Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
The shortages of supply and high demand may have increased the price of equipment, in particular respiratory protection such as N95 respirators. The ongoing COVID-19 pandemic has created worldwide shortages of personal protective equipment (PPE)-helping protect Health Care Provider (HCP) from potentially infectious patients and materials, toxic medications, and other potentially dangerous substances used in healthcare delivery. Due to recent SARS-CoV-2 pandemic in 2019 and 2020, the Center for Disease Control and Prevention (CDC) has recommended healthcare personnel to use N95 respirators being recommended for protection due to its capacity of filter media against the air-borne pathogen Staphylococcus aureus (S. aureus). Results showed that silver ALD successfully functionalized the N95 mask at 90 and 120 °C for two different numbers of ALD cycles (1100 and 1500 cycles). The deposited silver nano-islands were stable on the N95 filter media against washing. The leaching of silver nano-islands was studied using inductively coupled plasma mass spectrometry of phosphate-buffered saline solution after soaking the mask in it over predetermined times. <9% of Ag was removed after a maximum time of 4 h that was investigated. Antimicrobial tests showed that for samples functionalized with 1100 ALD cycles of Ag, 76% reduction in S. aureus colony-forming units content was observed after 24 h of biofilm development on the mask surfaces.

Due to the COVID-19 outbreak, there has been increasing interest in tailoring, modifying and improving conventional personal protective equipment to increase their service life and make them more effective against viruses and bacteria. Here, atomic layer deposition (ALD) was used to functionalize the filter of N95 mask with nano-islands of silver. X-ray photoelectron spectroscopy and x-ray absorption fine structure were used for ALD silver characterization; microbiological assay was conducted to study the effectiveness of the deposited silver against the air-borne pathogen Staphylococcus aureus (S. aureus). Results showed that silver ALD successfully functionalized the N95 mask at 90 and 120 °C for two different numbers of ALD cycles (1100 and 1500 cycles). The deposited silver nano-islands were stable on the N95 filter media against washing. The leaching of silver nano-islands was studied using inductively coupled plasma mass spectrometry of phosphate-buffered saline solution after soaking the mask in it over predetermined times. <9% of Ag was removed after a maximum time of 4 h that was investigated. Antimicrobial tests showed that for samples functionalized with 1100 ALD cycles of Ag, 76% reduction in S. aureus colony-forming units content was observed after 24 h of biofilm development on the mask surfaces.

**ARTICLE INFO**

**Keywords:**
N95 Respirators
Silver
Atomic Layer Deposition
Biofilm
*Staphylococcus aureus*

**ABSTRACT**

Due to the COVID19 outbreak, there has been increasing interest in tailoring, modifying and improving conventional personal protective equipment to increase their service life and make them more effective against viruses and bacteria. Here, atomic layer deposition (ALD) was used to functionalize the filter of N95 mask with nano-islands of silver. X-ray photoelectron spectroscopy and x-ray absorption fine structure were used for ALD silver characterization; microbiological assay was conducted to study the effectiveness of the deposited silver against the air-borne pathogen Staphylococcus aureus (S. aureus). Results showed that silver ALD successfully functionalized the N95 mask at 90 and 120 °C for two different numbers of ALD cycles (1100 and 1500 cycles). The deposited silver nano-islands were stable on the N95 filter media against washing. The leaching of silver nano-islands was studied using inductively coupled plasma mass spectrometry of phosphate-buffered saline solution after soaking the mask in it over predetermined times. <9% of Ag was removed after a maximum time of 4 h that was investigated. Antimicrobial tests showed that for samples functionalized with 1100 ALD cycles of Ag, 76% reduction in S. aureus colony-forming units content was observed after 24 h of biofilm development on the mask surfaces.

1. Introduction

Personal protective equipment (PPE) is used every day by healthcare personnel to protect themselves, patients, and others providing care. PPE helps protect Health Care Provider (HCP) from potentially infectious patients and materials, toxic medications, and other potentially dangerous substances used in healthcare delivery. Due to recent SARS-CoV-2 pandemic in 2019 and 2020, the Center for Disease Control and Protection (CDC) has recommended healthcare personnel to use N95 respirator. Originally, N95 respirators were designed for single-use and were worn by HCP to reduce exposure to airborne infectious agents, including the SARS-CoV-2 virus. Unfortunately, the ongoing COVID-19 pandemic has created worldwide shortages of personal protective equipment, in particular respiratory protection such as N95 respirators. The shortages of supply and high demand may have increased the price significantly, causing consumers to tend to use them for an extended period of time. However, besides the discomfort feeling and the difficulty in adapting the wearing of face masks for a long time, the SARS-CoV-2 virus may oculate and remain viable on the outer side of the respirator for approximately 3–5 days, but can be as long as 14 days [1,2], thereby the extended use of respirators may increase the risk of infection besides causing unexpected health problems, as air-borne bacteria may also be intercepted by respirators’ filter media [2,3].

When using face masks, the host’s saliva and sweat may nurture and create an ideal environment for these bacteria and viruses to remain viable on the surface [3]. This survivability is of utmost concern because in addition to new pathogens, such as SARS-CoV-2, having the potential to coexist with a broad range of bacteria [4], the accumulation of air-borne bacteria, especially the commensal pathogen *Staphylococcus aureus* (S. aureus), may pose unexpected threats to the wearers’ health. *S. aureus* is a common and important well-known bacterium responsible for causing mainly opportunistic infections due to its high virulence, invasiveness and possibility to re-infection [5]. Hence, although the N95 respirators being recommended for protection due to its capacity of filter particles at the size of SARS-CoV-2 virus [6–8], it would be of great value the development of strategies that aggregate not only the reduction of...
the dangerous spread of airborne particulates of viral aerosols, but also the accumulation of smaller particles, such as bacteria that can potentially penetrate the filter’s mask component. 

Even though several decontaminated procedures, such as disinfection via ultra violet and 70 °C dry heat [9–11], have been proposed to extend the longevity of that PPE, these decontaminated procedures may negatively affect its function. Furthermore, these techniques are not able to control the accumulation of air-borne pathogens in the respirators’ filter media during the time that they are being worn. Once the microorganisms are attached, the re-aerosolization of the bacteria under sneezing or coughing may pose risk for the wearers’ health [12]. Thus, re-engineering or improving the existing N95 mask to result in antibacterial and antiviral properties is of huge interest to maximize the protection against pathogens [13–15].

Silver has been reported as an antimicrobial agent for centuries [16]. Currently, silver coatings are used to protect surgical tools and catheters from bacteria [17]. Recently, engineered silver nanoparticles (Ag-NP) are reported to effectively inactivate bacteria without showing cytotoxicity [18,19].

Ag-NPs have been successfully used in clothing during pre-production, leading to many patents in the US and EU (e.g., [20–23]). In most cases, Ag-NPs were produced and stabilized using a capping agent like polyvinyl alcohol or polyvinyl pyrrolidone, the latter being more recent and less toxic [13,24,25]. This step is important as it can control the size of the nanoparticles. These nanoparticles were then suspended in different monomers that can then be used to finish the clothing processing. Efforts have been made to increase the life and durability of such Ag-NP fabric. In some cases, the application of Ag NPs along with an oil/water repellent led to increased life of the fabric that was further used to make masks [26].

However, even with commercial processes that include silver, a disadvantage of pre-production techniques is decreasing antimicrobial activity with use. As the active silver in these materials detaches from the fiber over time, there is notable loss of anti-microbial activity [27]. The detachment can come from either washing, inhalation, or absorption into the skin. Since the particle sizes for these AgNPs are < 30 nm, it is possible that these particles are absorbed in the skin when sweat interacts with the silver nanoparticles [28]. Therefore, a post processing step is needed to not only make regular surgical masks antimicrobial but also to enhance the life of the antimicrobial masks available in the market.

Ag-NPs are usually suspended in an aqueous solution and N95 masks are hydrophobic which makes the application difficult. Surgical polypropylene and polyester masks were functionalized with starch as a capping agent and soaking the mask in the prepared Ag-NPs colloidal solution [24]. However, even short-term contact to starch can cause skin irritations. In one recent report, researchers functionalized particulate respirator with Ag-NP that could inhibit the growth of S. aureus and Pseudomonas aeruginosa in real time [13]. But prior to coating N95 fabric with Ag-NP, surface wettability of N95 was modified by immersing the samples in sodium oleate overnight.

The concern that these post-production processes can pose is the inhalation and removal of Ag-NP during and after use since most of these postproduction techniques involve the use of a capping agent that adheres to both, the metal and fiber. Our proposed solution-free atomic layer deposition (ALD) method of spatial Ag in the form of nano-islands has this capability to uniformly functionalize substrates without immersing the samples in any solution; this feature is of interest especially for solvent-sensitive samples. Furthermore, by using ALD, the nano-islands of silver thus formed on the N95 would adhere to the surface by chemisorption rather than relying on its immobilization on a polymer matrix. The use of ALD also eliminates the need for capping agents since it offers precise control to grow islands of desired subcytotoxicity size. Previously, nanostructures of silver formed using electrochemical deposition or physical vapor deposition have been used for making the substrate surface enhanced Raman spectrometer compatible [29,30]. Nano-islands were preferred over immobilization of metallic particles on a solid due to the chemical bonding between the substrate and silver in the former. This strong adhesion was essential in implementing these nano-island deposits onto the N95 since they would not be removed or inhaled easily. In our work, the effect of wash test was also studied to investigate any potential detachment of Ag nano-islands from the N95 filter along with a leaching test to study the leachate solutions after dipping the N95 in a phosphate-buffered saline (PBS) solution.

ALD is a cyclic vapor phase thin film growth technique. Each cycle of ALD consists of four consecutive steps, precursor pulse, precursor purge, co-reactant pulse, and co-reactant/byproducts purge, followed by a chemical reaction on the surface [31,32]. Due to the self-limiting nature of the ALD chemisorb reaction on the surface, the thin films thus formed are conformal and highly uniform in contrast to other vapor phase thin film growth methods [33]. In contrast to other functionalization methods which involve solutions and subsequent drying, the ALD surface functionalization is performed at the gas/solid interface. This feature makes ALD favorable more specifically for substrates which have 3D, complex microstructure and are resorbable in solutions.

Recently, we have successfully functionalized collagen membranes with TiO2 at room temperature [34] and with Ag via ALD. Polypropylene (PP) and polyesters are being used as the main material for the filter of N95 respirators [35]. The microstructure of such PP filter

![Fig. 1. a) Photograph of the custom-built ALD system and b) schematic of the lining arrangements from top view.](image-url)
media is fibrous and similar to the microstructure of collagen membrane. In our earlier studies, coating collagen membrane with TiO₂ was successfully performed and documented [34,36]. Our results showed that via ALD one can deposit uniform and conformal thin film of TiO₂ on the surface of fibrous collagen and successfully promote the growth of osteoblast and mesenchymal stem cells [34,36].

Due to the complex 3D microstructure of the filters of the N95 mask (PP), ALD can be an effective technique to uniformly functionalize its surface, and engineer N95 masks (or help create other masks) with longer usage life and antibacterial as well as COVID-19 inactivation properties. There are silver-containing materials/pads that are used for the local management of superficial wounds, minor burns, abrasions, and lacerations (e.g., Silverlon™). Yet, such masks are not intended as personal protective equipment (PPE) for healthcare providers in clinical settings where the infection risk level is high (e.g., high inhalation exposure or aerosol-generating procedures), do not provide liquid barrier protection, and are not substitutes for filtering face piece respirators or surgical face masks. The aims of the proposed study were: to conduct the functionalization of N95 respirators by using ALD Ag with a recently patented ALD system [37,38] achieving nanometer length scale films; to characterize the functionalized samples chemically and morphologically; to obtain process – structure – performance inter-relationships of the resulting systems; and to study the antimicrobial properties of the functionalized samples.

2. Materials and methods

2.1. Silver thin film deposition on N95 mask

N95 mask from 3 M was cut into ~ 4 cm × 2 cm pieces and used as received. All ALD processes were carried out in a custom-built ALD reactor and in all experiments silicon wafer from WAFERPRO (item number: C04007) was used as a control sample [37]. A photograph and schematic of the linings representation of the ALD system is shown in Fig. 1. Due to the nature of the precursor and deposition required, the co-reactant used in this work was a borane complex to reduce the precursor to metallic silver. Thus, the ALD cycle consisted of alternate pulse/purge of the silver precursor and borane complex from the bubblers.

Ag(fod)(Pet3) (Ag(C₃F₇-COCHCOC₂H₅)(CH₂CH₃)₃, CAS #165461-74-5) from Strem Chemicals Inc. was used as the silver precursor and it was maintained at 96 °C during all depositions. Borane dimethylamine complex ((CH₃)₂BH, CAS #74-94-2) from Sigma Aldrich was used as a reducing agent/co-reactant to react with Ag(fod) (Pet3) and deposit metallic silver on the substrate [39-41]. The borane bubbler was kept at 52 °C. Four experimental groups of samples were prepared for this study on N95 filters: 1100 cycles Ag ALD deposited at 90 °C, 1500 cycles Ag ALD deposited at 90 °C, 1100 cycles Ag ALD deposited at 120 °C, and 1500 cycles Ag ALD deposited at 120 °C. As-received and non-coated N95 filter was used as control.

2.2. Material characterisation

Residual gas analysis (RGA) was done to study the thermal stability of the N95 mask filter using an RGA200 from Stanford Research System connected to a Pfeiffer turbomolecular pump. The base pressure of the system was 7.3x10⁻⁸ Torr. To evaluate the chemical composition of the surfaces, an X-ray photoelectron spectrometer (XPS; Kratos AXIS-165, Kratos Analytical Ltd., United Kingdom) equipped with a monochromatic Al Kα (1486.6 eV) x-ray source was used with operation at 15 kV and 10 mA. High resolution spectra of Ag 3d was collected using stepping size of 0.1 eV, and dwell time of 200 ms. The Ag K-edge x-ray absorption fine structure (XAFS) of deposited Ag was performed at the 5 BMD Sector of the Advanced Photo Source facility in Argonne National Laboratory using X-ray fluorescence detection. The images of the Ag coating and roughness profile were acquired by atomic force microscopy (AFM) with WITTEC-300RA Raman-AFM using silicon wafer as substrate. Water contact angle (WCA) were conducted to study the surface wettability using a Rame-hart C.A. goniometer (model No. 100-00). For this, a micro-syringe was used to place 5 μL of deionized water droplet onto sample surfaces according to sessile drop method. To calculate the WCA values, captured photos were analyzed via “contact angle” Plugins of ImageJ software. To observe the surface morphology of the N95 fibrous interlayer, the micrographs at 1,000 × magnification were acquired with scanning electron microscopy (SEM; JSM-6610LV, JEOL Ltd., USA) using the secondary electrons detector operating at 12 keV of beam energy.

2.3. Microbiological assay

To test the effect of the developed surfaces on N95 mask filter in controlled bacterial growth, the air-borne pathogen S. aureus was used as a model strain for microbiological assay. For this, the experiment was carried out in triplicate in two separate occasions to investigate the effect of developed surfaces on both stages of bacterial adhesion and biofilm formation (n = 6). Hence, prior to the experiments, S. aureus ATCC 14,458 strain was grown on Mannitol Salt Agar (MSA, BD Difco) plates at 37 °C for 24 h. Then, loopfuls of MSA-grown colonies were inoculated into a tube containing 5 mL of sterilized Tryptic Soy Broth (TSB, BD Difco), and grown overnight aerobically at 37 °C. After this period, overnight culture (1 mL) was added to 5 mL of fresh TSB and incubated until it had reached the exponential growth phase, with an optical density of 0.3 at 550 nm. Bacterial cells were then diluted 1:10 in TSB to approximate 10⁷ cells/mL. Subsequently, the N95 mask filter discs (6 mm in diameter) previously sterilized by autoclaving process (121 °C for 15 min) [13] were placed in a 96-well microplate and aerobically incubated with the bacteria inoculum in TSB (1:10 v/v) for 2 h and 24 h at 37 °C. Finally, after each period of bacterial adhesion (2 h) and biofilm formation (24 h), the discs were gently washed in 0.9% NaCl, and a serial dilution of a vortexed suspension (0.9% NaCl, 1 min) [42] was plated in duplicate on Brain Heart Infusion agar (BD Difco). The agar plates were incubated aerobically at 37 °C for 24 h for colony-forming unit (CFU) counts. Data were expressed as the logarithm of CFU per milliliter (log10 CFU/mL), and the antibacterial efficiency of the biofilm-formed samples was calculated using equation 1 [43]:

\[
R\% = \frac{B - A}{A} \times 100/B
\]

where R is the ratio of bacterial reduction (%), A is the mean number of viable bacterial colonies after contacting with uncoated control N95 mask filter, and B is the mean number of viable bacterial colonies after contacting with coated N95 mask filter samples.

2.4. Leaching of Ag nano-islands

To study the effect of biological fluids on removing Ag from our ALD Ag treated masks, leaching tests were performed. In these studies, 5 mm discs from as-deposited N95 masks were cut out and each disc was submerged into separate 1 mL of PBS solutions (Gibco, ThermoFisher Scientific Inc) for soaking times of 5, 30 and 240 min. Along with these, two 5 mm disc samples from undeposited and as-deposited N95 masks were prepared without PBS soaking in order to serve as negative and positive controls, respectively.

The control samples underwent microwave digestion prior to analysis using a Perkin Elmer Titan MPS microwave sample digestion system where HNO₃ and H₂O₂ were added to the 1 mL PBS solution containing the sample and digested at a temperature of 165 °C. This was sufficient to digest the sample for ICP-MS based on the literature available for polypropylene.[44] After cooling, the samples were diluted by 10x with DI water and introduced into the ICP along with the standard solution. The PBS leaching solutions underwent similar chemical digestion and dilution procedure before characterization.

The analysis was performed using a Perkin Elmer NexION 2000 ICP-
MS equipped with an Elemental Scientific (ESI) prepFast sample introduction system where the prepared solutions were delivered using a peristaltic pump at a flow rate of 300 μL/min. A PFA ST3 Type C nebulizer was used along with a RF Power of 1600 W to ensure robust plasma. The mass spectral data was acquired by Kinetic Energy Discrimination (KED) mode with Helium cell gas. The data analysis was conducted using Syngistix software (v2.5).

2.5. Statistical analysis

The influence of the ALD functionalization of N95 mask on the number of CFUs was statistically evaluated using GraphPad Prism version 8.0.0 for Windows (GraphPad Software, San Diego, CA), and final CFU graphs were prepared with the same software. After checking the normality with the Shapiro-Wilk test, the number of CFUs was analyzed using a one-way analysis of variance (ANOVA) (factor = surface functionalization). The Tukey honestly significant difference (HSD) test was used as a post hoc technique for multiple comparisons. The mean difference with statistical significance was set at p value < 0.05.

3. Results and discussion

3.1. Material characterisation

Ag ALD was conducted on silicon wafer and N95 mask filter. Surface topography was characterized using AFM. The AFM images shown in Fig. 2 clearly demonstrate the deposition of silver as a nano-island thin film on the silicon wafer surface. After silver deposition, the roughness of the surface increased from 563 pm for pristine Si wafer to 2.33 nm after silver ALD which is attributed to the island growth mechanism of silver on the surface. Therefore, these results showed the physical dimensional aspects of deposition from thermal ALD of silver was successfully performed on Si wafer sample (Fig. 2).

To investigate the feasibility of using this Ag ALD process on N95 masks, potential thermal degradation of PP at 120 °C was investigated. For all atomic masses, after about half hour of degassing at 120 °C, no out-gassing products were observed from N95 mask samples in the mass range of 12 to 44 above the RGA background (typically ~ 10^{-9} to 10^{-11} torr). Therefore, comparing the residual gas data of the background and those of the as received N95 filter sample at 120 °C, we find out that 0.5 h of degassing is sufficient to remove contaminants prior to silver
deposition at 120 °C, while the N95 filter remains intact.

PP is hydrocarbon and its molecular structure consists of carbon (C) and hydrogen (H) atoms (Fig. 3a). Fig. 3b-c shows the layers of 3 M™ N95 mask and SEM of the fibrous morphology of the mid-layer, respectively. XPS of pristine N95 mask was done prior to the Ag deposition. As it can be seen in Fig. 3d, XPS of pristine N95 filter media shows C–C bond (from the backbone of the polypropylene chain) at 285.0 eV which is consistent with literature results [45]. This N95 filter has not been washed prior to deposition and used as received, therefore, the O 1s peak at 529.2 eV could in part be related to ambient contamination (Fig. 3d). After 1500 cycles ALD on N95 mask, in addition to the 2 peaks of pristine N95, the silver peak (Ag 3d) was observed on the top layer of the N95 mask. As presented in Fig. 3d, the results on the mid-layer of N95 mask show that, due to the fibrous nature of the N95 filter media (Fig. 3c), silver was able to diffuse into the bottom layer and also coat the mid-layer. We previously reported such infiltration in our study on another polymer [46].

Generally, the respirators in the market do not have antibacterial properties and using them beyond their service life shows lower efficiency and provides a suitable environment for growth and proliferation of bacteria, especially on the mid-layer [13]. Therefore, it is highly preferable to focus on the N95 mid-layer for silver treatment to promote its antibacterial properties. XPS results of the silver deposition on N95 mid-layer show that, due to the fibrous nature of the N95 filter media (Fig. 3c), silver was able to diffuse into the bottom layer and also coat the mid-layer. We previously reported such infiltration in our study on another polymer [46].

Our XAFS result confirmed that our thermal ALD resulted in amorphous metallic silver as indicated by the broader and less intense radial peaks with no evidence of Ag₂O [48]. Due to the hydrophobic nature of silver, upon Ag ALD water contact angle of the samples remained unchanged suggesting similar wetting properties as the pristine filter media (Fig. 4a).

XPS analysis confirmed the formation of silver on the filter media of N95 on all four groups of samples: 1100 cycles Ag deposited at 90 °C, 1100 cycles Ag deposited at 120 °C, 1500 cycles Ag deposited at 90 °C, and 1500 cycles Ag deposited at 120 °C. As presented in the survey spectra, in addition to O and C, silver peak (Ag 3d) was observed on all our samples.

3.2. Wash test

Our proposed practical method to study the adherence of Ag ALD nano-islands onto N95 filter media is the washing test. Data for the sample with 1500 cycles Ag deposited at 120 °C was selected for the wash test and the corresponding results. Two washing methods (normal wash with running DI water and washing under sonication in DI water) each one for 1 min and 10 min were chosen. To investigate the stability of Ag nano-islands on N95 filter media, XPS characterization was done and compared for functionalized samples before and after the wash test. Our wash test results (Fig. 6) revealed that neither washing with running DI water nor sonication in DI negatively affected the adhesion between ALD Ag nano-islands on the N95 filter media. This attractive feature makes this functionalization method a potential candidate for other surfaces which may be sensitive to washing during usage.
Fig. 4. a) Water contact angle measurements of four groups of Ag coated N95 mask filter. b) XPS spectra of silver ALD on mid-layer of N95 mask and high-resolution Ag 3d peak on mid-layer N95 for sample which was functionalized with from top to bottom: 1100 cycles Ag ALD at deposited at 120 °C, 1100 cycles Ag ALD deposited at 90 °C, 1500 cycles Ag ALD deposited at 120 °C, and 1500 cycles Ag ALD deposited at 90 °C.

Fig. 5. XAFS on Ag ALD coated N95 filter media after 1500 ALD cycles compared with the reference Ag metal foil.
3.3. Leaching of Ag Nano-islands on N95 masks

Previously reported leaching of solution deposited Ag-NP from different textile fabrics indicate that the leaching concentration of Ag-NP was highest from wool which was reduced over time [49]. A recent study indicated that vaporized hydrogen peroxide and ultraviolet irradiation seem to be the current standard for N95 respirator decontamination [50]. Washing mask with water, soap, and alcohol may reduce particle filtration efficiency significantly [51].

In this study, leaching of Ag nano-islands deposited on PP masks via ALD was investigated. These nano-islands are expected to adhere to PP masks better than NPs due to greater surface contact area nano-islands deposits have over NPs as indicated in a previously reported work on chemical bonding through the Volmer-Weber island growth (e.g., [52,53]). To examine the degree of Ag nano-islands adhesion on the PP mask material, an analysis of the silver content of the leachate solutions by ICP MS was performed and results are presented in Table 1. About 96.8 µg/L was found in the positive control and only 8.5% of that was measured in leachate solution after submerging the as-deposited disc sample in PBS for 4 h. These leaching results are less than the values reported for the first wash of fabrics (cotton and wool) loaded with nanoparticles [49] and indicate potentially promising Ag functionalization method especially when realizing the average size of the Ag-nano-

![Figure 6](image)

**Fig. 6.** XPS of the functionalized sample with 1500 cycles Ag ALD at 120 °C before wash test and after 1 min and 10 min washing with running water and washing under sonication.

| Sample                  | Ag Content (µg/L) | % Ag leached |
|-------------------------|-------------------|-------------|
| Negative control        | 0.06              | –           |
| 1500AgNI Positive control | 96.80            | –           |
| 1500AgNI 5 min          | 1.30              | 1.35        |
| 1500AgNI 30 min         | 2.50              | 2.58        |
| 1500AgNI 240 min        | 8.22              | 8.49        |

### Table 1

ICP MS data for elemental analysis of Ag in negative and positive control, and PBS wash solutions exposed to the as-deposited mask for 5, 30 and 240 min.

![Figure 7](image)

**Fig. 7.** Microbiological assay for control and experimental samples using *S. aureus*. A) Average CFU counts (log₁₀ CFU/mL) after 2 h of bacterial adhesion and B) 24 h of biofilm formation on surfaces. C) Bacterial reduction (%) demonstrate the antibacterial efficiency of treated surfaces in relation to control against *S. aureus* in 24 h biofilm. **p < 0.01, ***p < 0.001, and ****p < 0.0001, using Tukey HSD test. In panel A, # indicate significant differences between 1100 90 °C vs. 1100 120 °C (p < 0.05) and 1500 120 °C (p < 0.0001), and $ indicate significant differences between 1500 90 °C vs. 1100 120 °C (p < 0.01) and 1500 120 °C (p < 0.0001). The error bars indicate standard deviations.
islands in this work is ~16 nm which is below the reported cytotoxicity level of silver (~30–100 nm) [18].

3.4. Bacterial adhesion behavior and antibacterial properties of Ag-coated N95 mask filter for different treatment parameters

Because surface properties are known to influence bacterial attachment behavior, we investigated whether the modification of the N95 mask filter surfaces with different silver ALD treatment parameters would influence the bacterial adhesion (2 h) of the airborne pathogen S. aureus. Interestingly, when the surface treatment was performed at 120 °C, a statistically significant increase on bacterial adhesion was observed for both Ag ALD number of cycles: 1100 (p < 0.01) and 1500 (p < 0.0001) compared to the control group. These N95 mask filter surfaces also showed higher S. aureus loads compared to those functionalized at 90 °C (p < 0.05). Conversely, no significant difference in viable cell count was noted between control and the groups treated at 90 °C for 1100 cycles (p = 0.99) and 1500 cycles (p = 0.98); this suggests that early on biofilm development on these surfaces had no effect on the adherence of bacterial cells to the N95 mask filter samples (Fig. 7A).
Once the biofilm buildup begins with bacterial adhesion to the surface and it is followed by the co-aggregation and proliferation of bacterial cells into cell clusters [54], an approach providing an antibacterial control during this time would be of great relevance to respiratory wearers’ health. Thus, to further evaluate the antibacterial properties of the developed surfaces under biofilm establishment, it was investigated by CFU counts after a culture period of 24 h. As shown in Fig. 7B, for the sample which was functionalized at 120 °C for 1500 cycles, surface continued allowing more S. aureus to adhere and proliferate than the control group (p < 0.001). In contrast, all other experimental surfaces reduced the average viable CFU counts. According to the microbiological results, besides the 1100 cycles deposited at 90 °C does not influence 2 h bacterial adhesion, it was also able to significantly reduce the viable bacterial count after 24 h of biofilm development than the control surface (p < 0.01). Qualitatively, the S. aureus CFU content was reduced by about 76% (Fig. 7C). Altogether, it indicates that the sample which was functionalized for 1100 Ag ALD cycles at 90 °C was potent for maintain initial bacteria adherence and suppress subsequent biofilm formation.

Considering our XPS and antibacterial data, results showed improvement in suppressing the biofilm formation on N95 samples with smaller size silver. Ag is well known for its antimicrobial activity [57,58]. It is also mentioned that the ability of Ag in “pits” formation on the cell membrane is a crucial factor [59]. Smaller particles have a larger surface area per unit mass and can diffuse easier through the thicker membrane wall destroying the internal cellular components of bacteria. Our results showed that 2 h was not sufficient time for an efficient bacteria/Ag contact, while after longer time (24 h), effective contact happened which resulted in reduction of biofilm formation. According to our proposed mechanism in Fig. 8, lower number of cycles (in this case 1100 Ag ALD cycles) resulted in smaller size silver nano- islands on the surface and consequently more effective against biofilm formation after 24 h. Our previous work on surface treatment of collagen membrane with similar Ag ALD demonstrated the island growth on the fibers of collagen too [60].

In the current work, the antibacterial properties of Ag deposited N95 was studied only against S. aureus. The properties of ALD functionalized N95 will be studied against other microbiological assays in future studies. Also, there are reports in the literature on the efficient effect of copper on killing COVID19 virus. In future work, copper ALD-modified masks will be fabricated and the antivirus properties against COVID19 may be investigated.

In the current work, the antibacterial properties of Ag deposited N95 was studied only against S. aureus. Further, ALD overall is a relatively slow vapor phase deposition method, except for some increasingly attractive modified versions, like spatial ALD (S-ALD). [61].

4. Conclusions

The current study presented a new silver functionalization method on non-woven polypropylene. Ag metal nano- islands were deposited on filter of N95 mask using Ag(fod)(Pet3) precursor and borane complex reducing agent. This method is solvent-free and efficient in achieving respirators without blocking the pores and negatively affecting air permeability of the mask. XPS and XAFS confirmed the ALD Ag on N95 samples and that it has amorphous structure with no evidence of Ag₂O. This functionalization did not change the wetting properties of the samples and adhesion of Ag nano-islands were tested by washing and soaking. Wash test results showed that the achieved adhesion between silver and N95 filter media is stable against washing methods of running water and sonication. After exposing the as-deposited sample to PBS for an extended period of 4 h, only 8.5% of deposited silver was leached into the solution. For the sample which was functionalized with 1100 Ag ALD cycles at 90 °C, 76% reduction in biofilm formation was observed after 24 h.

The potential applications of such Ag ALD coated filter media can be as antibacterial wound dressings, face masks or other PPEs with the additional target of longer-term service life. This novel functionalization method is also a potential candidate for surface treatment of surfaces which are sensitive to washing.

CRediT authorship contribution statement

Sarah Hashemi Astaneh: Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Funding acquisition, Visualization, Writing – original draft, Writing – review & editing. Harsheep Bhatia: Methodology, Investigation, Visualization, Writing – review & editing. Bruna Egumi Nagay: Methodology, Investigation, Formal analysis, Visualization, Writing – review & editing. Valentin Adelino R. Bariao: Resources, Funding acquisition, Visualization, Writing – review & editing. Gregory Jorsich: Formal analysis, Resources, Supervision, Writing – review & editing. Cortino Sukotjo: Resources, Project administration, Supervision, Writing – review & editing. Christos G. Takoudis: Resources, Visualization, Funding acquisition, Writing – review & editing, Project administration, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

Partial funding for this project was provided by the National Science Foundation [grant number DMR-1309114]. Part of this work was performed at the DuPont-Northwestern-Dow Collaborative Access Team (DND-CAT) located at Sector 5 of the Advanced Photon Source (APS). DND-CAT is supported by Northwestern University, the Dow Chemical Company, and DuPont de Nemours, Inc. The authors are grateful for the help of the APS staff scientist Qing Ma. This research used resources of the Advanced Photon Source; a U.S. Department of Energy (DOE) Office of Science User Facility operated for the DOE Office of Science by Argonne National Laboratory [Contract number DE-AC02-06CH11357]. The ICP-MS / PBS analyses were carried out at the Research Resources Center of the University of Illinois at Chicago.

References

[1] N. Linton, T. Kobayashi, Y. Yang, K. Hayashi, A. Akhmetzhanov, S. Jung, B. Yuan, R. Kinoshita, H. Nishihura, Incubation Period and Other Epidemiological Characteristics of 2019 Novel Coronavirus Infections with Right Truncation: A Statistical Analysis of Publicly Available Case Data. J. Clin. Med. 9 (2020) 538, https://doi.org/10.3390/jcm9020538.
[2] C. for D.C. and P. CDC, Recommended Guidance for Extended Use and Limited Reuse of N95 Filtering Facepiece Respirators in Healthcare Settings, (n.d.).
[3] H. Mittal, C. for D.C. and P. CDC, Survival of Microorganisms on HEPA Filters, Appl. Biosaf. 16 (2011) 163–166, https://doi.org/10.1177/153567601101600305.
[4] R. Mirzaei, P. Goodarzi, M. Asadi, A. Soltani, A. Solhani, H. Ali Abraham Aljanaie, A.S. Jeda, S. Dashtbin, S. Jalalifar, R. Mohammadzadeh, A. Teimoori, K. Tari, M. Salari, S. Ghiasvand, . Kazemi, R. YousefiShahoul, H. Keyvani, S. Karampoo, Bacterial co-infections with SARS-CoV-2, IUBMB Life. 72 (2020) 2097–2111, https://doi.org/10.1002/iub.2552.
[5] S. Deinhardt-Emmer, K.F. Haupt, M. Garcia-Moreno, J. Geraci, C. Forstner, M. Pletz, C. Ehrhardt, B. Loffler, Staphylococcus aureus Pneumonia: Preceding Infection Paves the Way for Low-Virulent Strains, Toxins (Basel). 11 (2019) 734, https://doi.org/10.3390/toxins11120734.
[53] P. Shrestha, D. Gu, N. Tran, K. Tapily, H. Baumgart, G. Namkoong, Investigation of Volmer-Weber Growth during the Nucleation Phase of ALD Platinum Thin Films and Template Based Platinum Nanotubes, ECS Trans. 33 (2019) 127–134, https://doi.org/10.1149/1.3485249/XML.

[54] W. Yin, Y. Wang, L. Liu, J. He, Biofilms: The Microbial “Protective Clothing” in Extreme Environments, Int. J. Mol. Sci. 20 (2019) 3423, https://doi.org/10.3390/ijms20143423.

[55] J.S. Kim, E. Kuk, K.N. Yu, J.-H. Kim, S.J. Park, H.J. Lee, S.H. Kim, Y.K. Park, Y.H. Park, C.-Y. Hwang, Y.-K. Kim, Y.-S. Lee, D.H. Jeong, M.-H. Cho, Antimicrobial effects of silver nanoparticles, Nanomedicine Nanotechnology, Biol. Med. 3 (2007) 95–101, https://doi.org/10.1016/j.nano.2006.12.001.

[56] A.F. Wady, A.L. Machado, C.C. Foggi, C.A. Zamperini, V. Zucolotto, E.B. Moffa, C.E. Vergani, Effect of a Silver Nanoparticles Solution on Staphylococcus aureus and Candida spp, J. Nanomater. 2014 (2014) 1–7, https://doi.org/10.1155/2014/545279.

[57] T. Hamouda, A. Myc, B. Donovan, A.Y. Shih, J.D. Reuter, J.R. Baker, A novel surfactant nanemulsion with a unique non-irritant topical antimicrobial activity against bacteria, enveloped viruses and fungi, Microbiol. Res. 156 (2001) 1–7, https://doi.org/10.1078/0944-5513-00069.

[58] P. Dibrov, J. Dzioba, K.K. Gosink, C.C. Hase, Chemiosmotic Mechanism of Antimicrobial Activity of Ag+ in Vibrio cholerae, Antimicrob. Agents Chemother. 46 (2002) 2668–2670, https://doi.org/10.1128/AAC.46.8.2668-2670.2002.

[59] I. Sondi, B. Salopek-Sondi, Silver nanoparticles as antimicrobial agent: a case study on E. coli as a model for Gram-negative bacteria, J. Colloid Interface Sci. 275 (2004) 177–182, https://doi.org/10.1016/j.jcis.2004.02.012.

[60] S. Hashemi Astaneh, Surface Functionalization and Enhancement of Properties of Biomaterials through Atomic Layer Deposition, Univ, Illinois Chicago, 2020. Ph.D. Thesis.

[61] P. Poodt, D.C. Cameron, E. Dickey, S.M. George, V. Kuznetsov, G.N. Parsons, F. Roozeboom, G. Sundaram, A.d. Vermeer, Spatial atomic layer deposition: A route towards further industrialization of atomic layer deposition, Undefined 30 (1) (2012) 010802, https://doi.org/10.1116/1.3670745.