

Research Article

Antibacterial Coating for Elimination of *Pseudomonas aeruginosa* and *Escherichia coli*

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A polymer antibacterial surface has been successfully developed. The coating system used silane as binder and Ag particles as antibacterial agent. The silver was synthesized using precipitation method. X-ray diffraction (XRD), field emission scanning electron microscopy (FESEM), Brunauer-Emmett-Teller (BET) tests, energy-dispersive X-ray spectroscopy (EDX), and X-ray photoelectron spectroscopy (XPS) were carried out to evaluate the silver particles. Antibacterial properties of the coating system were tested against gram-negative bacteria, namely, *Pseudomonas aeruginosa* and *Escherichia coli*. Different amounts of Ag were used in the coating to optimize its usage. The Japanese International Standard, JISZ2801, was used for bacteria test and the surface developed complies with the standard being antibacterial.

1. Introduction

Pseudomonas aeruginosa is present in our surroundings. It causes no harmful effects to healthy person but can cause serious illness and even fatal infections to those with weak immunity. *Pseudomonas aeruginosa* infections usually occur in hospitals because patients with weak immune systems due to some chronic illness are warded there. Apart from that, it is also a post-surgical infection. According to one study conducted in Seoul National University Hospital in 2002 [1], 78.7% of the pseudomonas cases were hospital acquired and the 30-day mortality rate was 39%. A study at Hospital of Kaunas University of Medicine [2], Lithuania, in 2010 using 5-year data showed that 58.8% of cases occurred in intensive care unit with its overall mortality rate of also 58.8%. *Escherichia coli* on the other hand is a bacteria known to cause food poisoning. Poor hygiene in the food storage and preparation area is a key to its spreading.

Application of silver as an antibacterial agent is well established and its usage can be traced to the ancient Ayurvedic

alternative medical practices. Since then, the interest in silver has never been receded. The breakthrough in nanotechnology and the outbreak of bird flu worldwide have awoken the world community to the presence of dangerous bacteria around us. This has further resulted in tremendous interest in antibacterial properties especially over the past two decades. Its antibacterial properties have also become a trademark for home appliances. One limitation in using silver is its high cost. Numerous techniques [3–8] have been developed to synthesize it and one of the simple and cheap routes is the chemical precipitation method. With this method, different sizes of particles, morphologies, and crystallite sizes can be synthesized using silver nitrate as precursor.

The sol-gel method is one of many viable techniques [9–11] for coating preparation. It allows materials to be developed at low temperatures. Its versatility in fabricating a wide range of materials with different properties found its applications in numerous fields such as corrosion and coating, development of sensor, catalysis, membranes, and optoelectronics. Developing antibacterial coating based on the sol-gel

technique enables different types of polymers to be used as binder [12, 13]. Amongst the binders, silane has been widely used due to its simplicity, durability, strength, and transparency.

Although antibacterial coatings using polymer as binder have been extensively investigated, the usage of silane as binder as well as its performance towards “hardy” bacteria is still limited. Moreover, the simplicity in applying the coating to a surface as well as retaining its transparency level is also our focus. Herein, we report our success in developing antibacterial coating for elimination and inhibition of the bacteria. XRD, BET, and FESEM were used to evaluate the crystal structure, surface area, and morphology of the particles, respectively. UV-Vis spectroscopy was used for optical characterization of the coating.

2. Methodology

2.1. Synthesis of Ag Particles. 0.2 mL of ethylene diamine was added to the 40 mL of 0.1 M silver nitrate solution and stirred for 5 minutes. 0.1 g of dispersing agent cetyltrimethylammonium bromide (CTAB) was added to the solution and stirred for another 5 minutes before 10 mL of hydrazine hydrate was added. The stir was continued for another 15 minutes. The particles were separated from the solution by centrifuging the solution at 5000 rpm for 20 minutes. The particles were washed from the reactants chemicals by repeating the centrifuging process with deionized water and lastly with ethanol. The particles gathered were dried in desiccators for 24 hours. Ball milling process with weight ratio of ball to sample being 120 : 1 was carried out for 4 hours at 300 rpm. XRD, BET, and FESEM were used to characterize the Ag particles.

2.2. Preparation of Coating. Methyltrimethoxysilane ($\text{Si-CH}_3\text{-(OCH}_3\text{)}_3$) and n-propanol were mixed (weight ratio of 1 : 1) in a beaker. Ag particles (0.5% wt) were added to the mixture and were vigorously stirred. Nitric acid was diluted (5%) to obtain the pH of 0.1 and was used as catalyst which was later added to the system by 10% wt and stirred. The sol-gel produced was then applied onto glass substrates using only a sponge and allowed to dry before transparency measurement was carried out using UV-Vis spectroscopy. FESEM and EDX were used to study the morphology and distribution of the Ag on the surface.

2.3. Antibacterial Testing. The antibacterial testing for coatings was carried out according to Japanese International Standard, JISZ2801, by an accredited laboratory SIRIM QAS Sdn. Bhd., Malaysia. Generally, there are few steps involved in the tests [14].

- (a) Sample bacteria were suspended in nutrient broth cultivate medium and were incubated. After the cultivation, the suspension was diluted with some amount of sterilized water to obtain a sample suspension whose bacteria concentration was in the range of 1.0×10^5 – 1.0×10^6 cfu/mL (colony forming unit per milliliter).

- (b) A sample suspension of 0.040 mL was dropped on the surface of a specimen, which was horizontally placed on a sterilized Petri dish, and the droplet was covered with a sterilized polyethylene (PE) film to prevent the suspension from drying up.
- (c) The specimens were subsequently placed in an incubator and bacteria were incubated for 24 hours at 37°C and relative humidity of 90%. After the incubation, the surface of the specimen and the PE film were rinsed to harvest bacteria exposed to the sample. To count the total viable bacteria, a plate counting technique using standard agar medium was adapted.

In order to obtain control data, a sample suspension of 0.040 mL was dropped on a sterilized Petri dish, and the droplet was covered with a sterilized PE film. Such a specimen is designated as control. For control specimens, the following processes, that is, incubation, harvest, and counting the total viable count, were the same as those for the sample specimens and were carried out simultaneously. The number of bacteria in our control sample was reported to increase from 3×10^5 to more than 1.0×10^8 after 24 hours of incubation.

3. Results and Discussions

Figure 1(a) shows the sharp XRD peaks of the Ag particles indicating its crystallinity. All the peaks in XRD pattern can be indexed to a face-centered cubic structure of Ag (JCPDS, file number 04-0783). Figure 1(b) reveals the relatively uniform roundish morphology. The sizes of particles are approximated to be in the range of 50 nm to 300 nm and agglomeration of the nanosized crystallite can be seen quite clearly.

Nitrogen sorption (BET) was used to determine the specific surface area of the Ag nanoparticles. Nitrogen sorption isotherms are displayed in Figure 2. BET characterization gives specific surface area of the as-prepared samples value as $2.8 \text{ m}^2/\text{g}$. Its total pore volume is $1.137 \times 10^{-2} \text{ cm}^3/\text{g}$ for pores smaller than $498.4 \times 10^{-10} \text{ m}$ in diameter. The average pore diameter is $162.5 \times 10^{-10} \text{ m}$.

It is believed that antibacterial performances can be improved by increasing the specific surface area of the materials [15]. Hence, the materials with nanostructures would provide more available surface sites for bacteria to have contact with silver.

Elemental analysis was carried out on the synthesized Ag using EDX. The presence of C in EDX test could probably come from the carbon tape used to hold the sample (Table 1). To confirm this inference, we carried out XPS test on the sample. XPS has been widely used to determine the oxidation state of the outer layer of nanoparticles [16, 17]. XPS characterization provides surface elemental analysis which in our case will eliminate the element of C should the inference is true. This is because the XPS has lesser energy penetrating depth (~8–10 nm) as compared to EDX (10 μm). From XPS, no C was detected which confirmed that the presence of C in the EDX which came from the carbon tape. On the other

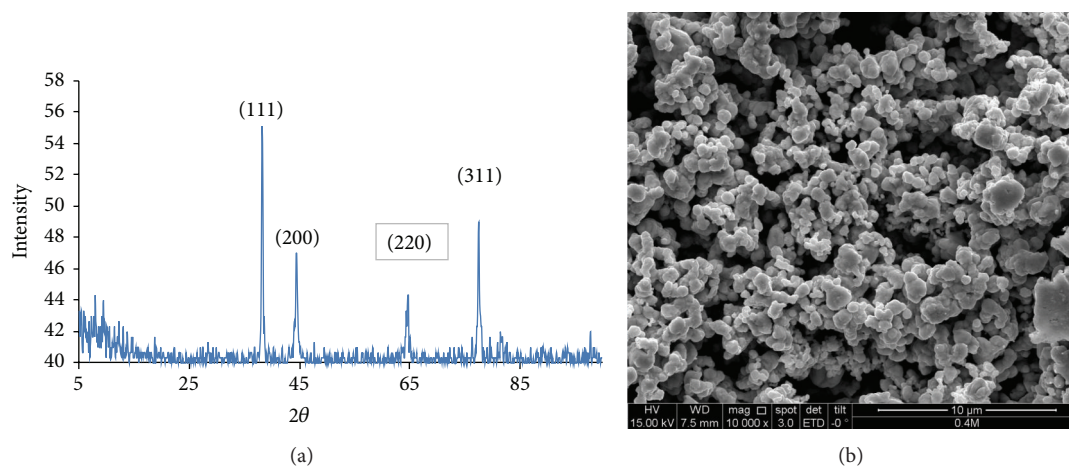


FIGURE 1: XRD and FESEM of the Ag nanoparticles.

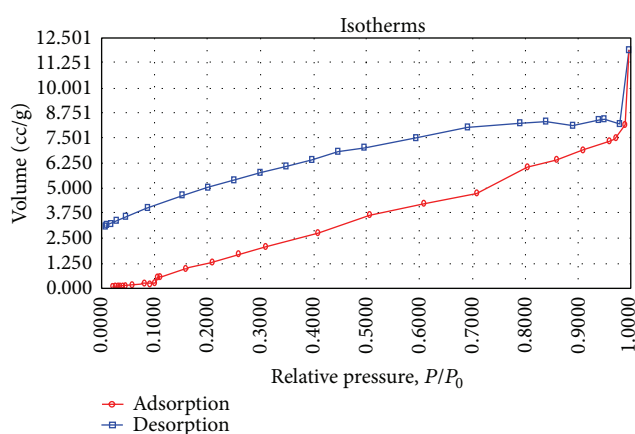
FIGURE 2: N₂ adsorption-desorption isotherms of the synthesized Ag.

TABLE 1: Ag element detected using EDX and XPS.

Elements	Detected with EDX (%)	Detected with XPS (%)
O	4.89	51.11
Ag	80.25	48.89
C	14.86	—

hand, the increase in the percentage of O shows that the outer layer of the Ag is oxidized.

Transparency of the coating was evaluated using UV-Vis spectroscopy in the visible spectrum range of 300 nm to 900 nm. Figure 3 shows that the presence of Ag particles in the coating has slightly reduced light transmission. The visual appearances of the blank glass and Ag-polymer coated glass are shown in Figure 3.

One of the requirements for coating to be antibacterial is the ability to expose the active antibacterial ingredients on the surface, which in our case are the Ag particles. Figure 4 reveals the coating when viewed using FESEM. The distribution of Ag can be seen scattered on film, which was confirmed

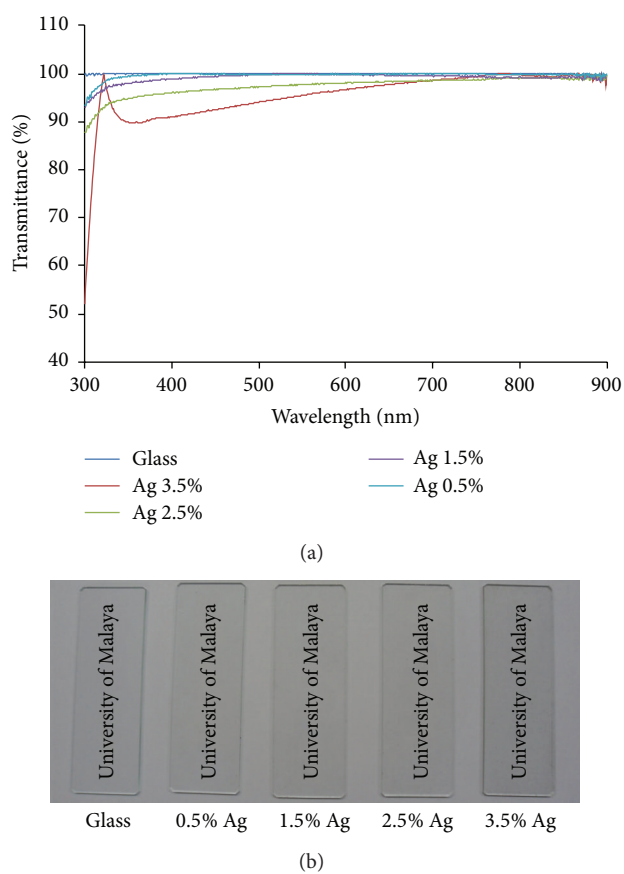
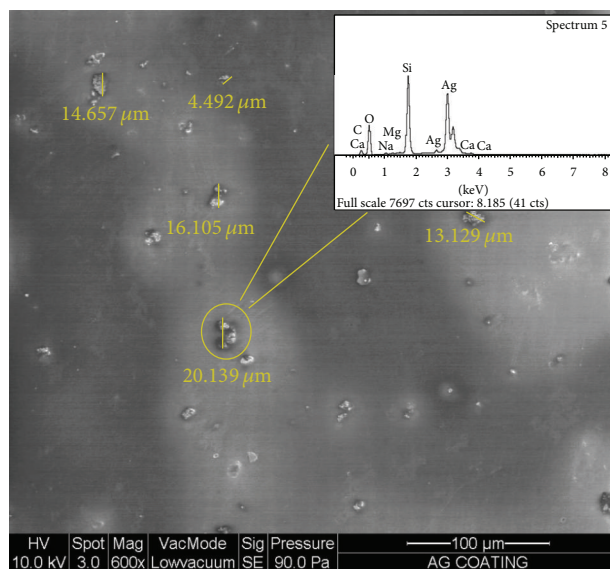


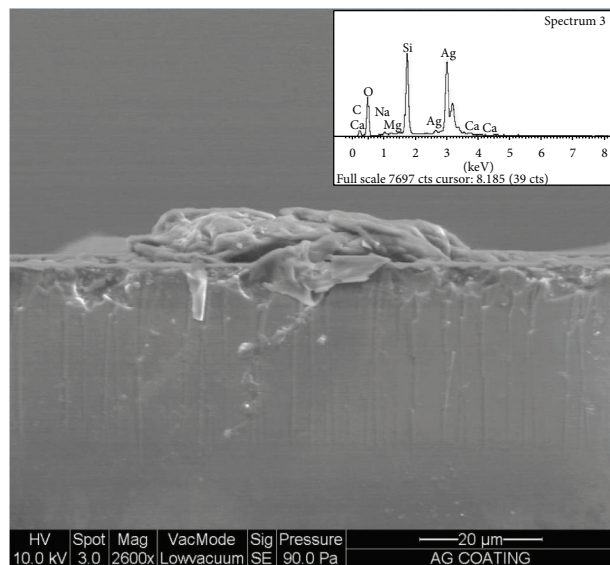
FIGURE 3: (a) Optical properties of the coatings by UV-Vis spectroscopy of the coatings and (b) optical (eye) comparison.

by EDX. The AgNPs were dispersed and exist as “clumps” and do not exist as continuous layer of AgNPs covering the whole surface. This was purposely done due to several reasons:

- covering the whole surface with AgNPs will affect the transparency level and interfere in the original properties (e.g., colour and beauty) of the surface;



(a)



(b)

FIGURE 4: FESEM images showing (a) top view of the polymer coating and (b) cross-sectional view of the coating showing Ag particles with EDX spectra.

(b) to minimize the amount of Ag in the coating system but still effective in eliminating the bacteria. Continuous layer of Ag is not necessary as it will only result in a waste and expensive coating due to high price and redundant use of Ag.

Figure 5 shows result of the antibacterial test against *Pseudomonas aeruginosa* and *Escherichia coli* bacteria. In this test, bacteria were allowed to breed on the Ag coated slides for a period of 24 hours. Number of bacteria on the Ag coated slides at zero hours and after 24th hour was counted. To be accepted as an antibacterial coating, the number of living bacteria cells after the 24th hour must be lesser than the one at

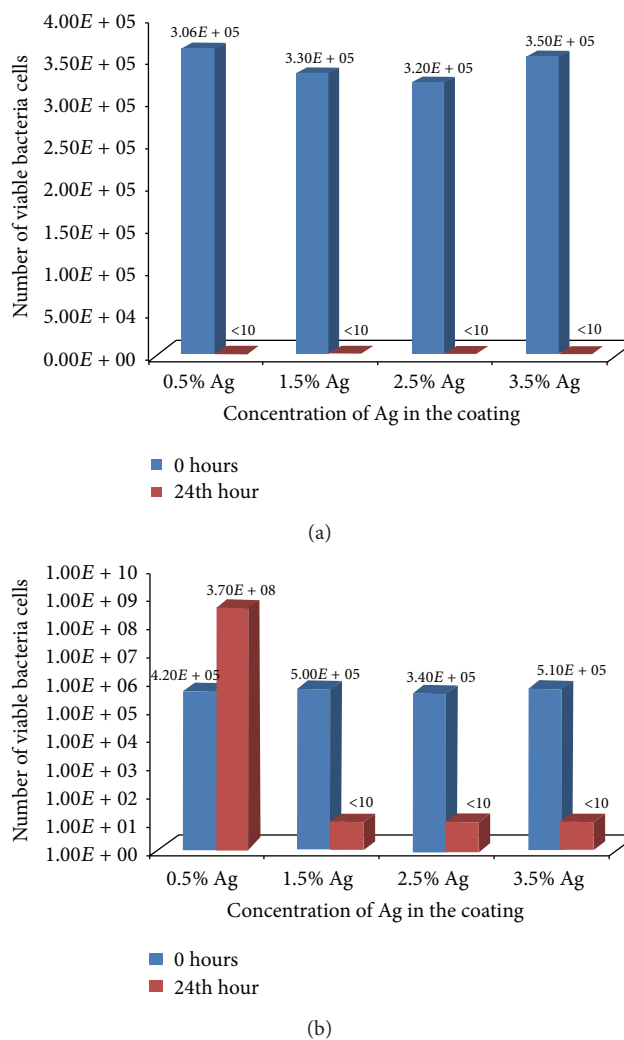


FIGURE 5: Graph depicting number of viable bacteria cells at 0 and after 24 hours. (a) *Pseudomonas aeruginosa* and (b) *Escherichia coli*.

zero hours. This would indicate the reduction or elimination of the bacteria by the coated slides.

All the Ag coated glass samples resulted in approximately total elimination (<10) of the *Pseudomonas aeruginosa* bacteria after the 24th hour. For *Escherichia coli* tests, except for the coating with 0.5% Ag, the other samples proved to inhibit the bacteria almost in total as well as (<10) after the 24th hour. To establish the fact that the antibacterial properties come solely from the presence of Ag in the coating systems, tests for blank and polymer-coated glass were carried out. For both cases, the amount of bacteria increased from approximately 3×10^5 to more than 1.0×10^8 after 24 hours of incubation. The bacteria were not eliminated. This indicates that the antibacterial properties of the coating system are contributed solely by the Ag particles. Therefore based on the tests, the coating system has been proven to be able to eliminate these two bacteria.

Nanosilver (Ag nanoparticle) is one of the most commonly used nanomaterials because of its strong antimicrobial activity. Several studies [18, 19] have proposed the mechanism

in which Ag particles eliminate bacteria. These are based on (i) dissolved silver ions interaction and (ii) reactive oxygen species (ROS). In the former, dissolved ions interrupt the bacteria cell's ability to form bonds essential for its survival. These bonds produce the cell's physical structure and therefore when bacteria meet silver ions they literally fall apart. Silver also disrupts multiple bacterial cellular processes, including disulfide bond formation, metabolism, and iron homeostasis resulting in increase of permeability of the cell wall [18]. For the latter, a recent study suggested that ROS generation by Ag nanoparticles or Ag⁺ ions could also be responsible for the strong antibacterial agents [19]. There are three types of ROS commonly associated with Ag nanomaterials: singlet oxygen ¹O₂, hydroxyl radical [•]OH, and peroxide radical O₂^{•-}. Among these, ¹O₂ is said to be the most detrimental to cells. In our case, although Ag particles are used instead of its ionic form (Ag⁺), it has been reported that metallic silver can be oxidized to silver ion [19] especially if they are in nanosize. On the other hand, study on ROS generation by Xu et al. [20] showed that the usage of Ag NP does produce ROS when tested against *Escherichia coli*. Therefore we believe that the antibacterial property of our sample can be attributable to both mechanisms.

Antibacterial coating can provide bacteria-free environment especially in hospitals and are useful to prevent infections by multidrug-resistant bacteria. Specifically, the coatings could potentially be used for walls in the operation theatre and ICU or any surface. they could possibly reduce the number of mortalities for cases related to bacteria acquired from these two rooms. Therefore, overall mortality cases could be reduced.

4. Conclusion

Antibacterial coating based on sol-gel technique has been successfully developed and proven to be able to effectively eliminate and inhibit the growth of gram-negative bacteria, namely, *Pseudomonas aeruginosa* and *Escherichia coli*. The coating showed a high and tolerable level of transparency which is important so that the material coated can retain its original properties.

Conflict of Interests

The authors declare that they have no competing interests regarding the publication of this paper.

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