

Polymer thin film with *in situ* synthesized silver nanoparticles as a potent reusable bactericide

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Silver nanoparticles are well-established antibacterial agents. However, an effective design and formulation that ensures: (i) ease of synthesis and fabrication, (ii) amenability to deployment over large surfaces of variable shape, (iii) high efficacy and (iv) multiple reuses with the possibility of periodic monitoring, is yet to emerge. A nanocomposite thin film of poly(vinyl alcohol) with silver nanoparticles generated within, through a soft-chemical *in situ* synthesis, is shown to be a good candidate to fulfil most of the above requirements. Efficient antibacterial activity, multiple reuses and facile monitoring of the film through spectroscopy and microscopy are demonstrated. Preliminary studies demonstrate the effective bactericidal action of the thin film coating on stirring rods.

Keywords: Bactericide, *Escherichia coli*, polymer nanocomposite, silver nanoparticle, thin film.

Introduction

SERIOUS concerns have been raised about the efficacy of synthetic antibiotics as the action of many are limited to specific targets and several bacteria have developed antibiotic resistance¹. The bactericidal effect of noble-metal nanoparticles has attracted considerable attention in this context. Significant advantages of metal nanoparticles include the ease of fabrication and incorporation in different matrices, tunability of characteristics, possibility of functionalization and targeted delivery². Bactericidal efficiency of gold and copper nanoparticles has been demonstrated^{3–7}. Nano silver is perhaps the most extensively studied because of the low cost, ease of preparation of stable formulations, and activity against a wide spectrum of prokaryotes while being relatively harmless to eukaryotes; it may be noted though that its cytotoxicity and genotoxicity in human cells have been discussed⁸. Antibacterial efficacy of silver and its salts has been known for a long time^{9,10}. In recent years, there has been extensive research on the use of nanometric silver particles as antibacterial agents¹¹. Silver nanoparticles have been deployed in a wide variety of matrices and formula-

tions, for example, as composites^{12–16}, colloids^{17–22}, fibres^{23–25}, gels^{26,27}, coatings^{1,28–30}, membranes³¹ and thin films^{32–37}. Both extracellular and intracellular mechanisms have been proposed to explain the antibacterial action of silver nanoparticles^{38–42}.

Efficient design of a bactericidal agent based on silver nanoparticles should ensure low cost of fabrication and high efficacy. An agent which is easy to deploy and amenable to efficient multiple reuse will be highly valuable in this regard; the feasibility of monitoring the agent through the multiple use cycles is advantageous from the point of view of design and optimization. Possibility of fabricating the bactericidal coating over large-area surfaces of varying shape would be an added benefit. Review of the extensive literature in the area of silver nanoparticle-based bactericides indicates that these fundamentally important aspects are rarely met in a single design. Polymer–metal nanocomposite thin films are of special interest in this context, as they facilitate efficient immobilization of the nanoparticles in the polymer matrix, and are amenable to coating on surfaces of varying shapes and sizes, extensive reuse and monitoring through successive action cycles. *In situ* generation of metal nanoparticles within polymer films is a particularly efficient approach to such nanocomposite thin films⁴³. The simple *in situ* technique which we have developed earlier⁴⁴ for the fabrication of silver nanoparticle-embedded poly(vinyl alcohol) (Ag-PVA) thin film involves an environmentally benign fabrication protocol and provides free-standing films. The highlights of the method include the use of aqueous medium for the fabrication process, deployment of the bio-compatible and bio-degradable polymer itself as the reducing agent, and soft-chemical synthesis involving mild thermal annealing used to generate the metal nanoparticles *in situ* inside the polymer matrix which serves as the stabilizer as well. Utility of these nanocomposite thin films in a wide range of areas, including optical limiting^{44,45}, microwave absorption⁴⁶, random lasers⁴⁷, e-beam lithography⁴⁸, non-volatile memory devices⁴⁹, catalysis⁵⁰ and sensing⁵¹ has been demonstrated; significance of multiple reuse has been especially demonstrated in the catalytic application.

Among the several avenues where bactericides find utility, purification of drinking water is one of the most

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critical and important. The antibacterial agent used should be non-toxic to humans at the concentrations being used, effective against a wide spectrum of pathogens, and cheap and easy to fabricate. One of the efficient and convenient ways to deploy the agent in a domestic setting would be as a coating on stirring devices. The methodology we have developed for the fabrication of silver nanoparticle-embedded polymer thin film meets most of these demands. Experiments on *Escherichia coli* establish that Ag-PVA film can be used efficiently and repeatedly, allowing monitoring of the film by spectroscopy and microscopy between uses. The minimum inhibitory and minimum bactericidal concentrations (MIC and MBC) in terms of the weight of silver nanoparticles in the PVA film used to treat unit volume of the *E. coli* bacterial medium are found to be 4.5 and 6.5 $\mu\text{g}/\text{ml}$ respectively. These values are appreciably lower than most of the values reported earlier^{27,39,52–58}. It may also be noted that the bacterial concentrations used in many of these reports are lower than those used in the present study.

Experimental

Fabrication of Ag-PVA film

The Ag-PVA film was fabricated following the general procedure developed earlier^{43,44}. Aqueous solutions of silver nitrate (22 mg in 0.5 ml) and PVA (70 mg in 1 ml) were mixed to yield an Ag/PVA weight ratio of ~ 0.20 . Millipore MilliQ purified water was used in all operations. The AgNO_3 -PVA solution was either spin-coated on quartz plates (at 500 rpm for 10 s followed by 1000 rpm for 10 s, using a Laurell Technologies Corporation model WS-400B-6NPP/LITE/8K photoresist spinner), or dip-coated on glass rods. The coated film was subjected to thermal annealing at 130°C for 1 h, whereupon the nanoparticles were generated within the film. The Ag-PVA film coated on quartz plate was characterized by its surface plasmon resonance (SPR) spectrum (recorded on a Varian model Cary 100 UV-Visible spectrometer), and the film thickness was measured using a profilometer (Ambios Technology XP-1). The film morphology was examined using an NT-MDT model Solver Pro M atomic force microscope (AFM) in non-contact mode using a cantilever with force constant of 12 N/m. The exact silver content in the film was estimated using inductively coupled plasma-optical emission spectroscopy (ICP-OES); a Varian model Liberty Series ICP-OES was used. Film samples for transmission electron microscope (TEM) imaging were prepared as follows. The substrate was prepared by first spin-coating a few drops of a solution of polystyrene (PS, average molecular weight (M_w) = 280 kDa) in toluene (1 g in 8 ml) on a glass plate at 1000 rpm for 10 s, followed by drying in a hot-air oven at 85–90°C for 20 min. The AgNO_3 -PVA solution was

spin-coated on top of the PS layer (at 8000 rpm for 10 s to obtain a very thin film) and subsequently heated. The film was then cut and peeled off the glass and placed on a 200 mesh TEM copper grid and dipped in toluene, whereupon the PS alone dissolved out. Imaging was carried out using a Tecnai G2 FEI F12 TEM at an accelerating voltage of 200 kV.

As the antibacterial studies are conducted in aqueous medium, choice of PVA in terms of its M_w and the extent of hydrolysis (% hydrolysis) is critical, since these parameters influence its solubility and swelling in water. PVAs with $\geq 98\%$ hydrolysis were chosen because of their limited solubility and appreciable swelling in water. Because of the low solubility at room temperature, the aqueous solutions of PVA for fabricating the film had to be prepared in water at 60–70°C, and then cooled to ambient temperature to yield a viscous but homogeneous solution; this was mixed with the AgNO_3 solution. It may be noted that the thermal treatment of the AgNO_3 -PVA film makes the final Ag-PVA film completely insoluble in water. We have carried out preliminary experiments with Ag-PVA thin films prepared using three types of commercially available (Aldrich) PVA with M_w and percentage hydrolysis values, 16 kDa and 98, 31–50 kDa and 98–99, and 85–146 kDa and 99+ respectively. Based on the strongest and quickest bactericidal effects observed, the last polymer (85–146 kDa, 99+) was chosen for all the experiments discussed below.

Antibacterial action of the Ag-PVA film

Antibacterial action of Ag-PVA on *E. coli* (NCIM No. 2931, ATCC No. 25922) was examined; control experiments were conducted in all cases. Test tubes and Milli Q water used in the experiment were autoclaved and the tubes were opened under sterilized conditions in a laminar flow system. The bacterial culture was prepared by adding 2 μl glycerol stock of the *E. coli* to 2 ml of Luria-Bertani (LB) broth and incubating at 37°C in a shaker operated at 120 rpm for 12 h. LB broth and ultra pure water (inoculated with known concentrations of *E. coli*) as well as ordinary tap water were studied. The pH of the various media used were in the range 6.8–7.4. Experiments on antibacterial action of Ag-PVA involved shaking the test sample with the film coated on a 6 cm^2 quartz plate at a specified temperature for a specified time period. MIC is the lowest concentration of an antibacterial agent required to inhibit the visible growth of a microorganism after overnight incubation, and MBC is the lowest concentration of the agent that will prevent the growth of the organism after subculture on a bactericide-free medium⁵⁹. Ag-PVA films of different areas coated on a 6 cm^2 quartz plate were used in the studies for determining the MIC and MBC values. In the experiments on ordinary tap water, the test sample at ambient temperature was stirred

using a glass rod coated with Ag-PVA for a specified time period. All test samples were analysed for the bacterial content or growth by either monitoring the optical density at 600 nm or plating on an LB agar plate, incubating under specified conditions and counting the bacterial colonies produced. Details of the experiments, including the conditions employed in each case are provided at the appropriate points in the following section.

Results and discussion

Silver nanoparticles in the Ag-PVA thin film which act as the bactericidal agent are formed through a simple *in situ* soft-chemical synthesis, wherein the precursor silver ions from AgNO_3 are reduced by the hydroxy groups of the PVA macromolecule (Scheme 1). The process involved is an example of chemistry carried out inside a solid polymer matrix, rather than in solution in a beaker. Due to the low concentration of silver ions and limited diffusion of ions and atoms inside the polymer film, the silver atoms formed by reduction aggregate to form silver nanoparticles. The inherent antibacterial efficacy of silver augmented by the large surface-to-volume ratio of the nanoparticles leads to the potent bactericidal action of the Ag-PVA nanocomposite thin film.

Characterization of the Ag-PVA film

Basic characterization of the spin-coated Ag-PVA film is shown in Figure 1. The electronic spectrum shows the well-defined SPR extinction peak due to the silver nanoparticles at 418 nm. The film has an average thickness of 0.75 μm . The AFM image shows the homogeneous, smooth structure of the film surface; the average roughness is found to be 1.2 nm. ICP-OES analysis shows the silver content in a 6 cm^2 film to be 108 μg , in good agreement with the average value of 112 μg expected based on the Ag/PVA weight ratio in the film. The TEM image reveals the silver nanoparticles present in the film with sizes in the range 7–9 nm, distributed uniformly throughout the film. The Ag/PVA weight ratio of 0.2 implies that only about 4% of the alcohol groups in the PVA film are oxidized when all the silver ions are reduced. As the chemical modification is relatively minor, the polymer characteristics of PVA are hardly affected; this is evident in the good thermal and photo stability displayed by these films.



Scheme 1.

Minimum inhibitory concentration and minimum bactericidal concentration

MIC and MBC are usually determined by examining the growth of bacteria in samples treated with different concentrations of the bactericidal agent prepared through serial dilution. Since the present bactericidal agent is a thin film, samples with different areas and hence Ag nanoparticle content serve this purpose. The 0.75 μm thick Ag-PVA films with areas in the range 1.5–6.0 cm^2 (Ag content = 27–108 μg) coated on quartz plates of equal size (6.0 cm^2) were immersed in samples of 15 ml LB broth inoculated with $\sim 10^7$ – 10^8 CFU of *E. coli*. These were shaken for 30 min at 37°C and the plate removed immediately thereafter. The bacterial growth was monitored by measuring the optical density at 600 nm at regular time intervals for up to 12 h. Control experiments were conducted with *E. coli* samples not treated with Ag-PVA film, as well as one in which a pure PVA film (area 6.0 cm^2) coated on quartz plate was introduced. The observations are presented in Figure 2. Normal bacterial growth is observed in both the control experiments. Ag-PVA film with the smallest area of 1.5 cm^2 shows partial inhibition of bacterial growth, proving clearly the effect of the silver nanoparticles present. Ag-PVA film with area $\geq 3.8 \text{ cm}^2$ is found to suppress the bacterial growth effectively for up to 12 h or more. This corresponds to the MIC value of this bactericidal agent; in terms of the silver concentration, the MIC works out to be 4.5 $\mu\text{g}/\text{ml}$.

In order to examine the MBC, 1 ml each of the samples treated with Ag-PVA film of area 3.0 cm^2 or higher in the previous experiment, was diluted a million times through serial dilution, and 100 μl of the final diluted solution was spread on LB agar plates and incubated for 12 h at 37°C. Examination of the plates under a microscope showed that there was no growth of bacteria in the case of samples treated with Ag-PVA films $\geq 5.3 \text{ cm}^2$ in area. This value thus signifies the MBC; the corresponding silver concentration is 6.5 $\mu\text{g}/\text{ml}$. We have examined the reusability of the Ag-PVA film by repeating the experiment with a 6.0 cm^2 film. Observations made up to 8 h shown in Figure 3, indicate that the film can be used twice but the inhibition does not last for 8 h in the third use. Spectroscopy and microscopy of the used film indicate signs of silver nanoparticle aggregation and morphology changes in the film. It appears that the nutrient medium present in this experiment and the interaction with the bacteria affect adversely, the thin film as well as the silver nanoparticles, precluding multiple reuses. This situation may be contrasted with that observed with pure water samples in the next experiment.

Bacteria introduced in ultra pure water

In the previous experiment, we have used very large concentrations of bacteria, which are typically much higher

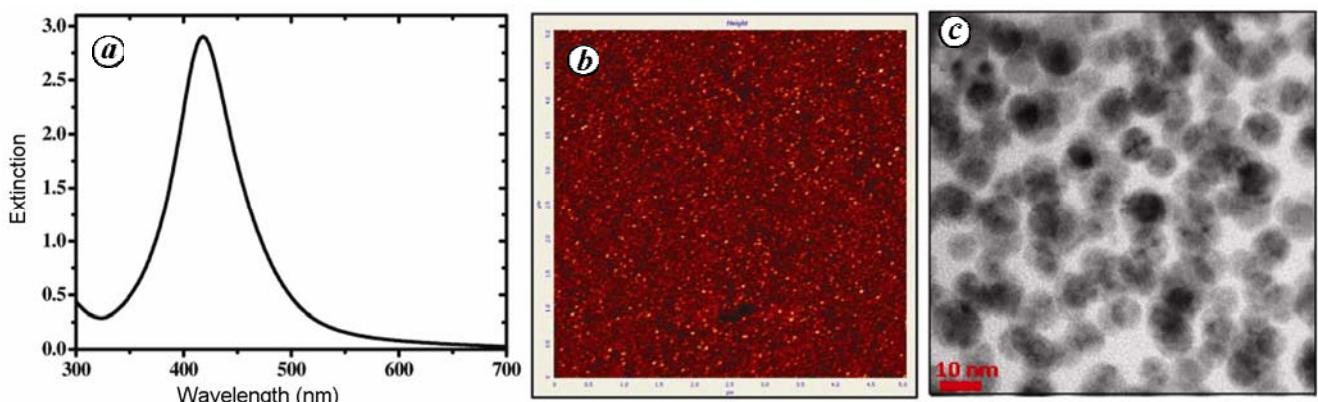


Figure 1. *a*, Surface plasmon resonance (SPR) extinction spectrum, *b*, Atomic force microscope (AFM) topography image ($5 \mu\text{m} \times 5 \mu\text{m}$) and *c*, Transmission electron microscope (TEM) image (scale bar = 10 nm) of the silver nanoparticle-embedded poly(vinyl alcohol) (Ag-PVA) film.

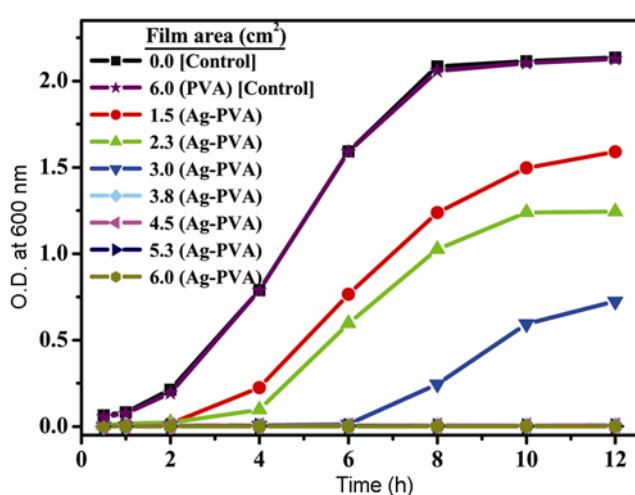


Figure 2. Bacterial growth reflected in the increase in optical density (at 600 nm) of the *Escherichia coli* suspension (in Luria-Bertani (LB) broth) with time; controls with no film and pure PVA film, and samples treated with Ag-PVA film having different areas and hence silver content are shown.

than that encountered in potable water. In spite of this, we have observed the effective bactericidal action of Ag-PVA films. We have considered slightly lower concentration of bacteria introduced in ultra pure water with no added nutrients; the time of treatment with Ag-PVA was also reduced. Approximately 10^5 CFU of *E. coli* was inoculated in 15 ml of water (Millipore Milli Q). Ag-PVA film coated on a 6 cm^2 quartz plate was immersed in this and shaken at 37°C for 15 min. After removing the film and washing it, the test was repeated using the same film in a new sample of bacteria-containing water; this was repeated altogether 19 times. Following each treatment, 200 μl of the treated water sample was spread on an LB agar plate and incubated for 12 h at 37°C . The bacterial colonies formed were observed under a microscope. Whereas the control sample of water showed ~ 2500 CFU/ml, not a single colony was detected in the experimental samples from up to the 20th use of the Ag-PVA film

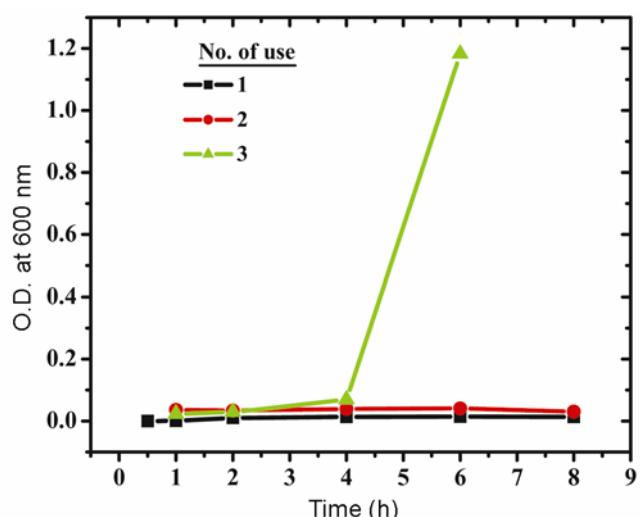


Figure 3. Bacterial growth reflected in the increase in optical density (at 600 nm) of the *E. coli* suspension (in LB broth) with time; samples treated with Ag-PVA film (area = 6.0 cm^2) in multiple uses 1–3 are shown.

(Figure 4). Electronic spectrum of the film was monitored through the repeated experiments; selected spectra of the films up to 20 uses are shown in Figure 5. Even though there is a gradual decline in the SPR extinction, the decrease even after 20 uses is only $\sim 13\%$; there is hardly any sign of particle aggregation. AFM images of the film surface show that the film remains intact through the multiple uses (Figure 6). These observations show that the Ag-PVA film is affected little under the conditions of this experiment through multiple cycles. Even with the fairly high bacterial concentrations, Ag-PVA film is effective as a reusable antibacterial agent for water purification. Based on the silver content in the initial Ag-PVA film and the extent of loss through the multiple uses as seen from the SPR spectra, the total amount of silver leached out into 300 ml (20 batches of 15 ml each) water can be estimated to be $\sim 14 \mu\text{g}$. Hence the maximum silver concentration in the treated water is $\sim 0.05 \mu\text{g}/\text{ml}$,

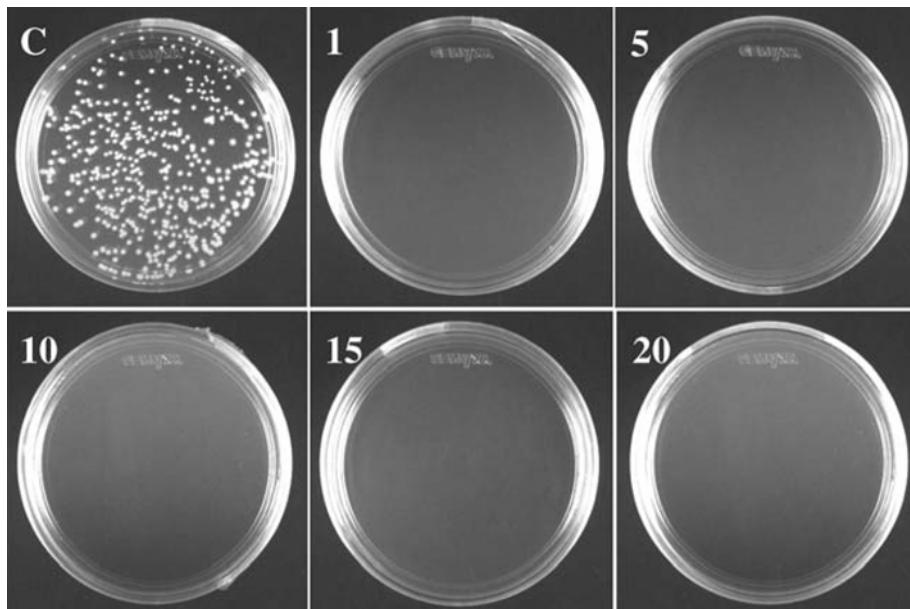


Figure 4. Photographs of petri plates (after 12 h incubation) spread with ultra pure water samples inoculated with 10^5 CFU *E. coli*. Control (C) and samples treated for 15 min with the same Ag-PVA film in multiple uses are shown; the number of uses is indicated.

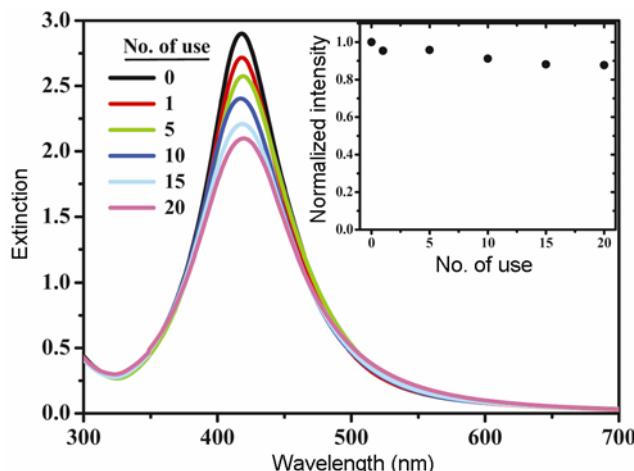


Figure 5. SPR extinction spectra of the same Ag-PVA film before and after multiple uses for 15 min each in ultra pure water inoculated with 10^5 CFU of *E. coli*. (Inset) Variation of the normalized integrated intensity of the SPR peak.

which is well below the upper limit of 100 ppb (0.1 μ g/ml) allowed in drinking water⁵⁸. The Ag-PVA film is particularly interesting for bactericidal application in view of the low extent of leaching and the low MIC/MBC values realized concomitantly.

Bacteria in ordinary tap water

Ordinary tap water (possibly containing a range of non-pathogenic and pathogenic bacteria) was treated by stirring with Ag-PVA-coated glass rods for 5 min at 27–

30°C. With 15 ml fresh samples of water, the process was repeated 21 times using the same Ag-PVA-coated rod. As in the previous experiment, the water was analysed for bacterial growth upon incubation for 12 h at 37°C. Whereas the control showed a bacterial count of ~ 250 CFU/ml, the 5-min-treated ones showed considerable reduction; even the sample subjected to the 20th reuse of the same rod showed ≤ 30 CFU/ml (Figure 7a). Similar reduction of bacterial concentration was observed in the case of a single treatment of ~ 200 –250 ml water (Figure 7b). Being a hydrogel, PVA is likely to swell in water, facilitating contact between the bacteria and the nanoparticles. The efficient multiple reuses indicate that there is little dissolution of the film coating or leaching of silver occurring while stirring with the glass rod. This experiment shows the significant application potential of Ag-PVA film as a coating on devices that can be used to stir water in order to kill the bacteria in it.

The different experiments described above demonstrate the antibacterial capability of the Ag-PVA film. The detailed mechanism behind the bactericidal action is not clear at the moment. Silver nanoparticles as well as Ag^+ ions are known to be effective bactericides^{40–42}. Antibacterial activity of silver nanoparticles has been attributed to their deleterious impact on the cell membrane^{39,40}. The bactericidal effect of Ag-PVA could result from the direct contact of bacteria with the nanoparticles or the interaction of bacteria with the silver ions released from the nanoparticles. The latter possibility is supported by the observation of antibacterial effects of a PVA film containing AgNO_3 , the precursor for the silver nanoparticle. However, it should be noted that unlike the Ag-PVA film,

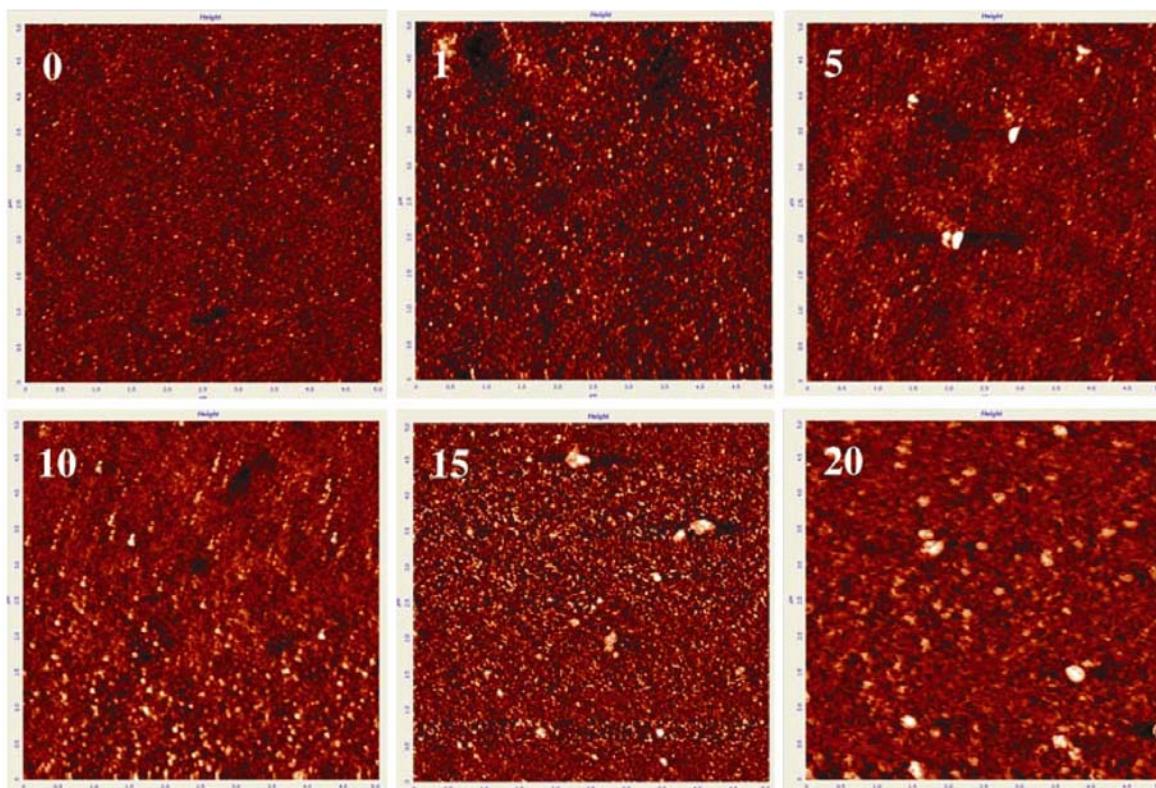


Figure 6. AFM images of the same Ag-PVA film before and after multiple uses for 15 min each in ultra pure water inoculated with 10^5 CFU of *E. coli*; the number of uses is indicated.

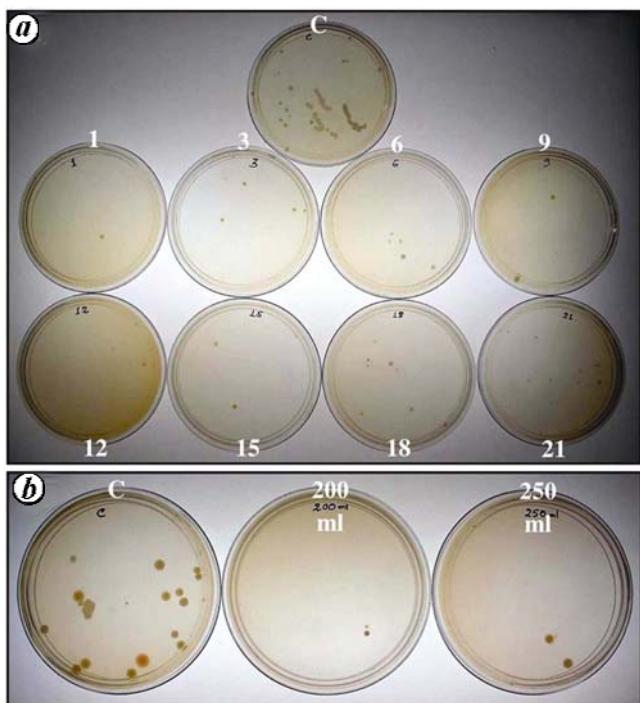


Figure 7. Photographs of petri plates (after 12 h incubation) spread with ordinary tap water: (a) 15 ml each of control sample (C) and samples treated for 5 min with the same Ag-PVA film-coated glass rod in multiple uses up to 21 times; the number of uses is indicated. (b) Control sample (C), and 200 and 250 ml samples treated for 5 min each with Ag-PVA film coated on glass rod.

the AgNO₃-PVA film is easily damaged upon a single bactericidal application and hence not amenable to repeated usage. Spectroscopy and microscopy experiments suggest that the Ag-PVA films are affected adversely when inserted in the LB broth with large bacterial content (10^7 – 10^8 CFU). In the case of water samples with relatively lower amounts of bacteria (10^5 CFU) the films are intact; the small decrease in the SPR spectral intensities suggests mild leaching of the nanoparticles. These observations imply that the nanoparticles within the film and their controlled leaching could both be involved in the bactericidal activity of the Ag-PVA film. Contact of the bacteria with the silver nanoparticles inside the Ag-PVA film and controlled leaching of the nanoparticles or silver ions could be facilitated by the hydrogel character of PVA and its swelling in the aqueous medium⁴⁵. Further experiments and optimization of the PVA film matrix are needed to improve the efficacy of the thin film bactericidal agent and to draw deeper insights into the mechanism of action involved.

Several unique features of the *in situ* synthesized Ag-PVA films make them superior bactericidal agents of interest in various applications, including water purification. As the nanoparticles are generated inside the film rather than physically incorporated, problems of inhomogeneous distribution and leaching are minimal. The polymeric nature and the specific fabrication method make the

thin film bactericidal agent highly adaptable for applications as coatings on a variety of surfaces. The antibacterial effect can be realized by simple stirring or even static contact, without employing forced flow or filtration. Convenience of carrying out spectroscopic and microscopic studies of the thin film through its usage cycles is particularly relevant for detailed investigations of the basis of the bactericidal efficacy.

Conclusion

The Ag-PVA film fabricated through a simple and facile *in situ* generation protocol, is shown to be an efficient bactericidal agent. Specifically its application in water purification is demonstrated. The major advantages are the extensive reusability of the film and the feasibility of monitoring the agent between multiple uses. Amenability of the material to fabrication as a coating on different surfaces is likely to enhance its application potential. The low cost and the feasibility of synthesizing the nanocomposite thin film even in a domestic environment makes it attractive, especially for applications in a rural scenario.

1. Vasilev, K. *et al.*, Tunable antibacterial coatings that support mammalian cell growth. *Nano Lett.*, 2010, **10**, 202–207.
2. Lee, D., Cohen, R. E. and Rubner, M. F., Antibacterial properties of Ag nanoparticle loaded multilayers and formation of magnetically directed antibacterial microparticles. *Langmuir*, 2005, **21**, 9651–9659.
3. Cioffi, N. *et al.*, Copper nanoparticle/polymer composites with antifungal and bacteriostatic properties. *Chem. Mater.*, 2005, **17**, 5255–5262.
4. Hsiao, M.-T., Chen, S.-F., Shieh, D.-B. and Yeh, C.-S., One-pot synthesis of hollow Au_3Cu_1 spherical-like and biomimetic botallackite $\text{Cu}_2(\text{OH})_3\text{Cl}$ flowerlike architectures exhibiting antimicrobial activity. *J. Phys. Chem. B*, 2006, **110**, 205–210.
5. Kim, Y. H., Lee, D. K., Cha, H. G., Kim, C. W., Kang, Y. C. and Kang, Y. S., Preparation and characterization of the antibacterial Cu nanoparticle formed on the surface of SiO_2 nanoparticles. *J. Phys. Chem. B*, 2006, **110**, 24923–24928.
6. Nath, S., Kaittanis, C., Tinkham, A. and Manuel Perez, J., Dextrans-coated gold nanoparticles for the assessment of antimicrobial susceptibility. *Anal. Chem.*, 2008, **80**, 1033–1038.
7. Zhao, Y., Tian, Y., Cui, Y., Liu, W., Ma, W. and Jiang, X., Small molecule-capped gold nanoparticles as potent antibacterial agents that target gram-negative bacteria. *J. Am. Chem. Soc.*, 2010, **132**, 12349–12356.
8. AshaRani, P. V., Mun, G. L. K., Hande, M. P. and Valiyaveettill, S., Cytotoxicity and genotoxicity of silver nanoparticles in human cells. *ACS Nano*, 2009, **3**, 279–290.
9. Friedenthal, H., Absolute and relative disinfection power of elements and chemical compounds. *Biochem. Z.*, 1919, **94**, 47–68.
10. Feng, Q. L., Wu, J., Chen, G. Q., Cui, F. Z., Kim, T. N. and Kim, J. O., A mechanistic study of the antibacterial effect of silver ions on *Escherichia coli* and *Staphylococcus aureus*. *J. Biomed. Mater. Res.*, 2000, **52**, 662–668.
11. Nowack, B., Krug, H. F. and Height, M., 120 Years of nanosilver history: implications for policy makers. *Environ. Sci. Technol.*, 2011, **45**, 1177–1183.
12. Kim, Y. H., Lee, D. K., Cha, H. G., Kim, C. W. and Kang, Y. S., Synthesis and characterization of antibacterial Ag– SiO_2 nanocomposite. *J. Phys. Chem. C*, 2007, **111**, 3629–3635.
13. Zhang, H. and Chen, G., Potent antibacterial activities of Ag/TiO₂ nanocomposite powders synthesized by a one-pot sol gel method. *Environ. Sci. Technol.*, 2009, **43**, 2905–2910.
14. Yuan, W. *et al.*, Deposition of silver nanoparticles on multiwalled carbon nanotubes grafted with hyperbranched poly(amoidoamine) and their antimicrobial effects. *J. Phys. Chem. C*, 2008, **112**, 18754–18759.
15. Niu, A., Han, Y., Wu, J., Yu, N. and Xu, Q., Synthesis of one-dimensional carbon nanomaterials wrapped by silver nanoparticles and their antibacterial behavior. *J. Phys. Chem. C*, 2010, **114**, 12728–12735.
16. Vijayakumar, P. S. and Prasad, B. L. V., Intracellular biogenic silver nanoparticles for the generation of carbon supported anti-viral and sustained bactericidal agents. *Langmuir*, 2009, **25**, 11741–11747.
17. Fabrega, J., Fawcett, S. R., Renshaw, J. C. and Lead, J. R., Silver nanoparticle impact on bacterial growth: effect of pH, concentration, and organic matter. *Environ. Sci. Technol.*, 2009, **43**, 7285–7290.
18. Nair, A. S. *et al.*, Organic-soluble antimicrobial silver nanoparticle polymer composites in gram scale by one-pot synthesis. *ACS Appl. Mater. Interfaces*, 2009, **1**, 2413–2419.
19. Eby, D. M., Schaeublin, N. M., Farrington, K. E., Hussain, S. M. and Johnson, G. R., Lysozyme catalyzes the formation of antimicrobial silver nanoparticles. *ACS Nano*, 2009, **3**, 984–994.
20. Kvitek, L. *et al.*, Effect of surfactants and polymers on stability and antibacterial activity of silver nanoparticles (NPs). *J. Phys. Chem. C*, 2008, **112**, 5825–5834.
21. Zhang, Y., Peng, H., Huang, W., Zhou, Y., Zhang, X. and Yan, D., Hyperbranched poly(amoidoamine) as the stabilizer and reductant to prepare colloid silver nanoparticles *in situ* and their antibacterial activity. *J. Phys. Chem. C*, 2008, **112**, 2330–2336.
22. Pang, M., Hu, J. and Zeng, H. C., Synthesis, morphological control, and antibacterial properties of hollow/solid Ag₂S/Ag heterodimers. *J. Am. Chem. Soc.*, 2010, **132**, 10771–10785.
23. Kong, H. and Jang, J., Antibacterial properties of novel poly(methyl methacrylate) nanofiber containing silver nanoparticles. *Langmuir*, 2008, **24**, 2051–2056.
24. Kong, H. and Jang, J., Synthesis and antimicrobial properties of novel silver/polyrhodanine nanofibers. *Biomacromolecules*, 2008, **9**, 2677–2681.
25. Kelly, F. M. and Johnston, J. H., Colored and functional silver nanoparticle-wool fiber composites. *ACS Appl. Mater. Interfaces*, 2011, **3**, 1083–1092.
26. Travani, A. *et al.*, Non-cytotoxic silver nanoparticle-polysaccharide nanocomposites with antimicrobial activity. *Biomacromolecules*, 2009, **10**, 1429–1435.
27. Jain, J., Arora, S., Rajwade, J. M., Omray, P., Khandelwal, S. and Paknikar, K. M., Silver nanoparticles in therapeutics: development of an antimicrobial gel formulation for topical use. *Mol. Pharm.*, 2009, **6**, 1388–1401.
28. Eby, D. M., Luckarift, H. R. and Johnson, G. R., Hybrid antimicrobial enzyme and silver nanoparticle coatings for medical instruments. *ACS Appl. Mater. Interfaces*, 2009, **1**, 1553–1560.
29. Huda, S., Smoukov, S. K., Nakanishi, H., Kowalczyk, B., Bishop, K. and Grzybowski, B. A., Antibacterial nanoparticle monolayers prepared on chemically inert surfaces by cooperative electrostatic adsorption (CELA). *ACS Appl. Mater. Interfaces*, 2010, **2**, 1206–1210.
30. Gottesman, R., Shukla, S., Perkas, N., Solovyov, L. A., Nitzan, Y. and Gedanken, A., Sonochemical coating of paper by microbiodical silver nanoparticles. *Langmuir*, 2011, **27**, 720–726.
31. Yu, D.-G., Lin, W.-C. and Yang, M.-C., Surface modification of poly(L-lactic acid) membrane via layer-by-layer assembly of silver

nanoparticle-embedded polyelectrolyte multilayer. *Bioconjugate Chem.*, 2007, **18**, 1521–1529.

32. Podsiadlo, P. *et al.*, Layer-by-layer assembly of nacre-like nanostructured composites with antimicrobial properties. *Langmuir*, 2005, **21**, 11915–11921.

33. Li, Z., Lee, D., Sheng, X., Cohen, R. E. and Rubner, M. F., Two-level antibacterial coating with both release-killing and contact-killing capabilities. *Langmuir*, 2006, **22**, 9820–9823.

34. Rhim, J.-W., Hong, S.-I., Park, H.-M. and Ng, P. K. W., Preparation and characterization of chitosan-based nanocomposite films with antimicrobial activity. *J. Agric. Food Chem.*, 2006, **54**, 5814–5822.

35. Wang, Q., Yu, H., Zhong, L., Liu, J., Sun, J. and Shen, J., Incorporation of silver ions into ultrathin titanium phosphate films: *in situ* reduction to prepare silver nanoparticles and their antibacterial activity. *Chem. Mater.*, 2006, **18**, 1988–1994.

36. Kubacka, A., Cerrada, M. L., Serrano, C., Fernández-García, M., Ferrer, M. and Fernández-García, M., Plasmonic nanoparticle/polymer nanocomposites with enhanced photocatalytic antimicrobial properties. *J. Phys. Chem. C*, 2009, **113**, 9182–9190.

37. Bai, H.-J., Gou, H.-L., Xu, J.-J. and Chen, H.-Y., Molding a silver nanoparticle template on polydimethylsiloxane to efficiently capture mammalian cells. *Langmuir*, 2010, **26**, 2924–2929.

38. Kyriacou, S. V., Brownlow, W. J. and Xu, X.-H. N., Using nanoparticle optics assay for direct observation of the function of antimicrobial agents in single live bacterial cells. *Biochemistry*, 2004, **43**, 140–147.

39. Gogoi, S. K., Gopinath, P., Paul, A., Ramesh, A., Ghosh, S. S. and Chattopadhyay, A., Green fluorescent protein-expressing *Escherichia coli* as a model system for investigating the antimicrobial activities of silver nanoparticles. *Langmuir*, 2006, **22**, 9322–9328.

40. Lok, C.-N. *et al.*, Proteomic analysis of the mode of antibacterial action of silver nanoparticles. *J. Proteome Res.*, 2006, **5**, 916–924.

41. Banerjee, M., Mallick, S., Paul, A., Chattopadhyay, A. and Ghosh, S. S., Heightened reactive oxygen species generation in the antimicrobial activity of a three component iodinated chitosan–silver nanoparticle composite. *Langmuir*, 2010, **26**, 5901–5908.

42. Jin, X. *et al.*, High-throughput screening of silver nanoparticle stability and bacterial inactivation in aquatic media: influence of specific ions. *Environ. Sci. Technol.*, 2010, **44**, 7321–7328.

43. Ramesh, G. V., Porel, S. and Radhakrishnan, T. P., Polymer thin films embedded with *in situ* grown metal nanoparticles. *Chem. Soc. Rev.*, 2009, **38**, 2646–2656.

44. Porel, S., Singh, S., Harsha, S. S., Rao, D. N. and Radhakrishnan, T. P., Nanoparticle-embedded polymer: *in situ* synthesis, free-standing films with highly monodisperse silver nanoparticles and optical limiting. *Chem. Mater.*, 2005, **17**, 9–12.

45. Porel, S., Venkatram, N., Rao, D. N. and Radhakrishnan, T. P., Optical power limiting in the femtosecond regime by silver nanoparticle-embedded polymer film. *J. Appl. Phys.*, 2007, **102**, 033107-1–033107-6.

46. Ramesh, G. V., Sudheendran, K., Raju, K. C. J., Sreedhar, B. and Radhakrishnan, T. P., Microwave absorber based on silver nanoparticle-embedded polymer thin film. *J. Nanosci. Nanotechnol.*, 2009, **9**, 261–266.

47. Meng, X., Fujita, K., Zong, Y., Murai, S. and Tanaka, K., Random lasers with coherent feedback from highly transparent polymer films embedded with silver nanoparticles. *Appl. Phys. Lett.*, 2008, **92**, 201112-1–3.

48. Abargues, R., Marqués-Hueso, J., Canet-Ferrer, J., Pedrueza, E., Valdés, J. L., Jiménez, E. and Martínez-Pastor, J. P., High-resolution electron-beam patternable nanocomposite containing metal nanoparticles for plasmonics. *Nanotechnology*, 2008, **19**, 355308–355400.

49. Leong, W. L. *et al.*, Organic memory with Au nanoparticles in a self-assembled block copolymer. *Adv. Mater.*, 2008, **20**, 2325–2331.

50. Hariprasad, E. and Radhakrishnan, T. P., A highly efficient and extensively reusable ‘dip catalyst’ based on silver nanoparticle-embedded polymer thin film. *Chem. Eur. J.*, 2010, **16**, 14378–14384.

51. Ramesh, G. V. and Radhakrishnan, T. P., A universal sensor for mercury (Hg, Hg^I, Hg^{II}) based on silver nanoparticle-embedded polymer thin film. *ACS Appl. Mater. Interfaces*, 2011, **3**, 988–994.

52. Baker, C., Pradhan, A., Pakstis, L., Pochan, D. and Shah, S., Synthesis and antibacterial properties of silver nanoparticles. *J. Nanosci. Nanotechnol.*, 2005, **5**, 244–249.

53. Panacek, A. *et al.*, Silver colloid nanoparticles: synthesis, characterization and their antibacterial activity. *J. Phys. Chem. B*, 2006, **110**, 16248–16253.

54. Lok, C.-N. *et al.*, Silver nanoparticles: partial oxidation and antibacterial activities. *J. Biol. Inorg. Chem.*, 2007, **12**, 527–534.

55. Zhu, C., Xue, J. and He, J., Controlled *in-situ* synthesis of silver nanoparticles in natural cellulose fibers toward highly efficient antimicrobial materials. *J. Nanosci. Nanotechnol.*, 2009, **9**, 3067–3074.

56. Wei, D., Sun, W., Qian, W., Ye, Y. and Ma, X., The synthesis of Chitosan-based Silver nanoparticles and their antibacterial activity. *Carbohydrate Res.*, 2009, **344**, 2375–2382.

57. Li, W. R., Xie, X. B., Shi, Q. S., Zeng, H. Y., Ou-Yang, Y. S. and Chen, Y. B., Antibacterial activity and mechanism of silver nanoparticles on *Escherichia coli*. *Appl. Microbiol. Biotechnol.*, 2010, **85**, 1115–1122.

58. Dankovich, T. A. and Gray, D. G., Bactericidal paper impregnated with silver nanoparticles for point-of-use water treatment. *Environ. Sci. Technol.*, 2011, **45**, 1992–1998.

59. Andrews, J. M., Determination of minimum inhibitory concentrations. *J. Antimicrob. Chemother.*, 2001, **48**, 5–16.

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