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Co-existing “spear-and-shield” air filter: Anchoring proteinaceous pathogen and self-sterilized nanocoating for combating viral pandemic

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ABSTRACT

Infectious pollutants bioaerosols can threaten human public health. In particular, the indoor environment provides a unique exposure situation to induce infection through airborne transmission like SARS-CoV-2. To prevent the infection from spreading, personal protective equipment or indoor air purification is necessary. However, it has been discovered that the conventional filter can become contaminated by pathogen-containing aerosols, meaning that advanced filtering and self-sterilization systems are required. Here, we fabricate a multilayered nanocoating around the fabric using laponite (LAP) with Cu2+ ions (LAP-Cu2+ nanocoating) two contradictory functions in one system: trapping proteinaceous pathogens and antibacterial effect. Due to the strong LAP-protein interaction, albumin and spike protein (S-protein) are trapped into the fabric when proteins are sprayed using a nebulizer. The protein-blocking performance of the nanocoated fabric is 9.55-fold higher than bare fabric. These trapping capacities are retained after rinsing and repeated adsorption cycles, showing reproducibility for air filtration. Even though the protein-binding occurred, the LAP-Cu2+ fabric indicates antibacterial effect. LAP-Cu2+ fabric has an equivalent air and water transmittance rate to that of bare fabric with a stability under physiological environment. Therefore, given its excellent “Spear-and-shield” functions, the proposed LAP-Cu2+ fabric shows great potential for use in filter and masks during the viral pandemic.

1. Introduction

With growing personal health risks globally, the demand and importance of air purification are gradually increasing for removing pollutants in the air. [1] The pollutants contain dust, virus, bacteria, or fungi in the form of an aerosol. [2] Especially, biological pollutants can be a significant factor in infection through airborne transmission. [3] Most of the biological pollutants have a variety of proteins on their surface and release their protein from aerosol occasionally. These aerosols (<5 μm in diameter by conventional terminology) with biological pollutants could be spread human-to-human by activities (breathing, talking, sneezing, coughing or spraying of liquids). This action could be accelerated in crowded indoor, where the higher transmission condition than the outdoor, and then lead to catastrophic pandemics. [4]

According to the previous reports, the concentration of airborne COVID-19-laden aerosols in outdoor environments is very low (1–3 RNA copy/m3 in a public area). [5–8] It means that the probability of airborne transmission through virus-containing aerosols in indoor environments could be higher than that in outdoor environments. Therefore, it is essential to maintain good indoor air quality within and around buildings and structures.

Recently, with an outbreak of severe acute respiratory syndrome coronavirus (SARS-CoV-2), it has been spread worldwide, causing 100 million infected people and 2.3 million related deaths until now. SARS-CoV-2 is spherical, 80–200 nm in diameter, with ~ 30,000 nucleotides in the core and four surrounding proteins: the spike (S), envelope (E), membrane (M), and nucleocapsid (N) proteins. [9,10] SARS-CoV-2 has an extremely contagious nature with an affinity for host cells 20 times higher than SARS-CoV. [11] Even worse, the biological activity of SARS-CoV-2 remains viable in aerosols for 3 h. [12] Therefore, it is essential to focus on personal hygiene and indoor air purification to prevent the
spread of the virus.

Once the virus or bacteria enter the human respiration pathway, the specific protein on its surface employs a significant role in recognition and attachment to the target host cell, the first step for infection.\[10\] The infection can be prevented by using proper personal protection equipment or air purification having excellent filtering and proteinaceous pathogens trapping capacities. In general, the filter in the purification membrane traps airborne contaminated substances by size exclusion.\[13\] The contaminated substances are physically captured on the surface of a fibrous or porous membrane, driven by straining, interception, diffusion, and inertial impaction mechanisms.\[14\] However, the overused filter rapidly decreases its filtration property; it is needed to be replaced frequently.\[13\] Otherwise, re-release of the filtered pollutant from the filter could occur. Furthermore, due to its physical adsorption mechanism, the filters can capture pollutants and proteins, but this may provide an environment in which bacteria can proliferate on filters over time. To avoid anti-proliferation of bacteria, the bacteria repellent nanocoating has been addressed;\[15\] however, it may concern re-contamination by releasing pathogens. Therefore, it is necessary for a technology that can prevent bacterial propagation by protein adsorption while inducing a deactivation of bioaerosol through the trapping proteins with specific interactions, not physically size sieving.

In the present study, we propose a newly designed filter for air purification that protects against proteinaceous pathogens with self-sterilization property in a simple preparation using laponite (LAP).

The LAP is a disc-shaped FDA-approved nanoclay\[16\] with a diameter of 20–30 nm diameter, a thickness of 1 nm, a positive edge charge, and negative face charge.\[17,18\] This nanoclay can be hydrated and dispersed in an aqueous solution to form stable electrical double layers with Na\(^+\) ions around the particles.\[19\] LAP also forms a stable protein complex via strong clay–protein interactions. By taking advantage of these characteristics of LAP, we develop a multilayered nanocoating for air filtration using LAP and the commercially available polymer poly (diallyldimethylammonium chloride) (PDAC) as negatively and positively charged materials, respectively. A layer-by-layer (LbL) assembly method, the multilayer fabrication technique with versatile, safe, inexpensive, and high throughput, is employed to produce an evenly distributed nanocoating on the fibers.\[20\] The LAP-incorporated fabric completely anchors incoming proteins in contaminated aerosol and protects from the penetration. The attached proteinaceous bacterial can be killed by the additionally addressed Cu\(^{2+}\) ions within the LAP layer. The LAP-Cu\(^{2+}\) fabric having 2 paradoxical “Spear and Shield” effects can provide decontaminated air with high-level of gas and water vapor transmittance (Fig. 1). Therefore, the LAP-Cu\(^{2+}\) nanocoatings can be applied to filter in face mask or air purifier to upgrade their filtration performance.

2. Materials and methods

2.1. Materials

PDAC (Mw 2,000,000), bovine serum albumin (BSA), and copper chloride (II) (CuCl\(_2\)) were purchased from Sigma-Aldrich. LAP (Na\(_x\)(Mg\(_{3}\)Li\(_{1}\)Si\(_4\)O\(_{10}\)(OH)\(_2\))\(_{n}\)H\(_2\)O) was obtained from BYK-ALTANA (Widnes, UK).\[25\] PET fabric from a commercially available product was used in the experiments.

Biotinylated SARS-CoV-2 S-protein (D614G), His Avitag™, Super

Fig. 1. Schematic illustration of 2 paradoxical effects on LAP-Cu\(^{2+}\)-coated fabric. When the contaminated aerosol containing bacterial or proteinaceous pathogens contacts the fabric, the LAP can trap the pathogens (Spear), and the Cu\(^{2+}\) ions kill the bacteria (Shield) over the nanocoating without interfering air and vapor transmission. Finally, the aerosol is decontaminated.
3. Stable trimer (MALS verified) was purchased from ACROBiosystems Inc (USA). FITC-conjugated BSA, a BCA Protein Assay Kit, and streptavidin-FITC were purchased from Thermo Fisher Scientific (Waltham, MA, USA).

2.2. LAP-Cu$_{2}^{+}$ nanocoating preparation on the substrate

A silicon wafer was used as the substrate for the LAP-Cu$_{2}^{+}$ nanocoating. The wafer was cleaned with Piranha solution ($\text{H}_2\text{SO}_4:\text{H}_2\text{O}_2 = 3:1$) for 5 min and sequentially treated with oxygen plasma (CUTE-1B) to negatively charge the surface. The PET fabric and commercially available surgical mask (Yuhan Kimberly Ltd., Korea) was washed with deionized (DI) water and ethanol and immersed in 5 M NaOH solution for 2 h at 60 $^\circ$C to generate carboxylic acid via the hydrolysis of the fabric. PDAC (1 mg/mL at pH 4) and LAP solutions (5 mg/mL at pH 9–10) were prepared.

The LAP-Cu$_{2}^{+}$ nanocoating was generated using the following steps. First, the substrate was immersed in PDAC solution for 5 min and washed in pure DI water twice for 1 min to remove unbound polymers. The substrate was then treated with the LAP solution in the same way as for the previous layer. In order to ensure an ordered arrangement of LAP within the layer, 50 mM CuCl$_2$ aqueous solution was used at washing step instead DI water.[25] This layering cycle was repeated until the desired number of bilayers had formed. Once the coating process had ended, the fabrication of the filter was completed.

![Fig. 2](image-url) - (a) Schematic illustration of the materials used for the proposed LbL coating. Due to the LAP and Cu ions, the two functions that attacking proteinaceous contaminants are adsorbed onto LAP (Spear) and bacterial are killed by Cu$^{2+}$ ions (Shield) are co-existing on filter. (b) Photographic and SEM images of pristine PET fabric and the proposed LAP-Cu$_{2}^{+}$ nanocoated fabric. (c) Atomic deposition analysis LAP-Cu$_{2}^{+}$ nanocoated fabrics using EDS.
coated substrate was dried using nitrogen gas and stored at room temperature.

2.3. Characterization of the LAP-Cu\textsuperscript{2+} nanocoating

The surface morphology of the samples was examined using SEM (IT-500, JEOL, Japan) and atomic force microscopy (AFM; X-10, Park Systems, Suwon, Korea). The chemical composition of the bare fabric and the Lap-Cu\textsuperscript{2+}-coated fabric was analyzed using SEM-EDS (7610F-Plus, JEOL, Japan).

2.4. Air permeability and water vapor transmittance rate

The air permeability of the bare and coated fabrics was measured with an air permeability tester (TQD-G1, Labthink Instruments Co., Ltd., Jinan, China) at a pressure of 15 MPa, and the water vapor permeability of the nanocoating was assessed using a water vapor permeability tester (W3/031, Labthink Instruments Co., Ltd., Jinan, China) at a temperature of 38 \(^\circ\)C and a relative humidity of 90%.

2.5. BSA trapping tests

To determine the protein-trapping ability of the bare and LAP-Cu\textsuperscript{2+}-coated fabric, they were immersed in 10 mL of BSA solution (1 mg/mL), with 100 \(\mu\)L of the BSA solution subsequently collected at set time intervals. The total BSA in these aliquots was quantified using BCA assays.

To test the performance of the fabrics under flow conditions, 1 mL of 0.1 mg/mL BSA solution (prepared using 1 \(\times\) phosphate-buffered saline [PBS]) was placed on the bare or LAP-Cu\textsuperscript{2+}-coated fabric (See Fig. 4(b)) and let BSA solution penetrate. Once the solution had penetrated the fabric, the solution was collected after each penetration step. The change in the concentration of the BSA solution was monitored using BCA assays.

BSA-FITC solution (0.1 mg/mL) was also prepared using 1 \(\times\) PBS. BSA-FITC aerosols were generated with a nebulizer (NB-152U, CAS, Korea) at a flow rate 5 L/min for 0.5, 1, 2 and 3 min. To determine whether the fabrics were genuinely trapping the protein, rather than it superficially collecting on the surface, the change in fluorescence after washing the fabrics was analyzed with photoluminescence (PL) spectroscopy (FP-8300, Jasco, Japan) and confocal microscopy (LSM 980, ZEISS Microscopy, Germany).

2.6. S-protein trapping tests

S-protein solution was prepared at a concentration of 2 \(\mu\)g/mL in 1 \(\times\) PBS. S-protein aerosols were generated in a customized chamber, and the fabrics were exposed to these for 3 min. The fabrics were subsequently washed after aerosol contact and then dried under ambient conditions. The samples were then analyzed using confocal microscopy.

2.7. Antibacterial test on the LAP-Cu\textsuperscript{2+} nanocoating

The pathogens \textit{S. aureus} (gram-positive, NCCP14570) and \textit{P. aeruginosa} (gram-negative, NCCP14780) used in this study were provided by the National Culture Collection for Pathogens. Both strains were cultured in tryptic soy broth (Soybean-casein digest media; Becton, Dickinson, and Company; BD, NJ, USA) at 37 \(^\circ\)C under aerobic conditions. After culturing overnight, both strains were assessed at OD\textsubscript{600} = 0.1. The fabrics prepared in 1 cm\textsuperscript{2} were immersed in the bacteria-inoculated medium in a 12-well plate and incubated at 37 \(^\circ\)C under aerobic conditions for 6 h. Following treatment, the optical density of the bacterial solution was measured at 600 nm to assess the bacterial viability.

3. Results and discussion

As illustrated in Fig. 2a, negatively charged LAP can be deposited on the fibers as a multilayered nanocoating with positively charged PDAC via electrostatic interaction. Prior to the LbL assembly process, the
Fabric which was composed mainly of polyethylene terephthalate (PET), was negatively charged to attract PDAC as the primary layer (see the experimental section). The LAP was then sequentially accumulated to form the multilayered nanocoating to trap proteinaceous pathogens onto the LAP by protein-clay interactions. In the present study, we also utilized a unique characteristic of LAP – self-assembly under high ionic concentrations – to deposit LAP within each layer.[19] Generally, LAP disperses well in water due to the resulting stable electrical double layer formed by Na\(^+\) ions. However, by increasing the ionic strength during particle dispersion, the surrounded Na\(^+\) ions are screened, leading to the face–edge aggregation of LAP in a “house of cards” structure.[19] To provide a self-sterilization fabric, we have used CuCl\(_2\) as the reagent to stack LAP within a multilayer. Using a CuCl\(_2\) solution during the rinsing process, aggregations of Cu\(^{2+}\) and LAP were formed and can retain the stable Cu\(^{2+}\) ion with suppressing the oxidation of Cu\(^{2+}\). This can more effectively block proteinaceous pathogens and provide an antibacterial effect, in one LAP-Cu\(^{2+}\) nanocoating system.

Fig. 2b shows that 10 cycles of PDAC/LAP layers were deposited on the PET fabric. As observed in the photo images, the nanocoating changed the fabric color from white to blue because of the presence of the Cu\(^{2+}\) ions. The SEM image illustrates that the fibers in the pristine fabric were entirely smooth. On the other hand, the LAP-Cu\(^{2+}\) nanocoating was present both on single fibers and between fibers, leading to a rougher surface morphology. To confirm whether the LAP-Cu\(^{2+}\) nanocoating on the fibers was stable, we mapped the element composition using SEM with an energy-dispersive X-ray spectrometer (SEM-EDS; Fig. 2c). The bare fabric primarily consisted of C and O, representing pure PET (Figure S1). With the LAP-Cu\(^{2+}\) nanocoating on the fibers, high levels of Mg, one of the components of LAP, were observed and evenly distributed around the fibers. In addition, Cu was found to overlap with Mg, indicating that copper ions are stacked within the LAP interlayers.

Figure S2a indicates the thickness of the LAP-Cu\(^{2+}\) nanocoating as the number of bilayers increased, showing a consistent linear relationship for the first 10 bilayers (Phase I). Then the association became slightly steeper, indicating that several laponite layers are stacked, resulting in enhanced potential for protein trapping action when proteinaceous pathogen passes through the multilayer film.

Next, we have confirmed the protein trapping capability of LAP-Cu\(^{2+}\) nanocoating as a filter. We mimicked the spread of viruses through aerosols sized 100 nm to 5 μm generated by human activities such as conversations, sneezing, and coughing using a nebulizer (Fig. 3a top). Fig. 3a presents a schematic of the experimental setup showing fluorescence-labeled protein-containing aerosols injected into the inlet chamber with the nebulizer and passing through pristine or LAP-coated fabric on their way into the outlet chamber. Similarly, Figure S3 presents an image of the actual setup. To confirm the protein-trapping effect by the nature of LAP, the LAP-nanocoated fabric without Cu\(^{2+}\) was used. The protein-blocking ability of fabric can be measured based on the levels of protein trapped in the fibers. In the present study, BSA was sprayed as an aerosol toward the fabric at a high concentration (0.1 mg/mL), thus the levels of filtered BSA were not significantly different for the plain and LAP-coated fabrics (Figure S4). The washing step, designed to mimic a vigorous condition for filter washing or continuous human respiration, led to any weakly bound BSA being removed from the bare fabric; as a result, there was a dramatic decrease in the PI intensity of the trapped BSA after washing. However, the LAP-Cu\(^{2+}\) nanocoating more strongly retained the trapped protein, with a trapping capacity that was 3.72 times higher than the plain fabric (Fig. 3b), with Fig. 3c showing images of fluorescence-labeled BSA fixed on the surface of the fibers. In addition, the trapping efficiency did not depend on the nebulizer treatment time (Figure S5).

We also investigated the trapping efficiency of the proposed LAP nanocoating for S-protein, which is the outermost protein of SARS-CoV-2. S-protein plays a vital role in receptor recognition and membrane fusion for invading host cells.[27] S-protein was entirely trapped over the fibers when aerosolized at a low concentration (2 μg/mL) with a nebulizer. As shown in 3-dimensional images, the trapped S-protein was dramatically reduced for pristine fabric. On the other hand, the S-protein did not detach from the LAP-coated fabric. In fact, the LAP nanocoating provided 9.55 times higher filtering ability in comparison to bare fabric (Fig. 3e). The protein has several domains, which are containing positive, negative charge area, and non-polar area. The structure and charged potential of BSA and S-protein is illustrated in Fig. 3b and d. LAP platelet shows a negative charged face due to the Na\(^+\) ions and positive charged edge due to the Mg. Also, the hydroxyl group in the edge can be responsible for hydrogen bonding. Therefore, the approaching proteinaceous pathogens are spontaneously bound to LAP via (1) hydrogen bonding and (2) electrostatic interaction (Fig. 2a, Spear effect). Therefore, we believe that the proposed LAP-nanocoated fabric can prevent proteinaceous pathogens from entering the human body due to the strong binding affinity between the LAP and protein. The protein adsorption property of LAP-Cu\(^{2+}\) nanocoating could be also realized on flat substrate, the silicon wafer. When immersing the LAP-Cu\(^{2+}\) nanocoating on wafer in excess BSA solution, the nanocoating trapped approximately 1.7 mg/cm\(^2\) in 1 h, representing an excellent protein-
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trapping ability (Figure S6). The BSA trapped in the nanocoating was also not readily released again, with a 99.75% trapping efficiency.

We have also confirmed the excellent S-protein trapping capacity of LAP-coated fabric even after rinsing step (Fig. 4a). Indeed, continuous air and water flow including respiration through the fabric can lead to the release of contaminants from the fabric. Overall, the strong trapping efficiency of the LAP-Cu\(^{2+}\) nanocoating dramatically enhances the blocking properties of the filter. To further investigate the reusability and durability of the LAP-Cu\(^{2+}\) nanocoating on the fabric under several trapping cycles, BSA solution was passed through the fabric in cycles under a steady flow (Fig. 4b). The trapping efficiency was calculated by comparing the concentration of the initial protein solution and the protein solution after passing through the fabric. It was found that the trapping efficiency of the LAP-Cu\(^{2+}\)-coated fabric was 6–10 times higher than that of the pristine fabric (Fig. 4c). It was thus confirmed that the proposed LAP-Cu\(^{2+}\) nanocoating can effectively prevent the influx of protein toward the inside of the fabric, whereas most of the protein can pass through the bare fabric. In addition, the trapping efficiency of the LAP-Cu\(^{2+}\) nanocoated fabric was maintained after seven cycles of the protein solution, verifying its potential reusability and durability.

Next, the shield antibacterial effect of the LAP-Cu\(^{2+}\) nanocoating was assessed. In general, Cu\(^{2+}\) ions can easily enter a bacterium and disrupt its metabolism and membrane integrity. Therefore, a portion of the inserted Cu\(^{2+}\) ions in the LAP-Cu\(^{2+}\) nanocoating can be released and kill any bacteria on the fabric (Fig. 5a). Bacteria in contact with the LAP-Cu\(^{2+}\)-coated fabric induce acidic conditions. With lowering pH conditions, Cu\(^{2+}\) ions can be released from LAP-Cu\(^{2+}\) nanocoated fabric due to weakened electrostatic interaction between LAP and Cu\(^{2+}\) ions. [30] Fig. 5b shows that the viability of gram-positive (Staphylococcus aureus) and gram-negative (Pseudomonas aeruginosa) bacteria on the LAP-Cu\(^{2+}\)-coated fabric was 73% and 75% lower, respectively, compared to the bare fabric.

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**Fig. 5.** “Shield effect: Antibacterial by Cu\(^{2+}\) ions.” (a) Schematic illustration of the antibacterial mechanisms associated with the LAP-Cu\(^{2+}\)-coated fabric. (b) Relative bacteria viability on bare and LAP-Cu\(^{2+}\)-coated fabrics.

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**Fig. 6.** (a) Air permeability of the fabric (L/min/cm\(^2\)). (b) Water vapor transmission rate (WVTR) for the fabric. (c) Stability of the LAP-Cu\(^{2+}\) film against physiological condition. (d) Film morphology and roughness changes before and after immersion in physiological solution.
Finally, the air and water vapor permeability were tested. Fig. 6a and b show that the transmittance level was not significantly changed before and after LAP-Cu\(^{2+}\) nanocoating addressed, meaning that the nanocoating did not completely fill the gaps between the fibers. It was concluded that LAP-Cu\(^{2+}\) coating can be applied for filters without disrupting airflow, even breathing for face mask. We have also observed that surface morphology and roughness of the coating did not change even after exposure to physiological condition and after BSA-trapping (Fig. 6c, d). This confirmed that the proposed LAP-Cu\(^{2+}\)-nanocoated fabric would remain functional. It is also revealed that a highly rough and stable LAP-Cu\(^{2+}\) nanocoating was not changed, with magnified images showing LAP discs present on top of the nanocoating after stability test. Line profiling of the LAP-Cu\(^{2+}\) nanocoating revealed the presence of repeating 14–16 nm grooves (Figure S7). Finally, from a practical perspective, the LAP-Cu\(^{2+}\) nanocoating can also be easily applied to commercially available masks. Overall, it was confirmed that LAP-Cu\(^{2+}\)-nanocoated masks were not inconvenient and did not suffer from aesthetic problems in everyday use (Figure S8). Because of the robust stability and protein-trapping property under physiological conditions, the trapped proteinaceous pathogens are not able to be released out during use. The copper ions are tightly bound to LAP when the nanocoating is dried state. Moreover, even if the LAP-Cu\(^{2+}\) nanocoating is detached by the physical stimuli, we believe that the impact for human threat may be negligible as the LAP and copper ions are well-known safe materials.[16,31] The LAP-Cu\(^{2+}\) nanocoating can be prepared easily on the various substrates for the desired purpose, not only in the viral pandemic but also in daily usage.

4. Conclusion

In this study, we developed a filter for face mask or air purifier that is able to efficiently block proteinaceous pathogens and self-sterilization property using LAP nanocoating. Because LAP has excellent protein binding characteristics due to the strong protein–clay interactions, we designed an LAP-containing LbL nanocoating for the fabric. To increase the amount of LAP on the fabric and produce a strong antibacterial effect, we added Cu\(^{2+}\) ions to the LAP nanocoating. The excellent protein-trapping efficiency of the LAP-Cu\(^{2+}\)-nanocoated fabric indicates that it can prevent SARS-CoV-2 from penetrating the fabric. The nanocoated fabric exhibited reproducibility and stability even after several protein penetration cycles. In addition, the bacteria attached to the LAP-Cu\(^{2+}\)-nanocoated fabric were efficiently killed via the oligodynamic effect of the embedded copper ions. The LAP-Cu\(^{2+}\) nanocoating was found to be highly stable under physiological conditions. It was also confirmed that the LAP-Cu\(^{2+}\) nanocoating did not affect the water vapor or air permeability of the fabric, thus it does not interfere with respiration. Thus, the proposed LAP-Cu\(^{2+}\)-nanocoated fabric exhibits significant potential for air decontamination and multi-functional protection against airborne transmission like the COVID-19 pandemic.

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.

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