Silver nanoparticle-coated silica hybrid particles as antibiofouling agent on filter materials

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ABSTRACT

Pathogenic microorganisms filtered on the medium can proliferate and be a source of contamination when an appropriate nutritional condition is attained by the filtered dust materials (*i.e.*, biofouling). Silica microparticles with coated AgNPs can be a promising antibiofouling agent for filter-materials because the microparticles are well dispersible without ligands and easily collectable by a conventional centrifugation and furthermore, the nanoparticles coated on the microparticle can exhibit nano-properties. Here we introduce silver nanoparticle-coated silica (AgNPs@SiO_2) hybrid particles synthesized by seed-mediated growth method and their applications as a biocidal coating material for air and water filtration systems. The AgNPs@SiO_2-immobilized filter-materials for both air and water exhibit excellent antibacterial activity toward gram-negative bacteria, *Escherichia coli*, and gram-positive bacteria, *Staphylococcus epidermidis* and *Pseudomonas aeruginosa*.

Keywords: silver nanoparticle, silica hybrid particle, antibiofouling, filter-material, biocidal coating

1 INTRODUCTION

Pathogenic bacteria and viruses cause health problems and occasionally, lead to pandemic. Thus, global demand for clean air and safe water is increasing. Filtering systems have been utilized to provide clean air and water. However, the pathogenic microorganisms filtered on the medium can proliferate and be a source of contamination when an appropriate nutritional condition is attained by the filtered dust materials (*i.e.*, biofouling) [1,2]. Recent advances in nanoscience and nanotechnology afford nanoparticles (NPs) that can exhibit antimicrobial activity. However, those NPs have some drawbacks in practical utilization as an antibiofouling agent. For example, silver nanoparticles (AgNPs) exhibit antimicrobial effects most effectively when their surface is exposed to the microorganisms because AgNPs exhibit antimicrobial effects by particle itself and by releasing Ag^+ ions [3,4]. However, the AgNPs without ligands aggregate seriously upon their contact, limiting their utilization as an antibiofouling coating material for a filter medium. *In situ* production and coating of naked AgNPs in a gas phase needs complicated equipment and high cost. Besides, the nanoparticles can be released to the environment and exhibit toxicity.

Silica hybrid microparticles with coated AgNPs can be an alternative because the microparticles are well dispersible without ligands and easily collectable by a conventional centrifugation and furthermore, the nanoparticles coated on the microparticle can exhibit nano-properties [5]. In this report, we introduce silver nanoparticle-coated silica (AgNPs@SiO_2) hybrid particles synthesized by seed-mediated growth method and their applications as a biocidal coating material for air and water filtration systems.

2 EXPERIMENTAL SECTION

2.1 Instrumentation

The TEM images were recorded using a CM30 (Philips, 200 kV). The SEM images were recorded using a FEI XL30-ESEM or NOVA200 NOVA-SEM. All of the SEM samples were coated with Pd/Pt for 20 sec (HITACHI, E-1010 Ion Sputter). UV-Vis spectra were recorded using a Perkin Elmer Lambda 25 with a 1 cm-cell.

2.2 Synthesis

Synthesis of AgNPs@SiO_2 [6]. Briefly, aminopropyl (AP)-functionalized silica particles (diameter 406 nm, 34 g) prepared by a Stöber-like process were dispersed in de-ionized water (DW) and pH of the solution was adjusted ~4 using HCl, yielding 0.5 L solution A. Into 0.01 M NaOH solution (4L), 1.7 mL tetrakis(hydroxymethyl)phosphonium chloride was added and after stirring for 2 min, 160 mL of 1% $AgNO_3$ solution was poured and the solution was stirred for 15 min, yielding Ag seed solution. The Ag seed solution was combined with solution A and the combined solution was gently swirled at times for 2 h for homogeneous seeding of Ag seeds on AP-functionalized silica. The extra Ag seeds were removed by centrifugation. The solid was dispersed in 0.5 L of DW and poured into

$Ag(NH_3)_2^+$ solution which was prepared by dissolving 21 g of $AgNO_3$ and 42 mL of NH_4OH in 35 L of DW at ~12 °C. The mixture was stirred for 30 min for sorting-out of Ag seeds, *i.e.*, the remaining Ag seeds became more spacious through sorting-out process. Then, 10 mL of formaldehyde (in 1 L of DW) and 20 mL of formaldehyde (in 0.2 L of DW) was added to the reactor for 3 h and for 2 h, respectively, through 3 channels with gentle stirring. After further stirring for 2 h, the product was collected and rinsed with DW using centrifugation and then, dispersed in DW to make l L of AgNPs@SiO_2 solution (yield ~50 g).

Air Filter Coating with AgNPs@SiO_2. Air filter coating followed the previous aerosol method [6] using medium filters (Fabriano®, 4 × 4 cm^2) and a 1.3% AgNPs@SiO_2 solution with a clean air flow rate of 2 L min^{-1}. Particle concentratons of upstream and downstream from the filter samples were 17.8 × and 1.75 × 10^4 # cm^{-3}, respectively. The coating areal density was calculated from these particle concentratons and coating time variables. For the SEM images of trapped bacteria on the coated air filter, 50 mL of the bacterial solution (optical density of 0.1 at a wavelength of 600 nm) was aerosolized and deposited onto the coated air filter sample that was prepared with a coating areal density of 2 × 10^8 # cm^{-2} using the same aerosol method.

AgNPs@SiO_2-Immobilized Membrane. A polyamide thin film composite (PA TFC) membrane prepared by a published method [7] was functionalized with cysteamine to utilize thiol moieties for immobilization of AgNPs@SiO_2. First, PA TFC membrane was treated with acyl chloride (Trimesoyl chloride) in a base solution (pH = 12 using 0.122 g of DMAP, 2.08 g of K_2CO_3, 5 M KOH in 20 mL of DI water) to make amide bonds and expose acyl chloride moieties. Immediately, the cysteamine aqueous solution was contacted with the acyl chloride functionalized-PA TFC membrane for 30 min to make amide bonds and expose thiol moieties. The thiol-exposed TFC membrane was rinsed with DI water several times. For immobilization of AgNPs@SiO_2 on the thiol-exposed TFC membrane, 0.1 L aqueous suspension of AgNPs@SiO_2 at 7.5 × 10^8 # mL^{-1} was agitated for 24 h for homogeneous dispersion in a glass jar with a lid. The thiol-exposed TFC membrane (5 × 5 cm^2) was attatched beneath the lid and after tightening, the jar was placed upside down for 24 h, giving 11 ± 1% of membrane surface coverage. After immobilization, excess and loosely-bound AgNPs@SiO_2 particles were removed by rinsing with DI water. The prepared membranes (pristine TFC and TFC-AgNP@SiO_2) were stored in DI water untill used.

2.3 Antibacterial Test

Different types of bacteria were selected for antibacterial tests: *Escherichia coli* (ATCC 11775, ATCC 47076), *Staphylococcus epidermidis* (ATCC 14990), *and Pseudomonas aeruginosa* (KCTC 2004). Bacterial solutions were prepared by liquid culture, in which the desired bacteria were suspended in BD® Difco™ Nutrient Broth. After incubation overnight at 37 °C, the bacterial media were diluted to 1 × 10^6 colony forming units (CFUs) mL^{-1} and used for following antibacterial tests.

An air filter sample (1 cm-diameter circular shape) was placed into the 10-fold diluted bacterial solution (10 mL) and incubated at 37 °C for 24 h. The incubated solution was serially diluted to obtain a countable number of colonies, and 100 μL of each diluted solution was spread onto the surface of 87 mm × 15 mm petri dishes containing 15 mL of nutrient agar. The plates were then cultured at 37 °C for 24 h and the number of CFUs was counted. The antibacterial efficacy was calculated based on the control experiment which does not include the coated air filter sample.

To investigate the antibacterial behavior of AgNPs@SiO_2 particles in aqueous solution, 5 mL of bacterial solution was mixed with 0.01 % AgNPs@SiO_2 solution and incubated at 30 °C for 10 min. Ten μL of incubated solution was dropped onto a silicone wafer and analyzed by SEM.

Each membrane sample of 2×2 cm^2 was placed into each bacterial solution and cultured at 37 °C for 2 h. The incubated membranes were cleaned with a PBS solution twice to remove the unattached bacteria. Then, the bacteria attached on the membrane surface were separated by sonication in 10 mL of a sterile PBS buffer solution for 7 min. The obtained bacterial solution was diluted with a PBS solution and spread on LB agar plates. The number of CFUs on each plate was counted after incubation at 37 °C for 12 h.

3 RESULT AND DISCUSSION

Aminopropyl-functionalized silica colloids with sub-micron sizes were used as a substrate material to coat AgNPs. The AgNP-coating was achieved by growing 1–3 nm-sized Ag seeds up to ~30 nm in size on the silica surface where the Ag seeds remained spaciously through seeding and sorting-out process. The aminopropyl moieties on the silica surface are bonded with silver seeds and embedded into the grown AgNPs. The SEM and TEM images in Figure 1 show the resultant AgNPs@SiO_2 particles with relatively homogeneously coated AgNPs on SiO_2 submicrospheres. The hybrid particles were collectable by a conventional centrifugation and redispersible. The hybrid structure was stable for more than one year when the sample was stored at a concentration of 1.3% and at dark place with a tightened lid, promising as a coating material for air and water filtration systems.

Figure 2 shows the UV-Vis spectrum of AgNPs@SiO_2 solution, together with that of AgNP solution. Compared to the typical plasmonic band of AgNP solution, AgNPs@SiO_2 solution displays an additional broad band at longer wavelength region, implicating higher order plasmonic interactions between closely placed AgNPs [8].

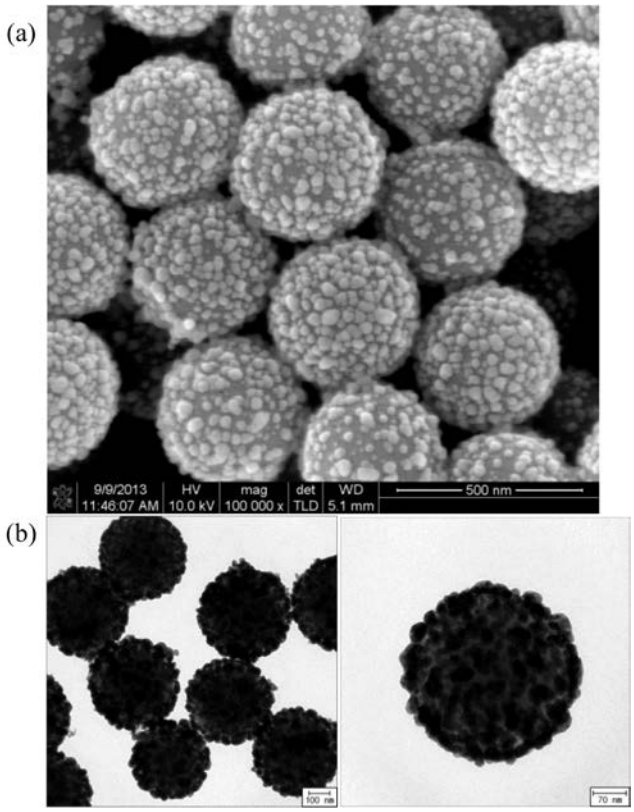

Figure 1. (a) SEM and (b) TEM images of AgNPs@SiO_2.

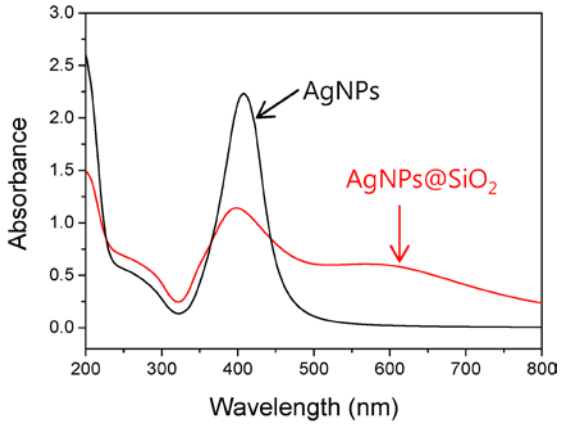

Figure 2. UV-Vis spectra of AgNP and AgNPs@SiO_2 solution.

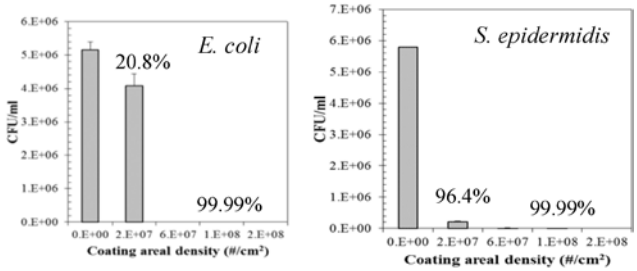

Figure 3. Antibacterial efficacy of AgNPs@SiO_2-coated air filter samples.

Figure 3 shows a significant antibacterial efficacy of coated air filter samples. Compared to the pristine air filter, the air filter sample coated with a coating areal density of 1 × 10^8 # cm^{-2} shows antibacterial efficacy of 99.99% for both *E. coli* and *S. Epidermidis*. The air filter sample coated with a coating areal density of 2 × 10^7 # cm^{-2} shows antibacterial efficacy of 20.8% for *E. coli* and 96.4% for *S. Epidermidis*.

It would be interesting to see how the bacteria were trapped and killed on the coated air filter medium. Figure 4 shows the SEM images of captured bacteria on the coated air filter fiber. The protruding AgNPs@SiO_2 particles on the smooth fiber seem to trap both types of bacteria firmly so that the trapped bacteria cannot escape.

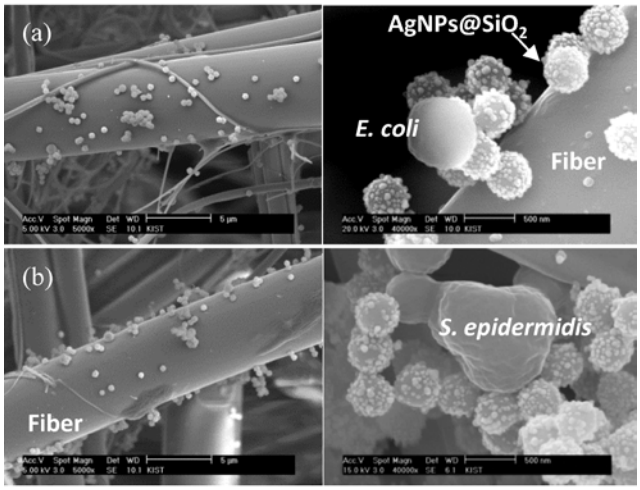

Figure 4. SEM images of (a) *E. coli* and (b) *S. Epidermidis* trapped on the coated air filter fiber.

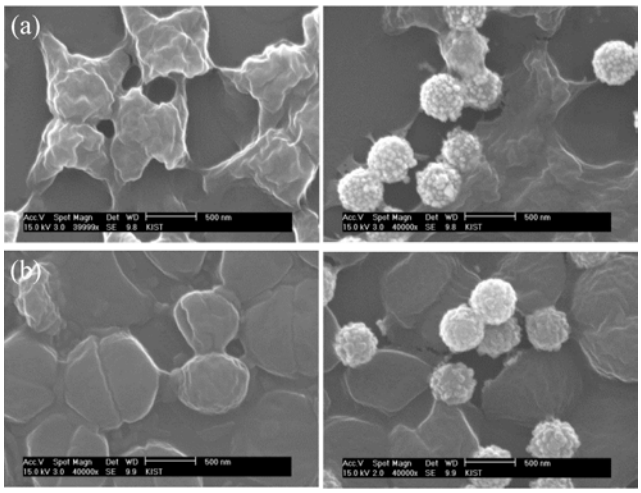

Figure 5. SEM images of (a) *E. coli* and (b) *S. Epidermidis* treated with AgNPs@SiO_2 solution for 10 min.

The fate of trapped bacteria was further investigated indrectly by culturing the bacteria with AgNPs@SiO_2 aqueous solution. The left images show the untreated bacteria. Only after 10 min, the bacterial membrane of E. coli directly contacted with AgNPs@SiO_2 particles were

completely ruptured. For the case of *S. Epidermidis*, the AgNPs@SiO_2 particles were firmly attached to the bacterial membrane or the membrane was torn down from the AgNPs@SiO_2 particles by the sputtering force for SEM sample preparation. It implicates that the trapped bacteria on the coated air filter cannot escape without rupturing their cellular membrane leading to death. The protluding AgNPs on the smooth silica sphere play a role as multiple pins that the contacted membrane can be ruptured. It has been suggested that AgNPs chemically adsorb Mg^{2+} and Ca^{2+} ions, which tightly hold the orgnanic molecules constituting cellular membrane of bacteria, and rupture the celluar membrane [9].

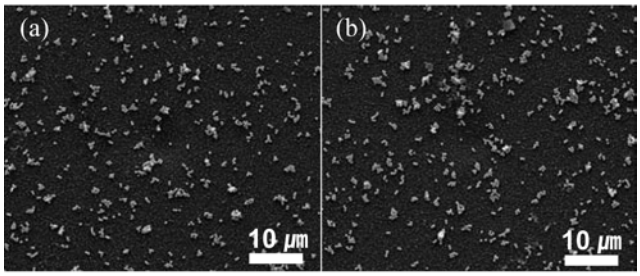

Figure 6. SEM image of AgNPs@SiO_2-immobilized TFC membrane (a) before and (b) after performance.

AgNPs@SiO_2-immobilized TFC membrane exhibited NaCl rejection of 98.8 ± 0.2% along with water flux of 29 ± 2 L m^{-2} h^{-1}. This performance is comparable to those of the pristine TFC membrane (99.0 ± 0.1%, 30 ± 2 L m^{-2} h^{-1}) and quite optimistic compared with the case of AgNP depositon, which reported a significant decline in NaCl rejection [10]. The tirvial loss in water flux can be attributable to the nice distribution and low surface coverage (11%) of the AgNPs@SiO_2 particles as shown in Figure 6(a). Most particles remained adhered to the membrane surface in Figure 6(b) after the performance test. The adhered structure seemed to be stable up to extened operaton time of 150 h, probably due to the multiple Ag–S bonds between particle and membrane.

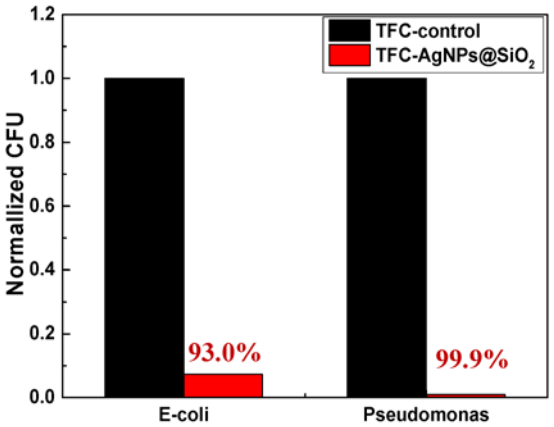

Figure 7. Antibacterial efficacy of AgNPs@SiO_2-immobilized TFC membrane.

AgNPs@SiO_2-immobilized TFC membrane exhibited a significant antibacterial efficacy of 93.0% and 99.9% for *E. coli* and *P. aeruginosa*, respectively, compared to the pristine TFC (Figure 7). This significant antbacterial efficacy is attributable to not only the released Ag^+ ions but also the closely distributed AgNPs on the silica submicrosphere, which rupture the bacterial cellular membrane upon contact.

4 CONCLUSION

Silver nanoparticle-coated silica (AgNPs@SiO_2) hybrid particles were synthesized by seed-mediated growth method and coated on the air and water filtration materials. The AgNPs@SiO_2-coated air filter and AgNPs@SiO_2-immobilized polyamide thin film composite membrane exhibit excellent antibacterial activities toward gram-negative bacteria, *Escherichia coli*, and gram-positive bacteria, *Staphylococcus epidermidis* and *Pseudomonas aeruginosa*.

Acknowledgment. This work was supported by the Future-based Technology Development Program (Green Nano Technology Development Program) through the National Research Foundation of Korea funded by the Ministry of Science, ICT & Future Planning (Grant No. 2014-060222) and the KIST institutional program (Project No. 2E26140).

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