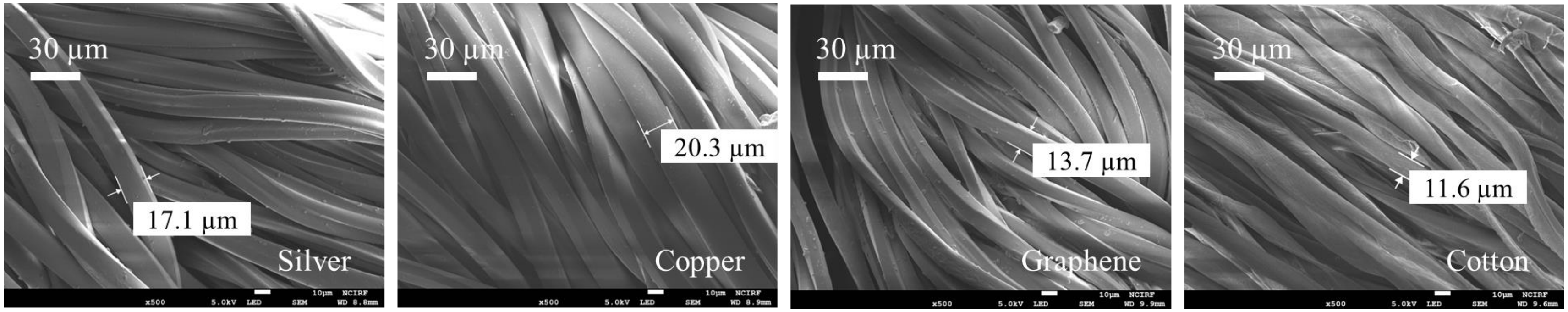
(d) Inhalation resistance according to the three washing methods and number of washing cycles.

Figure 4. Results of the four tests on reusable masks before and after applying the three washing methods for different washing cycles (0, 1, 2, 5, and 10 cycles). (a) Filtration efficiency according to the three washing methods and number of washing cycles. The dashed line indicates the N95 certification standards of NIOSH (over 95%; NIOSH, 1995); (b) Bacterial filtration efficiency according to the three washing methods and number of washing cycles. The dashed line indicates the ASTM F2100 and MFDS standards (over 95%; ASTM, 2019; MFDS, 2022); (c) Antibacterial activity according to the three washing methods and number of washing cycles; (d) Inhalation resistance according to the three washing methods and number of washing cycles. The dashed line indicates the N95 certification standards of NIOSH (under 35 mmH_2O; NIOSH, 1995).

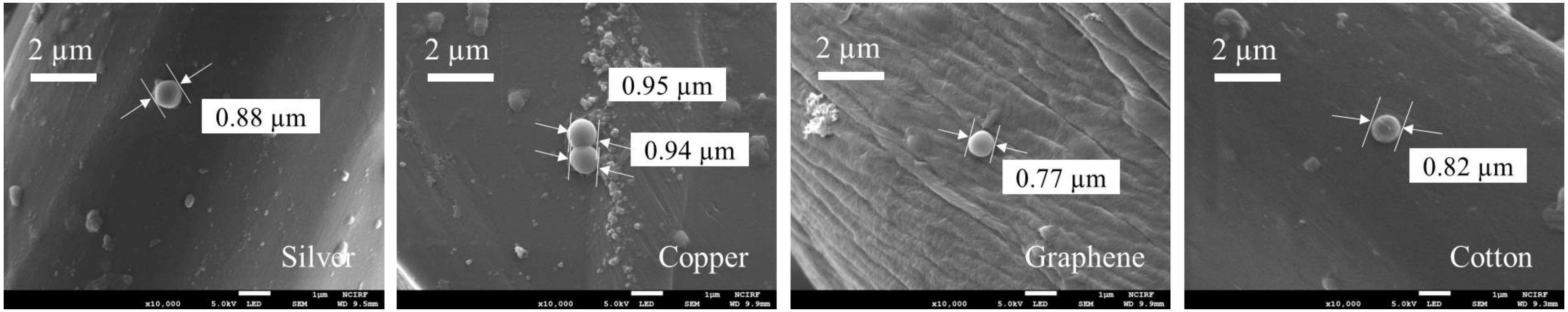
3.3 FE-SEM images of the surface of face masks

Fig. 5(a) shows the magnified images (500×) of mask fiber strands from each mask type before washing. The cotton mask is the thinnest and has an uneven width. Reusable antibacterial masks show a width of approximately 13–20 μm. Fig. 5(b) shows the magnified images of *S. aureus* (10,000×) attached to the fabric of an unwashed mask when sprayed with *S. aureus*. *S. aureus* is approximately 0.7–0.9 μm in size.

Fig. 6 shows the images of mask surfaces before washing and after one and ten washing cycles using Method A. All three reusable antibacterial masks and the cotton mask showed braided fabric. When washing was not performed, the fibers of the cotton mask were looser compared to those of the other reusable antibacterial masks (Fig. 6[a]). No significant difference was observed in loosening of the mask fibers between one washing cycle and no washing (Fig 6[b]). After 10 washing cycles, the yarn loosened and changed into a more twisted shape (Fig. 6[c]). When the masks were washed using Methods B and C, minimal differences in fiber loosening were observed compared to that before washing (Appendix 8).

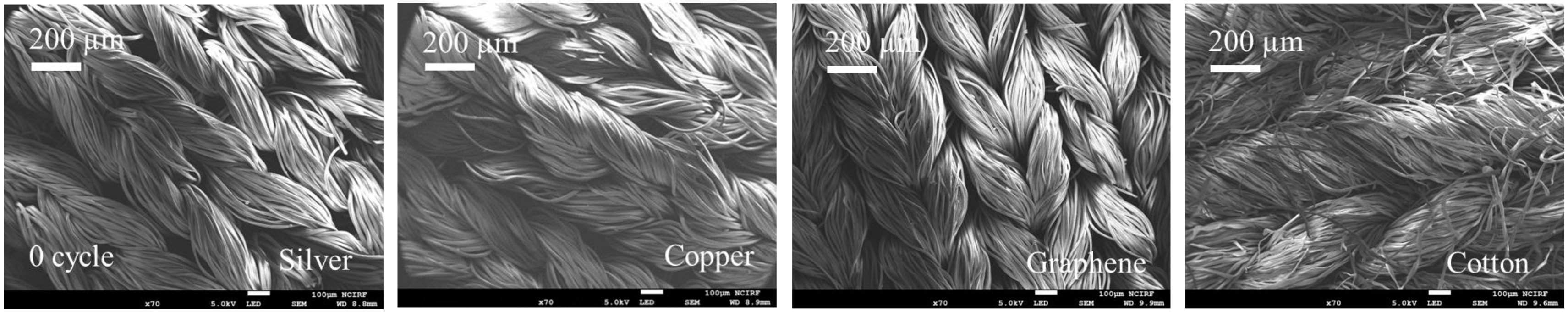


(a) Strands from the silver, copper, graphene, and cotton masks before washing (500× magnification).

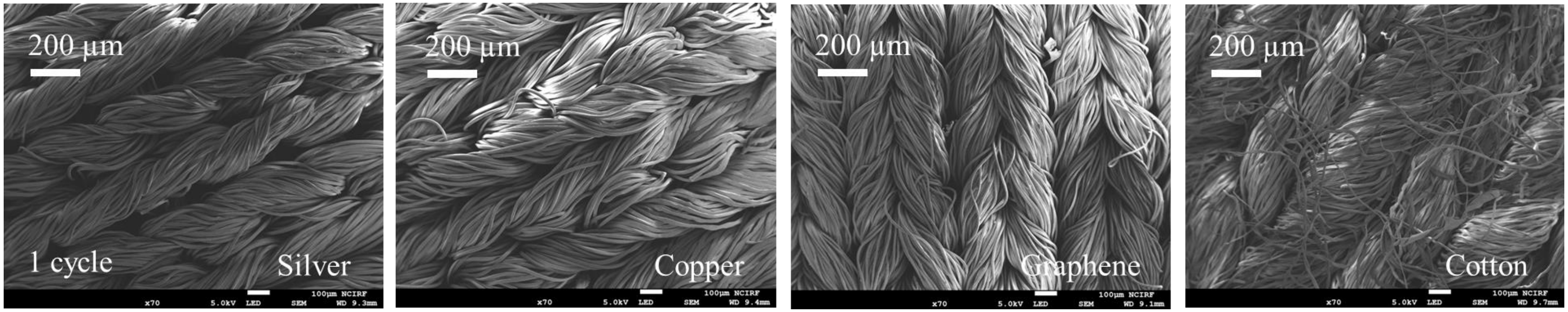


(b) Images obtained after *S. aureus* was sprayed on each mask type (10,000× magnification).

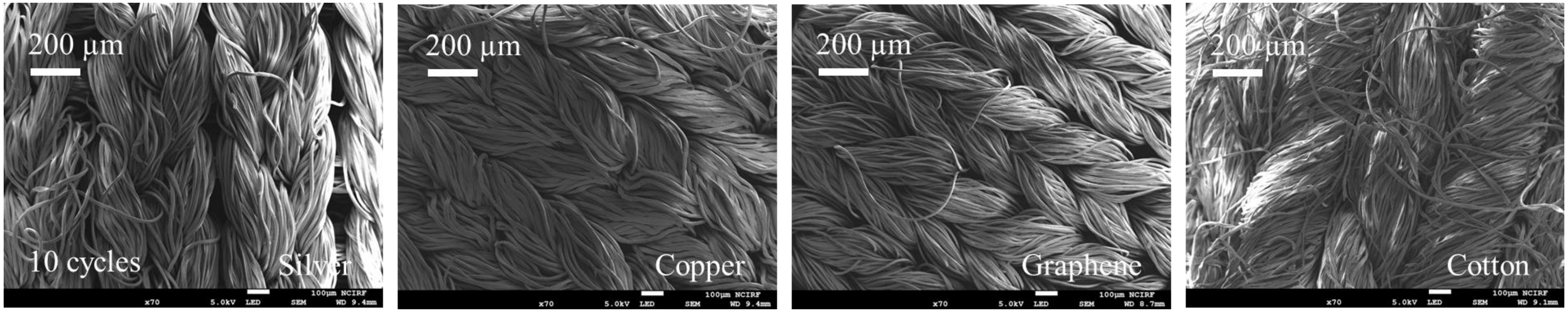
Figure 5. Images of the unwashed mask surfaces. (a) Strands from the silver, copper, graphene, and cotton masks before washing (500× magnification); (b) Images obtained after *S. aureus* was sprayed on each mask type (10,000× magnification).



(a) Surface images of the silver, copper, graphene, and cotton masks without washing (70× magnification).



(b) Surface images of the silver, copper, graphene, and cotton masks after one cycle of washing (70× magnification).



(c) Surface images of the silver, copper, graphene, and cotton masks after ten cycles of washing (70× magnification).

Figure 6. Surface images of silver, copper, graphene, and cotton masks observed via FE-SEM (70× magnification) after treatment with Method A. (a) Surface images of the silver, copper, graphene, and cotton masks without washing (70× magnification); (b) Surface images of the silver, copper, graphene, and cotton masks after one cycle of washing (70× magnification); (c) Surface images of the silver, copper, graphene, and cotton masks after ten cycles of washing (70× magnification).

3.4 Rate of performance change after each washing cycle

Tables 5, 6, 7, and 8 show the increase/decrease in performance from 0 (no washing) to 1 cycle, from 0 to 2 cycles, from 0 to 5 cycles, and from 0 to 10 cycles of washing using Methods A, B, and C. The data are expressed as percentages with their statistical significance between each cycle.

The change in the performance of silver masks for each number of washing cycles using Methods A, B, and C for the three tests is shown in Table 5. Silver masks exhibited almost no significant difference in their performance compared to the initial (no washing) value, irrespective of washing methods and number of cycles. Table 6 presents the rate of change in the performance of copper masks; the washing methods and cycles had a minimal effect on the change in their performance. Regarding graphene masks, significant difference was observed in their antibacterial properties when Methods B was used, compared to their initial performance without washing (Table 7). Significant difference was also observed for cotton masks in the intake resistance test, with the most significant difference being observed in Method C, corresponding to a washing method using high temperatures (90 °C; Table 8).

Appendix 9 presents the rate of change in the filtration efficiency and the statistical significance values after washing the four types of masks according to the three cleaning methods and different number of cycles. Cotton masks showed statistically significant changes in filtration efficiency in all washing methods, whereas three types of reusable antibacterial masks mainly showed significant changes after washing using Method C.

Table 5. Rate of performance change and statistical difference according to the washing methods and number of cycles of silver masks using the *t*-test.

Washing method	Washing cycle	Change rate (%)		
		Bacterial filtration efficiency	Antibacterial activity	Inhalation resistance
Method A	1	**102.34***	101.17	107.56
	2	100.00	101.17	97.49
	5	99.06	101.17	96.05
	10	99.61	101.17	111.91
Method B	1	100.46	101.17	73.20
	2	101.25	98.25	89.24
	5	101.36	101.17	77.53
	10	100.68	101.17	**73.63***
Method C	1	99.54	101.12	103.92
	2	101.81	101.17	107.86
	5	100.98	101.17	100.79
	10	101.83	101.17	92.01

*$P < 0.05$

Table 6. Rate of performance change and statistical difference according to the washing methods and number of cycles of copper masks using the *t*-test.

Washing method	Washing cycle	Change rate (%)		
		Bacterial filtration efficiency	Antibacterial activity	Inhalation resistance
Method A	1	99.32	102.00	90.39
	2	99.14	102.02	100.02
	5	97.13	102.02	93.20
	10	98.92	102.02	88.71
Method B	1	98.34	102.02	100.39
	2	**98.21***	101.70	98.99
	5	97.92	102.02	86.91
	10	99.10	102.02	81.60
Method C	1	98.74	102.02	100.00
	2	**96.67***	102.02	97.73
	5	98.89	102.02	93.84
	10	98.08	102.02	96.70

*$P < 0.05$

Table 7. Rate of performance change and statistical difference according to the washing methods and number of cycles of graphene masks using the *t*-test.

Washing method	Washing cycle	Change rate (%)		
		Bacterial filtration efficiency	Antibacterial activity	Inhalation resistance
Method A	1	98.98	112.11	109.92
	2	96.84	112.34	91.17
	5	96.28	112.34	104.72
	10	99.09	**112.39***	110.41
Method B	1	**96.73***	**112.39***	119.24
	2	99.60	**111.91***	108.01
	5	96.72	**111.00***	133.02
	10	96.06	**112.26***	**121.59***
Method C	1	98.91	99.89	112.34
	2	98.57	**111.83***	108.70
	5	97.73	101.55	130.30
	10	101.11	**110.86***	**135.06***

*P < 0.05

Table 8. Rate of performance change and statistical difference according to the washing methods and number of cycles of cotton masks using the *t*-test.

Washing method	Washing cycle	Change rate (%)		
		Bacterial filtration efficiency	Antibacterial activity	Inhalation resistance
Method A	1	100.15	110.29	89.54
	2	100.64	111.91	**138.80***
	5	**97.98***	118.02	**141.53***
	10	100.51	118.18	90.09
Method B	1	100.72	103.50	**151.40***
	2	101.06	102.02	128.68
	5	100.33	104.53	115.76
	10	101.14	124.05	134.95
Method C	1	101.22	102.90	**158.28***
	2	101.33	96.73	**164.43***
	5	100.97	111.27	**153.10***
	10	100.71	117.59	132.51

*$P < 0.05$

4. Discussion

The data indicate a difference in performance between reusable antibacterial masks and those certified by national and international standards. However, the irregular performance of reusable antibacterial masks according to washing limits the accurate determination of the effect of washing method and number of cycles on the change in mask performance.

As for the performance of the reusable antibacterial masks used in this study, when the filtration efficiency was tested using NaCl, the initial value before washing failed to satisfy the N95 mask standard of the NIOSH (NIOSH, 1995). After repeated washing, the filtration efficiency of the reusable antibacterial masks increased by up to approximately 6% (Fig. 4[a]); however, this increase was not confirmed to exceed the N95 test standard. The bacterial filtration efficiency values were below the N95 standard of 95% (ASTM, 2019), but the change was insignificant (Fig. 4[b]). The antibacterial activity either increased minimally or decreased before and after washing (Fig. 4[c]). The value of inhalation resistance was a minimum of 5 mmH_2O to a maximum of 16 mmH_2O before and after washing (Fig. 4[d]), satisfying the N95 standard (NIOSH, 1995). These results indicate that repeated washing for 10 times does not cause a significant change in the filtration performance of the antibacterial masks tested (Fig. 4[a] and [b]). Such a finding agrees with previous studies, reporting that the filtration efficiency of reusable masks does not change significantly when washed in a washing machine and that reusable masks can be reused several times after washing (Sankhyan et al., 2021; Whyte et al., 2022b).

Differences in mask manufacturers used in this study may lead to differences in performance between the mask types before and after washing, depending on fabric knitting method or material. Therefore, the mask performance changes according to the mask material and the comparisons are challenging. The filtration efficiency of particles varies depending on the type and structure of the fabric (Konda et al., 2020). Polyester fabrics have lower hygroscopicity and better electrostatic properties than natural fabrics, and polyurethanes have excellent elasticity and abrasion resistance (Perumalraj., 2015; Yang et al., 2020). By contrast, cotton masks have low fiber strength, low heat resistance, and high hydrophilicity (Felice et al., 2022; Whyte et al., 2022a). The greater changes in the filtration efficiency and inhalation resistance of cotton masks after washing compared with those of reusable antibacterial masks may be due to the cotton fibers exhibiting low strength. According to FE-SEM observations of the surfaces of the antibacterial masks and the cotton mask, their fiber arrangement structures showed a similar twisted shape (Fig. 6). However, because all three layers of the reusable antibacterial masks could not be separated, the surface difference between the layers could not be compared. Therefore, the inhalation resistance of reusable antibacterial masks remains below the N95 mask standard even after repeated washings presumably because their fabric has a lower density and stronger strength than that of cotton masks (Fig. 6).

In both the silver and copper masks used in this study, antimony was the metal with the highest content, at a concentration of approximately 1 μg/g. The content of antimony may be related to the use of flame-retardant formulations or polyester fibers in mask manufacturing and processing (Rujido-Santos et al., 2022). The heavy metal content detected in this study was 0.4 μg/g in silver masks and 8 μg/g in copper masks. These concentrations were lower than those of a previous study on metal leaching in face masks containing antibacterial ingredients, reporting up to 5.27 mg/g for silver and up to 1.11 mg/g for copper (Pollard et al. 2021). Silver and copper are commonly used as antibacterial agents, and copper can be used for textile dyeing; copper components can be therefore detected (Pollard et al. 2021; Rujido-Santos et al., 2022). The highest quantity of iron was detected in the graphene mask at 0.9 μg/g, followed by antimony at 0.6 μg/g. Iron may be included in masks to form a metal-composite dye or to be used as a catalyst (Rujido-Santos et al., 2022). It has been reported that the amount of leached metal after washing a metal-containing mask does not equal the amount present in the initial mask, meaning that metal components may be leaching during mask washing. Leaching of antimicrobial agents into mask fibers may pose a potential risk to the mask wearer (Pollard et al., 2021).

Most commercially sold masks produced by imparting static electricity to polypropylene fibers, such as N95, are fabricated using electrostatic filter media, which increase the particle collection efficiency of the filter (Viscusi et al., 2009). Cotton and nylon fabrics have high triboelectric charging ability, and such electrocharged fabrics can improve the filtration efficiency (Bandi, 2020). The NIOSH standard uses charge-neutralized NaCl aerosol to test the filtration efficiency (NIOSH, 1995). However, ASTM does not specify charge neutralization for *S. aureus* aerosol (ASTM, 2019). Particles that are not charge-neutralized have an electrostatic effect, causing a risk of overestimating the filtration efficiency of the mask (Rengasamy et al., 2017; Rule et al., 2020).

In this study, because the average particle diameter of bacteria collected in each stage of the Anderson sampler is different, the bacterial filtration efficiency of each stage was calculated. The lowest efficiency was observed at the 6th stage at the bottom, where bacteria with an aerodynamic diameter of 0.65 μm were collected (Fig. 3). The highest number of bacteria was observed in the bottom of the Anderson sampler at the 5th and 6th stages (Table 4) meaning that small-sized bacteria were collected more. In the bacterial filtration efficiency test of the silver masks, the total bacterial filtration efficiency of the total 6 stages was 93%, whereas the efficiency of the 6th stage alone reached 13% (Tables 3 and 4). This finding is completely different compared to the 99% filtration efficiency of bacteria collected only at the 6th stage of the sampler for the KF94 and N95 masks. Therefore, the filtration efficiency for bacteria and viruses may show a value higher than the filtration efficiency using NaCl. In the bacterial filtration efficiency test, bacterial particles of 0.65 to 7 μm are collected using the aerodynamic particle size unit of the bacterial aerosol. The NaCl aerosols used for the filtration efficiency measurements were particles with a count median diameter of 0.075 μm. Therefore, the particle sizes generated and captured by each device were difficult to compare directly. Because the filter efficiency may vary depending on the particle size, shapes, and properties, the affecting factors should be considered (Tcharkhtchi et al., 2021). In a previous study, the efficiency of the bacterial filtration efficiency method was higher than that of the filtration efficiency method using NaCl due to the differences in the test conditions, including aerosol size and velocity (Rengasamy et al., 2017).

The reusable antibacterial masks used in this study were selected based on products that are frequently purchased from the internet. Therefore, as the results of this study cannot represent all the masks currently available on the market, the performance of reusable masks cannot be simply generalized. In addition, instead of disposable masks that are not recommended to be washed, reusable cotton masks were used as a control group for comparison with masks consisting of antibacterial fibers. Applying reusable antibacterial masks to the certification standards targeting filtering masks may be inappropriate. However, the masks were evaluated by applying internationally accepted standard test methods, and the certified masks were also used in the experiment to obtain reliability in the research results. In future studies, the evaluation of the performance of reusable antibacterial masks needs to be verified through follow-up studies that include more diverse types of masks and performance changes after washing. In addition, because the washing methods of the multi-use masks are not standardized, their reusability should be discussed considering the suitability and stability of the masks after washing.

5. Conclusions

The three types of reusable antibacterial masks evaluated in this study did not show a consistent pattern in their performance change even when various washing methods were applied. Additionally, their performance changed irregularly as the number of washing cycles increased.

Reusable antibacterial masks have been proven to filter large particle-sized microorganisms and prevent or eliminate their growth. However, their filtration efficiency, which indicates the ability to prevent airborne aerosols, is extremely low. Therefore, certified masks are appropriate for use in places with a certain risk of infection. Based on the results of this study, wearing an appropriate mask should be considered in daily life.

References

Andrejko, K. L., Pry, J. M., Myers, J. F., Fukui, N., DeGuzman, J. L., Openshaw, J., ... & Case-Control Study Team. (2022). Effectiveness of face mask or respirator use in indoor public settings for prevention of SARS-CoV-2 infection—California, February–December 2021. Morbidity and Mortality Weekly Report, 71(6), 212.

American Society for Testing and Materials (ASTM International). (2019). ASTM F2101-19. Standard test method for evaluating the bacterial filtration efficiency (BFE) of medical face mask materials, using a biological aerosol of *Staphylococcus aureus*. https://www.astm.org/f2101-22.html (Accessed 10 January, 2023).

Bandi, M. M. (2020). Electrocharged facepiece respirator fabrics using common materials. Proceedings of the Royal Society A, 476(2243), 20200469.

Centers for Disease Control and Prevention (CDC). (1997). The National Personal Protective Testing Laboratory (NPPTL). 42 CFR Part 84 Respiratory protective devices, summary. https://www.cdc.gov/niosh/npptl/topics/respirators/pt84abs2.html. (Accessed 10 January, 2023).

Centers for Disease Control and Prevention (CDC). (2022a). Types of masks and respirators. https://www.cdc.gov/coronavirus/2019-ncov/prevent-getting-sick/types-of-masks.html (Accessed 10 January, 2023).

Centers for Disease Control and Prevention (CDC). (2022b). Use and care of masks. https://www.cdc.gov/coronavirus/2019-ncov/prevent-getting-sick/about-face-coverings.html (Accessed 10 January, 2023).

Charvet, A., Bardin-Monnier, N., Thomas, D., Dufaud, O., Pfrimmer, M., Barrault, M., ... & Grauby, O. (2022). Impact of washing cycles on the performances of face masks. Journal of Aerosol Science, 160, 105914.

European Committee for Standardization (CEN) (2001). EN 149:2001+A1:2009; Respiratory Protective Devices. Filtering Half Masks to Protect Against Particles - Requirements, Testing, Marking.

European Committee for Standardization (CEN). (2020). CWA 17553: Community face coverings - Guide to minimum requirements, methods of testing and use.

European Committee for Standardization.

International Organization for Standardization (ISO). (2013). ISO 20743. Textiles—Determination of antibacterial activity of textile products, 2nd ed.; ISO: Geneva, Swizerland, 2013.

Konda, A., Prakash, A., Moss, G.A., Schmoldt, M., Grant, G.D., & Guha, S. (2020). Aerosol filtration efficiency of common fabrics used in respiratory cloth masks. ACS Nano, 14(5), 6339-6347.

Korean Agency for Technology and Standards (KATS). (2021). Safety standard for consumer products subject to the supplier's declaration of conformity. https://kats.go.kr/content.do?cmsid=51. (Accessed 10 January, 2023).

Korean Agency for Technology and Standards (KATS). (2016). Test method for antibacterial activity of textile materials. Korean Standards & Certifications. Seoul: Korean Standards Association. https://standard.go.kr/KSCI/standardIntro/getStandardSearchView.do?menuId=919&topMenuId=502&upperMenuId=503&ksNo=KSK0693&tmprKsNo=KSK0693&reformNo=05 (Accessed 10 January, 2023).

Korean Ministry of Food and Drug Safety (MFDS). (2022). Korean Quasi-drug Codex. https://www.mfds.go.kr/brd/m_211/view.do?seq=14671&srchFr=&srchTo=&srchWord=&srchTp=&itm_seq_1=0&itm_seq_2=0&multi_itm_seq=0&company_cd=&company_nm=&page=1 (Accessed 10 January, 2023).

Kwon, M., & Yang, W. (2022). Mask-wearing behaviors after two years of wearing masks due to COVID-19 in Korea: a cross-sectional study. International Journal of Environmental Research and Public Health, 19(22), 14940.

Korea Disease Control and Prevention Agency (KDCA) (2020), Masks are a must now! I use it with prime minister Chung! Division of Infectious Control, Korea Disease Control and Prevention Agency: Cheongju, Korea, 7 November 2020. Available online: https://www.kdca.go.kr/board/board.es?mid=a20501010000&bid=0015&act=view&list_no=711084 (Accessed 10 January, 2023).

National Institute for Occupational Safety and Health (NIOSH) (1995). Approval of respiratory protective devices. 42 CFR Part 84. https://www.ecfr.gov/current/title-42/chapter-I/subchapter-G/part-84 (Accessed 10 January, 2023).

Nguyên, N.N., Van Loon, J., Du Bois, E., Verlinden, J., Verwulgen, S., & Watts, R. (2021). Experimental Comparison of CWA 17553:2020 Community face coverings to surgical masks and filtering facepiece respirators. In: Arezes, P.M., Boring, R.L. (eds) Advances in Safety Management and Human

Performance. AHFE 2021. Lecture Notes in Networks and Systems, vol 262. Springer, Cham. https://doi.org/10.1007/978-3-030-80288-2_20

Perumalraj, R. (2015). Characterization of electrostatic discharge properties of woven fabrics. Journal of Textile Science and Engineering, 6(1), 1000235.

Pollard, Z. A., Karod, M., & Goldfarb, J. L. (2021). Metal leaching from antimicrobial cloth face masks intended to slow the spread of COVID-19. Scientific reports, 11(1), 1-8.

Rengasamy, S., Shaffer, R., Williams, B., & Smit, S. (2017). A comparison of facemask and respirator filtration test methods. Journal of Occupational and Environmental Hygiene, 14(2), 92-103.

Rubio-Romero, J. C., del Carmen Pardo-Ferreira, M., Torrecilla-García, J. A., & Calero-Castro, S. (2020). Disposable masks: Disinfection and sterilization for reuse, and non-certified manufacturing, in the face of shortages during the COVID-19 pandemic. Safety Science, 129, 104830.

Rujido-Santos, I., Herbello-Hermelo, P., Barciela-Alonso, M. C., Bermejo-Barrera, P., & Moreda-Piñeiro, A. (2022). Metal content in textile and (nano) textile products. International Journal of Environmental Research and Public Health, 19(2), 944.

Rule, A., Ramachandran, G., & Koehler, K. (2020). Comment on aerosol filtration efficiency of common fabrics used in respiratory cloth masks: Questioning their findings. ACS Nano, 14(9), 10756-10757.

Sankhyan, S., Heinselman, K. N., Ciesielski, P. N., Barnes, T., Himmel, M. E., Teed, H., ... & Vance, M. E. (2021). Filtration performance of layering masks and face coverings and the reusability of cotton masks after repeated washing and drying. Aerosol and Air Quality Research, 21(11), 210117.

Seidi, F., Deng, C., Zhong, Y., Liu, Y., Huang, Y., Li, C., & Xiao, H. (2021). Functionalized masks: powerful materials against COVID-19 and future pandemics. Small, 17(42), 2102453.

Shin, N., Lee, K., & Kang, Y. (2021). A survey study of compliance with mask-wearing to prevent coronavirus infections among korean adults. Journal of Korean Academy of Fundamentals of Nursing, 28(3), 275-285.

Tcharkhtchi, A., Abbasnezhad, N., Seydani, M. Z., Zirak, N., Farzaneh, S., & Shirinbayan, M. (2021). An overview of filtration efficiency through the masks: mechanisms of the aerosols penetration. Bioactive Materials, 6(1), 106-122.

Viscusi, D. J., Bergman, M., Sinkule, E., & Shaffer, R. E. (2009). Evaluation of the filtration performance of 21 N95 filtering face piece respirators after prolonged storage. American Journal of Infection Control, 37(5), 381-386.

Wang, W., Chen, T., Li, Z., Tan, Q., Meng, Z., Qiu, H., ... & Zheng, J. (2022). Comparison of filtration efficiency and respiratory resistance of COVID-19 protective masks by multi-national standards. American Journal of Infection Control, 50(5), 516-524.

World Health Organization (WHO). (2021). Coronavirus disease (COVID-19) advice for the public: When and how to use masks https://www.who.int/emergencies/diseases/novel-coronavirus-2019/advice-for-public/when-and-how-to-use-masks (Accessed 10 January, 2023).

World Health Organization (WHO). (2022a). Weekly epidemiological update on COVID-19 - 3 August 2022. https://reliefweb.int/report/world/coronavirus-disease-covid-19-weekly-epidemiological-update-3-aug ust-2022 (Accessed 10 January, 2023).

World Health Organization (WHO). (2022b). Coronavirus disease (COVID-19): Masks https://www.who.int/news-room/questions-and-answers/item/coronavirus-disease-covid-19-masks (Accessed 10 January, 2023).

Whyte, H. E., Montigaud, Y., Audoux, E., Verhoeven, P., Prier, A., Leclerc, L., ... & Pourchez, J. (2022a). Comparison of bacterial filtration efficiency vs. particle filtration efficiency to assess the performance of non-medical face masks. Scientific Reports, 12(1), 1-8.

Whyte, H. E., Joubert, A., Leclerc, L., Sarry, G., Verhoeven, P., Le Coq, L., & Pourchez, J. (2022b). Reusability of face masks: Influence of washing and comparison of performance between medical face masks and community face masks. Environmental Technology & Innovation, 102710.

Yang, X., Pu, Y., Zhang, Y., Liu, X., Li, J., Yuan, D., & Ning, X. (2020). Multifunctional composite membrane based on $BaTiO_3$@ PU/PSA nanofibers for high-efficiency PM2.5 removal. Journal of Hazardous Materials, 391, 122254.

Appendix

Appendix 1. Operating conditions of ICP-MS analysis of heavy metals.

Parameter	Analytical conditions
Instrument	NexION 350D (PerkinElmer, Inc., Waltham, MA, USA)
Nebulizer	NEBULIZER-PEEK MIRA MIST 4000
Spray chamber	Glass cyclonic spray chamber
RF generator (W)	500–1,600
Argon flow rate	
Plasma gas (L/min)	18
Auxiliary gas (L/min)	1.2
Nebulizer gas (L/min)	0.99
Cone	
Sampler cone	Platinum
Skimmer cone	Platinum
Hyper-Skimmer cone	Akynubyn alloy
Data acquisition	Peak hopping, 1 reading 30 sweep, 3 replicates
Measurement mode	Quantification mode
Acid solution	8 mL of 70% HNO_3 (JKC, Cheonan-si, Chungcheongnam-do, Republic of Korea)
Heating block	160 °C, 5 h

Appendix 2. Limit of detection (LOD) and limit of quantification (LOQ) for heavy metals (μg/g).

	Ag	Co	Cr	Cu	Fe	Mn	Ni	Pb	Sb	Ti	Zn[3]
LOD[1]	0.0005	0.0005	0.0005	0.0005	0.0005	0.0005	0.0005	0.0005	0.0005	0.0005	0.0012
LOQ[2]	0.0017	0.0017	0.0017	0.0017	0.0017	0.0017	0.0017	0.0017	0.0017	0.0017	0.0039

[1]LOD = 3 × lowest level of the standard solution

[2]LOQ = LOD × 3.33

[3]To select the largest value of LOD of each element, the LOD of zinc (Zn) was calculated as 3 × S_y/m (S_y: standard deviation of a regression, m: slope of calibration curve).

Appendix 3. Filtration efficiency (%) obtained at 0, 1, 2, 5, and 10 cycles after washing using Methods A, B, and C for each mask type (*n* = 3).

Washing method	Washing cycle	Mask type			
		Silver mask	Copper mask	Graphene mask	Cotton mask
Control	0	10.07 ± 1.63[1]	13.95 ± 1.13	10.98 ± 1.17	47.88 ± 2.95
Method A	1	12.39 ± 2.40	15.20 ± 0.86	11.85 ± 0.43	55.45 ± 3.30
	2	9.08 ± 1.18	16.22 ± 0.16	13.05 ± 0.22	56.26 ± 1.27
	5	10.20 ± 1.30	16.92 ± 0.79	13.64 ± 0.96	56.04 ± 2.74
	10	12.15 ± 3.18	18.50 ± 1.32	13.53 ± 1.19	55.57 ± 2.17
Method B	1	11.21 ± 0.30	15.00 ± 0.71	12.91 ± 1.29	55.41 ± 2.74
	2	10.91 ± 0.06	15.89 ± 1.10	13.81 ± 1.35	56.25 ± 3.02
	5	7.23 ± 1.11	13.30 ± 0.95	11.73 ± 1.75	57.76 ± 0.57
	10	11.74 ± 0.94	16.28 ± 0.86	14.15 ± 1.32	58.13 ± 1.27
Method C	1	12.52 ± 0.43	15.60 ± 0.23	12.79 ± 0.70	55.03 ± 1.28
	2	12.41 ± 0.47	16.36 ± 0.87	12.68 ± 0.43	55.37 ± 0.61
	5	13.47 ± 1.08	16.40 ± 0.29	13.60 ± 0.54	60.30 ± 4.08
	10	14.04 ± 0.74	17.84 ± 0.32	16.09 ± 0.63	56.46 ± 1.57

[1]Mean ± standard deviation

Appendix 4. Bacterial filtration efficiency (%) obtained at 0, 1, 2, 5, and 10 cycles after washing using Methods A, B, and C for each mask type (n = 3).

Washing method	Washing cycle	Mask type			
		Silver mask	Copper mask	Graphene mask	Cotton mask
Control	0	93.49 ± 1.16[1]	98.35 ± 1.04	96.53 ± 1.14	97.89 ± 0.89
Method A	1	95.68 ± 0.67	97.68 ± 0.26	95.54 ± 0.79	98.04 ± 0.48
	2	93.50 ± 2.78	97.51 ± 1.21	93.47 ± 3.61	98.52 ± 0.48
	5	92.61 ± 2.37	95.53 ± 3.06	92.94 ± 4.45	95.92 ± 0.26
	10	93.13 ± 2.03	97.29 ± 1.71	95.65 ± 5.14	98.39 ± 1.43
Method B	1	93.92 ± 1.92	96.71 ± 0.50	93.37 ± 1.31	98.11 ± 0.72
	2	94.66 ± 2.09	96.59 ± 0.22	96.14 ± 1.63	98.48 ± 0.45
	5	94.77 ± 0.58	96.31 ± 2.47	93.36 ± 2.24	97.86 ± 0.43
	10	94.13 ± 2.36	97.46 ± 1.55	92.72 ± 3.97	97.80 ± 1.38
Method C	1	93.06 ± 1.89	97.11 ± 1.17	95.47 ± 0.31	99.09 ± 0.10
	2	95.18 ± 2.87	95.08 ± 0.50	95.15 ± 0.54	99.19 ± 0.33
	5	94.41 ± 0.81	97.25 ± 1.10	94.33 ± 3.25	98.84 ± 0.10
	10	95.21 ± 0.32	96.46 ± 0.96	97.59 ± 0.77	98.59 ± 0.26

[1]Mean ± standard deviation

Appendix 5. Antibacterial activity (%) obtained at 0, 1, 2, 5, and 10 cycles after washing using Methods A, B, and C for each mask type (*n* = 3).

Washing method	Washing cycle	Mask type			
		Silver mask	Copper mask	Graphene mask	Cotton mask
Control	0	98.84 ± 0.93[1]	98.02 ± 2.75	88.98 ± 3.03	84.54 ± 16.62
Method A	1	> 99.99	99.98 ± 0.03	99.75 ± 0.14	93.24 ± 5.99
	2	> 99.99	> 99.99	99.96 ± 0.05	94.61 ± 2.81
	5	> 99.99	> 99.99	99.96 ± 0.05	99.77 ± 0.21
	10	> 99.99	> 99.99	> 99.99	99.91 ± 0.15
Method B	1	> 99.99	> 99.99	> 99.99	85.50 ± 8.98
	2	97.11 ± 0.58	99.69 ± 0.54	99.58 ± 0.45	76.67 ± 10.78
	5	> 99.99	> 99.99	98.77 ± 1.70	83.58 ± 1.90
	10	> 99.99	> 99.99	99.89 ± 0.20	99.41 ± 0.71
Method C	1	99.95 ± 0.09	> 99.99	88.88 ± 6.22	99.95 ± 0.09
	2	> 99.99	> 99.99	99.51 ± 0.81	81.78 ± 1.83
	5	> 99.99	> 99.99	90.36 ± 11.20	94.07 ± 3.00
	10	> 99.99	> 99.99	98.64 ± 2.04	99.41 ± 0.85

[1]Mean ± standard deviation

Appendix 6. Inhalation resistance (mmH_2O) at 0, 1, 2, 5, and 10 cycles after washing using Methods A, B, and C for each mask type (n = 3).

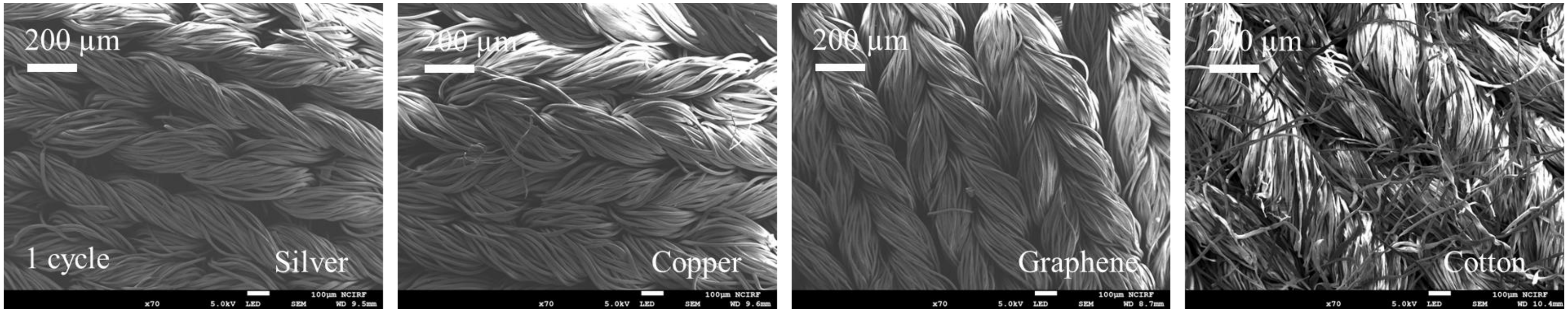
Washing method	Washing cycle	Mask type			
		Silver mask	Copper mask	Graphene mask	Cotton mask
Control	0	7.49 ± 0.92[1]	16.97 ± 1.63	5.14 ± 0.49	25.78 ± 1.88
Method A	1	8.06 ± 1.91	15.34 ± 1.10	5.65 ± 1.46	23.09 ± 4.39
	2	7.30 ± 2.06	16.98 ± 1.34	4.68 ± 1.19	35.79 ± 2.43
	5	7.20 ± 2.59	15.82 ± 0.73	5.38 ± 1.68	36.49 ± 2.99
	10	8.38 ± 1.87	5.67 ± 0.98	13.09 ± 7.91	23.23 ± 7.94
Method B	1	5.48 ± 2.56	17.04 ± 1.07	6.13 ± 0.60	39.03 ± 6.95
	2	6.69 ± 0.82	16.80 ± 1.47	5.55 ± 1.17	33.18 ± 7.80
	5	5.81 ± 1.25	14.75 ± 2.03	6.84 ± 1.02	29.84 ± 3.47
	10	5.54 ± 0.51	13.85 ± 1.74	6.25 ± 0.39	34.79 ± 10.04
Method C	1	7.79 ± 2.31	16.97 ± 1.20	5.77 ± 0.57	40.81 ± 4.00
	2	8.08 ± 3.77	16.59 ± 1.08	5.59 ± 0.40	42.39 ± 0.79
	5	7.55 ± 2.17	15.93 ± 2.05	6.70 ± 0.91	39.47 ± 4.26
	10	6.89 ± 2.15	16.41 ± 0.76	6.94 ± 0.29	34.16 ± 8.53

[1]Mean ± standard deviation

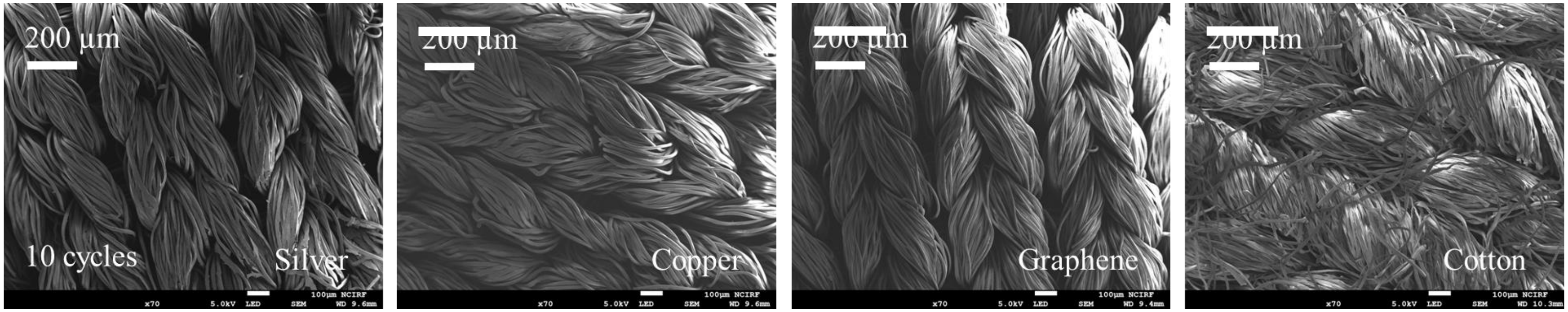
Appendix 7. Bacterial filtration efficiency (%) at each ACI stage (*n* = 3).

Stage (cut-off diameter)	Mask type					
	Silver mask	Copper mask	Graphene mask	Cotton mask	KF94 mask	N95 mask
1 (7 µm)	99.68 ± 0.32[1]	99.70 ± 0.51	99.70 ± 0.51	> 99.99	> 99.99	> 99.99
2 (4.7 µm)	99.82 ± 0.31	> 99.99	99.39 ± 1.05	99.38 ± 1.07	> 99.99	> 99.99
3 (3.3 µm)	99.27 ± 0.57	> 99.99	99.23 ± 1.34	99.48 ± 0.45	> 99.99	> 99.99
4 (2.1 µm)	97.20 ± 1.49	99.78 ± 0.38	98.68 ± 1.32	98.62 ± 0.69	> 99.99	> 99.99
5 (1.1 µm)	84.74 ± 2.23	95.24 ± 3.17	90.21 ± 3.91	93.65 ± 1.16	> 99.99	> 99.99
6 (0.65µm)	13.39 ± 15.88	73.53 ± 15.28	61.76 ± 13.48	78.13 ± 19.52	99.28 ± 1.26	99.28 ± 1.26

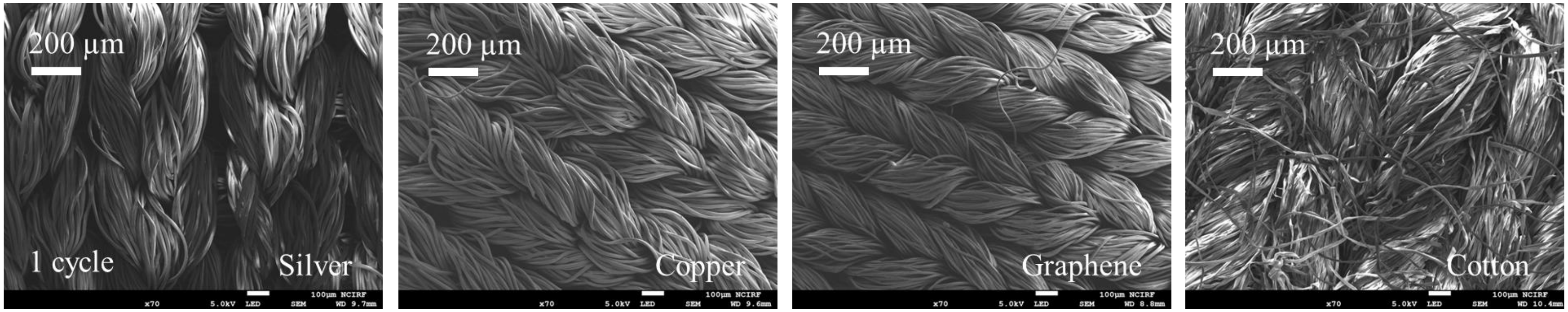
[1]Mean ± standard deviation



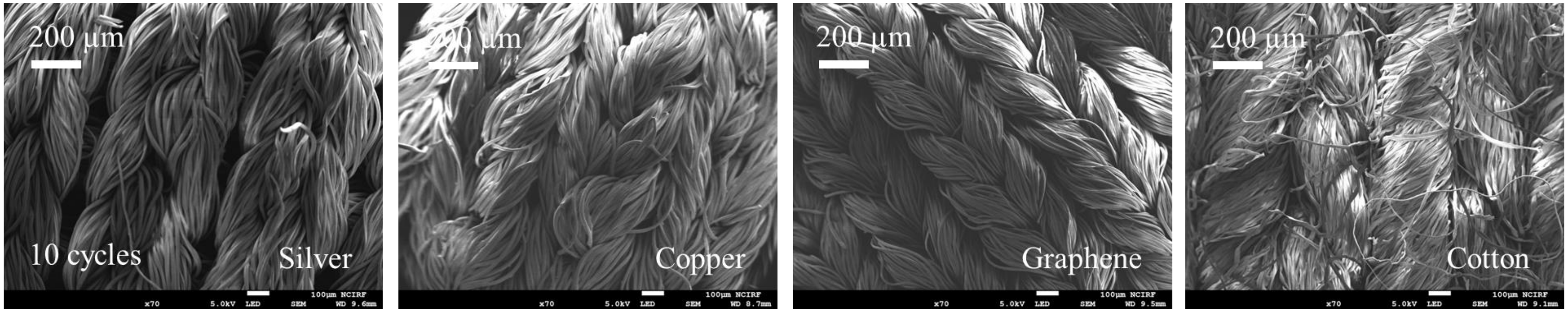
(a) Surface images of the silver, copper, graphene, and cotton masks after one cycle of washing after treatment with Method B.



(b) Surface images of the silver, copper, graphene, and cotton masks after ten cycles of washing after treatment with Method B.



(c) Surface images of the silver, copper, graphene, and cotton masks after one cycle of washing after treatment with Method C.



(d) Surface images of the silver, copper, graphene, and cotton masks after ten cycles of washing after treatment with Method C.

Appendix 8. Surface images of silver, copper, graphene, and cotton masks observed via FE-SEM (70× magnification) after treatment with Methods B and C. (a) Surface images of the silver, copper, graphene, and cotton masks after one cycle of washing after treatment with Method B; (b) Surface images of the silver, copper, graphene, and cotton masks after ten cycles of washing after treatment with Method B; (c) Surface images of the silver, copper, graphene, and cotton masks after one cycle of washing after treatment with Method C; (d) Surface images of the silver, copper, graphene, and cotton masks after ten cycles of washing after treatment with Method C.

Appendix 9. Rate of performance change and statistical difference according to the washing methods and number of cycles in filtration efficiency test using the *t*-test.

Washing method	Washing cycle	Mask type			
		Silver mask	Copper mask	Graphene mask	Cotton mask
Method A	1	122.96	108.96	107.93	**115.80***
	2	90.16	**116.26***	**118.87***	**117.50***
	5	101.28	**121.27***	**124.19***	**117.03***
	10	120.59	**132.60***	**123.26***	**116.06***
Method B	1	111.22	107.53	117.56	**115.72***
	2	108.30	113.85	**125.75***	**117.47***
	5	**71.75***	95.30	106.79	**120.63***
	10	116.57	**116.69***	**128.87***	**121.41***
Method C	1	**124.26***	**111.79***	107.93	**114.92***
	2	**123.19***	**117.23***	**118.87***	**115.63***
	5	**133.71***	**117.50***	**124.19***	**125.94***
	10	**139.33***	**127.87***	**123.26***	**117.91***

*$P < 0.05$

국문초록

재사용 항균성 마스크의 세척 후 여과효율, 흡기저항 및 항균성 평가

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연구 배경: 코로나19 판데믹 초기에는 인증된 마스크의 부족으로 일회용 마스크를 다회 사용하기도 하였다. 항균 섬유로 만들어진 재사용 마스크가 시중에 판매되고 있지만 이에 대한 성능과 항균성에 대한 과학적인 데이터는 부재한 실정이다. 따라서 본 연구에서는 재사용 가능한 항균 마스크에 대하여 세척 전후 여과효율, 흡기저항 및 항균작용의 지속성을 평가하고자 하였다.

연구 방법: 재사용이 가능한 은, 구리, 그래핀 항균 마스크와 면 마스크를 사용하여 세척 전후 성능 차이를 비교하였으며, 세척 전 성능은 KF94 및 N95 마스크와 비교하였다. 마스크를 세탁기에서 40 ° C로 세

탁(Method A), 60 ° C의 물에 15분(Method B), 90 ° C의 물에 15분 동안 침지하는 방법(Method C)을 활용하였다. 마스크는 10일 동안 매일 세척하여 1,2,5,10회 세척 후의 성능을 평가하였다. 마스크의 여과효율은 NaCl과 황색포도상구균을 활용하여 평가하였다. 또한 항균성과 흡기저항 시험을 시행하였다.

연구 결과: 재사용 항균성 마스크 3종의 분진 여과효율은 세척 전 10–13%로 낮았으며 세척 후에는 약 6% 증가했다. 세척 전 세균여과효율은 93–98%, 항균성은 88–98%로 나타났으나 세척을 반복할수록 성능이 불규칙하게 변화하였으며, 세척 전후의 성능에는 큰 차이가 없었다. 재사용 항균성 마스크는 무항균 마스크보다 높은 항균성을 보였다. 세척 전후 안면부 흡기저항은 5–17 mmH_2O로 적절한 통기성을 보여주었다.

결론: 재사용 항균성 마스크의 3종의 여과효율은 입자를 차단하는데 적합하지 않았다. 재사용 항균성 마스크의 성능은 세척 방법과 세척 횟수에 따라 불규칙하게 변화하였다. 따라서 일상생활에서는 호흡기 보호를 위하여 인증된 마스크를 착용하는 것이 권장된다.

주요어: 마스크 세척, 항균 마스크, 여과효율, 세균여과효율, 항균성, 흡기저항

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