Bio-inspired Polydopamine Treatment and Nanosilver Formation for Antibacterial Uses

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ABSTRACT

We employed mussels' adhesive secretion, dopamine, and its reducing ability toward silver ions, to create a novel antibacterial effect on a facial mask surface eliminating the need for harmful antiseptics and preservatives. Most past methods for depositing silver particles onto facial masks have lacked suitable processing conditions, and they require additives which limit their uses. Dopamine was adsorbed onto the nonwoven surface and then underwent a self-polymerization reaction to have silver nanoparticles (AgNPs) reduced on the surface. Surface characterization was carried out using microscopic analysis. Photospectrometers were employed to analyze size, deposition, and distribution of silver nanoparticles. For a facial mask design, the common substrates are nonwoven fabrics, which usually accumulate a lot of bacteria when immersed into nutrient-rich extracts and essential oils. Nanosilver for antibacterial uses has attracted much attention owing to its release of silver ions. With this simple strategy, we confirmed the antimicrobial performance of the facial mask surfaces against bacteria.

Keywords: mussels, polydopamine, silver nanoparticles, antibacterial, nonwoven fabric

1 INTRODUCTION

Mechanically, silver-based materials which account for their antibacterial properties are thought to release silver ions (Ag+) and attach to specific thiol (–SH) groups in a variety of structural and functional bacterial proteins [1-3]. Another possible mechanism involves an accumulation of reactive oxygen species (ROS), inducing a bacterial apoptosis-like response [4]. Therefore, AgNPs are being increasingly utilized in the medical industry due to their antibacterial properties. AgNPs can be synthesized in a variety of different ways; the most common methods of AgNP synthesis employ chemical reduction of silver salt (e.g., $AgNO_3$) using a reducing agent (e.g., sodium borohydride) [5]. However, reducing agents and organic solvents used in chemical reduction, such as N,N-dimethylformamide (DMF), hydrazine hydrate (N_2H_4), and sodium borohydride ($NaBH_4$), are highly reactive and pose potential environmental and biological risks [6]. So we focused on developing new green chemistry methods of AgNP synthesis with the advantage of using natural products and avoiding toxic reducing agents, organic solvents, and wasteful purifications with high cytotoxic residuals. With these methods, molecules produced by living organisms such as mussels can replace the reducing and capping agents. Polydopamine (PDA) is a biomimetic polymer that is based on the mussel's adhesive protein that is rich in L-3,4-dihydroxyphenylalanine (L-DOPA) and L-lysine and enables these animals to tightly attach themselves to wet surfaces. Dopamine, containing two hydroxyl groups at the orthopositions to one another, could reduce metal ions to metal nanoparticles, such as Au^+ and Ag^+. Under oxidative and alkaline conditions, dopamine provides a facile approach to surface modification in which self-polymerization of dopamine produces an adhesive PDA coating on a variety of substrates [7]. In this study, dopamine was coated onto nonwoven fabric. It performed a self-polymerization reaction to reduce silver nanoparticles and created a novel antibacterial effect on the facial mask surface, eliminating the need for harmful antiseptics and preservatives.

2 MATERIALS AND METHODS

2.1 Materials

Nonwoven fabric was ultrasonically cleaned in ethanol and deionized water for 15 min and dried in an oven at 60 ^{o}C before use. 3-Hydroxytyramine hydrochloride (dopamine/ HCl, 99%, Acros Co.), silver nitrate (99.9%, Alfa Aesar) and Tris hydrochloride (ultra-pure, from MP Biomedicals), were used as purchased. All chemical reagents were used as received and without further purification.

2.2 Dopamine Oxide Polymerization on Nonwoven Surface

3.152 g Tris–HCl was dissolved in 150 ml deionized water, mixed with 10 wt% NaOH aqueous solution to control pH value to 8.5, and then diluted to 200 ml with water for use as a 0.1 M alkaline Tris–HCl buffer solution. The nonwoven fabric was immersed in an alkaline Tris–HCl buffer solution of dopamine (1 g/L) for 15-240 min at room temperature. After a predetermined reaction time, the resulting nonwoven fabric was filtered and rinsed thoroughly with an ultrasonic cleaner for 3 h and dried in an oven at 60 ^{o}C. The obtained sample was denoted as nonwoven-PDA.

2.3 Electroless Deposition of Silver Coating on the Nonwoven-PDA Surface

The nonwoven-PDA substrate was incubated in aqueous solution of $AgNO_3$ (1-100 mM). The reduction was allowed to continue for 30 min at 70 ^{o}C. The resulting sample was separated by filtration, washed thoroughly with an ultrasonic cleaner for 3h and dried in an oven at 60 ^{o}C. The obtained sample is denoted as nonwoven-PDA/Ag in the discussion below. To systematically demonstrate the use of polydopamine in antibacterial applications, we developed a technique for producing a polydopamine-based coating containing nanosilver as seen in Figure 1.

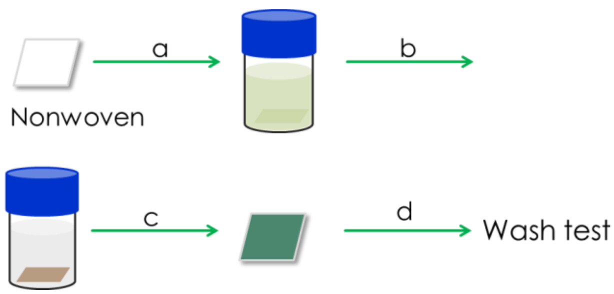

Figure 1. Preparation process for nonwoven-PDA/Ag. (a) Tris-HCl buffer solution of dopamine (1 g/L) (pH=8.5), room temperature, 15-240 min. (b) Aqueous solution of $AgNO_3$ (1-100 mM), 70 ^{o}C, 30 min. (c) Dried in oven, 60 ^{o}C. (d) Washed thoroughly with ultrasonic cleaner, 3h.

2.4 Microbiological evaluations

Antimicrobial behavior of the nonwoven surfaces was evaluated with aerobic plate count and fungi, yeast, and mold plate count. 1 g sterile nonwoven fabric was placed into a screw-cap test tube containing 1 ml sterile Tween 80 and the product was dispersed with a sterile spatula. 8 ml sterile modified letheen broth was added and mixed thoroughly to 10^{-1} dilution. The spread plate technique was used to facilitate recognition of different colony types. Duplicate sets of petri dishes, containing modified letheen agar or potato dextrose agar, were prepared and labeled. 0.1 ml of each dilution was mixed and pipetted onto surfaces of solid media in petri dishes, and inoculum was spread over the entire surface with a bent glass rod that was first sterilized by being dipped in 95% ethanol and quickly flamed to remove the ethanol. The medium was allowed to absorb the inoculum before the plates were inverted and incubated. All colonies in the plates were counted and average colony counts were obtained.

3 RESULTS AND DISCUSSION

3.1 Dopamine modified nonwoven fabric (nonwoven-PDA)

The dopamine solution was colorless and transparent. During the dopamine's oxidative self-polymerization, the color of the solution turned to deep brown. When the nonwoven fabric was immersed into dopamine solution for a longer period of time, the color of became darker (as shown in Figure 2). We characterized the surface morphology of nonwoven-PDA using SEM, and the results are shown in Figure 2. The longer the treatment time, the more uneven the fibers on the surface of the nonwoven fabric became. The elemental composition of the nonwoven fabric was investigated via EDX. Figure 3 shows the EDX patterns of the samples before and after dopamine modifications. It can be seen from Figure 2(b) that the nonwoven-PDA sample is composed of carbon, nitrogen, and oxygen elements. The results suggest the polydopamine was successfully deposited onto the nonwoven fabric.

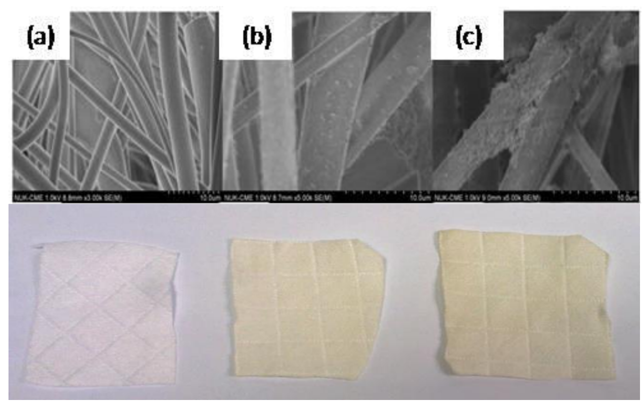

Figure 2. SEM images of polydopamine deposited on nonwoven polypropylene fabric with various reacting times: (a) 0 min, (b) 120 min, (c) 240 min.

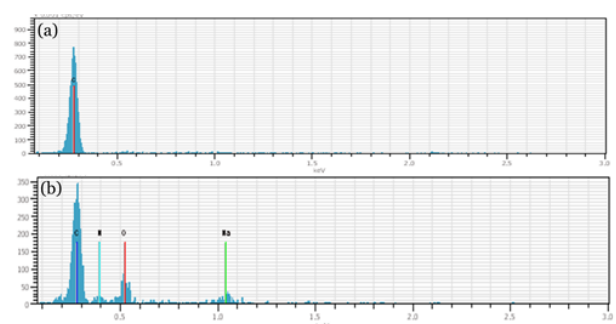

Figure 3. EDX patterns of (a) nonwoven fabric, (b) nonwoven-PDA fabric.

3.2 Nano-silver coated on the nonwoven-PDA surface (nonwoven-PDA/Ag)

The polydopamine on the nonwoven surface has been shown to be an effective functional layer for reduction of ionic silver. The silver-binding ability of the catechol and N-containing groups present in the PDA coating can make

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the reduced AgNPs attach on the surface of nonwoven fabric, as shown in Figure 4. The color becomes dark yellow after silver is coated on the nonwoven fabric. According to SEM analysis, the diameter of AgNPs were below 200 nm. We further confirmed the formation of AgNPs through emergence of a silver plasmon resonance, evident by an increase in absorbance around 420 nm by UV-vis spectrometer. The number and size of the AgNPs could be tailored via variation of immersion time with dopamine, as shown in Figure 5. In order to confirm that the AgNPs can bind well with the nonwoven-PDA, the nonwoven-PDA/Ag samples were washed with an ultrasonic cleaner for 3 h and investigated with a UV-vis spectrometer.The result shows 98 % of AgNPs can still be attached on the nonwoven-PDA.

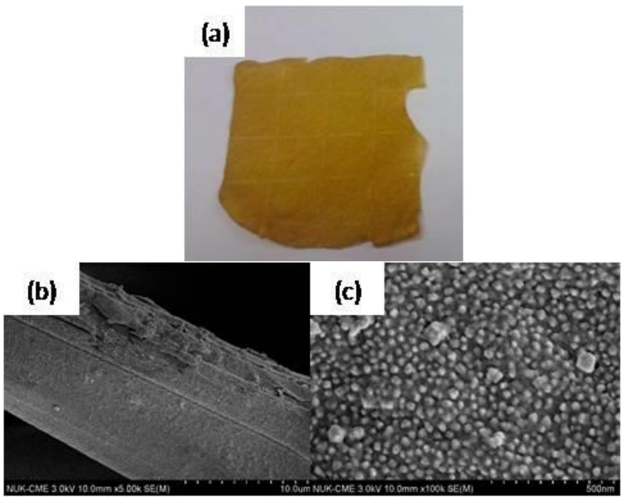

Figure 4. (a)Photograph of nonwoven-PDA/Ag. The SEM images show (b) the formation of nanoparticles on the fabric surfaces and its close-up look. (c) Using high magnification.

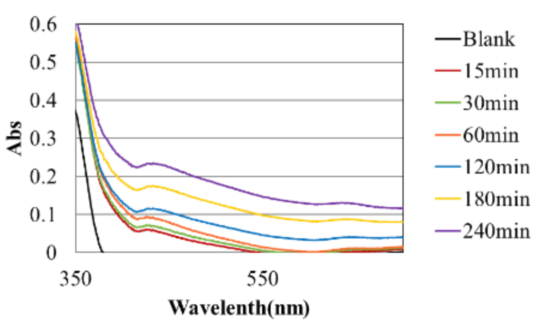

Figure 5. UV-vis spectrum of nonwoven fabric via variation of immersion time with dopamine solution. All samples were immersed in the same silver coating.

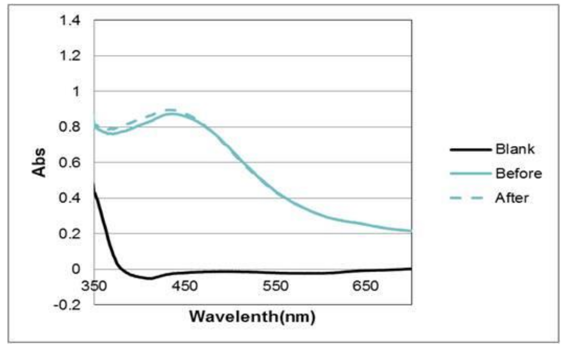

Figure 6. UV-vis spectrum of nonwoven-PDA/Ag sample before and after ultrasonic washing.

3.3 Bacterial Attachment and Death Assays

Antimicrobial behavior of the generated surfaces wa evaluated using the colony-forming unit (CFU) counting method. The results of antibacterial measurement are shown in Table 1. No CFUs were observed on the total bacterial counts or total fungi count tests (Figure 7b-c). Furthermore, we used chromogenic *E.coli*-coliform agar and chromogenic MRSA agar to detect *E. coli*, coli form and *Staphylococcus aureus* strains, and no CFUs were observed even after 72 h (Figure 7d-e). The result indicated that the *in situ* formation of AgNPs via exposure of PDA membranes to $AgNO_3$ solutions can ensure the complete inactivation of bacterial cells.

Table 1. Antibacterial measurement

Detection items		Methods	Results
1	total bacterial counts	Letheen Agar, Modified (MLA)	**0CFU/cm^2**
2	total fungi counts	Potato Dextrose Agar (PDA)	**0CFU/cm^2**
3	*E. coli*, Coli form	Chromogenic E. Coli /Coliform Agar	**N.D.**
4	*Staphylococcus aureus*	Chromogenic MRSA Agar	**N.D.**
Note	total bacterial counts：35 oC for 48~72 h total fungi counts：25 ^{o}C for 72~96 h test temperature：24.0 ^{o}C relative humidity：54 %		

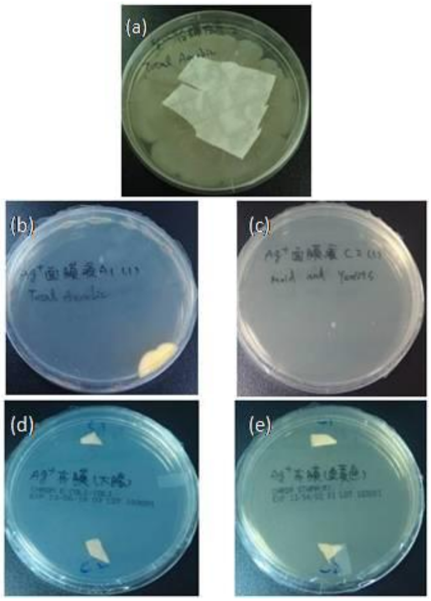

Figure 7. Photographs of antibacterial measurement. (a) total aerobic incubation with nonwoven fabric; nonwoven-PDA/Ag were incubated with (b) total aerobic incubation, (c) mold and yeasts, (d) *E. coli* and coliform, (e) *Staphylococcus aureus.*

4 CONCLUSION

The popular treatment of substrates for silver nanoparticle coating are physical adsorption of silver nanoparticles via vapor deposition methods, or electroless plating of silver salts, which still cause nanoparticle depletion from the surfaces and induce some toxic reactions. We demonstrated a novel immersion strategy for nonwoven fabric coatings that actively kill bacteria through silver ion release. When nanosilver comes into contact with bacteria, the silver ions can pass through the cell walls to react with RSH groups of proteins and therefore destroy the bacteria. The strategy is enabled by a polydopamine layer reducing silver nanoparticles from a silver salt solution onto nonwoven fabric. Finally, we confirmed the antimicrobial performance of the facial mask surfaces against both gram-positive and gram-negative bacterial strains. Thus, our approach represents a simple method that avoids toxic reducing agents and harmful antiseptics and preservatives; the results suggest significant potential for expansion of this concept to other coatings in the future.

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