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Effect of silver ion implantation on antibacterial ability of polyethylene food packing films

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Abstract: Bacterial adhesion on medical instruments' and food packages' surfaces causes implanted infections, food spoilage and human disease, therefore attracts a lot of attention in the field of medical and food applications. Containing the initial adhesion of bacteria on the surface of the material plays an important role in reducing potential safety hazards. In this work, we investigate the influence of silver ion implantation with different doses on the antibacterial performance of the polyethylene (PE) films. It is found out that silver ion implantation will not color the PE films but can improve their surface hydrophilicity. The silver-implanted PE films show the ability to inhibit bacterial adhesion and have the bactericidal effect, both of which can be improved with increasing silver implantation dose. This method also proves relatively safe, because the silver ions are relatively stable. The results will introduce potential applications for ion implantation in the food packing and food accessible materials.

Keywords: polyethylene; ion implantation; surface modification; bacterial adhesion; antibacterial ability; hydrophilicity.

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1. Introduction

Bacterial adhesion is the initial stage of pathogen biofilm formation and the formation of biofilms is the main cause of food-borne diseases (Erdem et al., 2017). During the processing of food, the food can be contaminated by microorganisms and become the source of cross-infection (Trachoo, Frank, & Stern, 2002). Moreover, microorganisms will be attached to food processing materials, conveying pipes, filter membranes and heaters to increase work resistance and reduce work efficiency (Miao et al., 2017), resulting in huge economic losses (Scharff, 2012; Wei, Helm, Corner-Walker, & Hou, 2006).

The biofilm formation process is postulated to have several major stages, including (I) initial reversible attachment, (II) a transition to irreversible attachment, (III) biofilm architecture development, (IV) mature biofilm formation, and (V) cell dispersion (Chao & Zhang, 2011; Fysun, Kern, Wilke, & Langowski, 2019; Renner & Weibel, 2011). Because mature biofilms have strong resistance to their living environment, and conventional fungicides cannot remove biofilms effectively (Bridier, Briandet, Thomas, & Dubois-Brissonnet, 2011; Escribano-Viana et al., 2018; Houari & Di Martino, 2007; Otter, Yezli, Salkeld, & French, 2013; Sav et al., 2018). Therefore, it is very important to inhibit bacterial adhesion and to control the biofilm formation in its early stage (Brecher & Hay, 2005; Lindsay & Von Holy, 2006; Shorten, Pleasants, & Soboleva, 2006; Zhang, Chao, Shih, Li, & Fang, 2011).

Ion implantation has become a hotspot in material modification methods in recent years (Li et al., 2019; Shiau et al., 2019; Xia et al., 2018; Zheng, Qian, & Liu, 2020). The ions generated by an ion source are shot to the surface of the materials at high speed. The energetic ions enter the surface, collide with the atoms in the solid, and finally stop in the materials and are embedded beneath the surface. During this process, some atoms are displaced, causing changes of the surface composition, structures, or properties of the materials, and thus serving purposes of modifications (Hu, Liu, Gan, & Long, 2019; Li et al., 2019). Especially, the ion implantation technology can adjust hydrophobicity of the surface (Price, Waters, Williams, Lewis, & Stickler, 2002; Tsuji et al., 2007) and improve the tissue compatibility by changing the chemical composition of the material surface (Wang et al., 2014), which can influence the materials' antibacterial adhesion. Hu et al. (Hu, Liu, Gan, & Long, 2019) have injected Fe^{3+} into graphene to improve the stability of the prepared material and its ability of inhibiting *E. coli*. Price C. et al. (Price, Waters, Williams, Lewis, & Stickler, 2002) have used argon-plasma bombardment for surface modification, which

has caused the incorporation of either hydrophilic or hydrophobic functional groups onto the surface. It turns out that the adhesion of Candida has been reduced. Zahran M. K. et al. (Zahran, Ahmed, & El-Rafie, 2014) have investigated antimicrobial activities by applying AgNPs–alginate composite on cotton fabric. The treated fabrics show the excellent antibacterial activity against *P. aeruginosa*, *S. aureus* and *E. coli*.

As a broad-spectrum antibacterial agent, silver is one of the most commonly used antibacterial agents at present (Marchetti et al., 2016; Sahai, Gaval, & Bhat, 2020; Sanchez-Valdes et al., 2018; Zheng et al., 2016). Tambur P et al. (Tambur, Bhagawan, Kumari, & Kasa, 2020) have decorated silver nanoparticles on functionalized multi-walled carbon nanotubes, which has proved to be effective against Bacillus subtilis, S. aureus, E. coli, P. aeruginosa. Oses J. et al. (Oses et al., 2014) have found that silver ions implanted into CrN layers have positive antibacterial effects on S. aureus and E. coli. However, there are concerns about whether silver can be applied in the food field efficiently and safely because it is a kind of heavy metal which can endanger human bodies (Marambio-Jones & Hoek, 2010; Zhang, Wang, & Levanen, 2013). Hadrup, N. et al (Hadrup, Sharma, Loeschner, & Jacobsen, 2020) evaluated silver to be genotoxic in vitro in mammalian cells. When silver ions are used in food packing or contact materials, there will be potential risks. Therefore, it is important to guarantee the antibacterial ability of silver ion fungicides and improve their safety at the same time. In this study, ion implantation was used to introduce silver ions at different doses in the food packing material – PE films (Figure 1). The treated films show the ability of inhibiting bacterial adhesion and bacterial growth on the surface. The safety of the implanted materials was evaluated with dissolution experiments.

2. Materials and methods

2.1. Materials

E. coli BL21, *S. aureus* 6538, and *P. aeruginosa* ATCC 9027 were purchased from China General Microbiological Culture Collection (CGMCC, Beijing, China). Luria Bertani (LB) agar and broth, M63 medium and tryptic soy broth (TSB) medium were purchased from Hepobio Company, Zhengzhou, Henan, China. All other chemical reagents (AR grade) were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China) and used as received.

2.2. Silver ion implantation

We used the ion implanter (High Voltage Engineering Europa B.V.,

Model B8385) at the Ion Beam Center, Helmholtz-Zentrum Dresden-Rossendorf, Germany. It can provide all kinds of stable ions with the energy from 10 to 500 keV. The fluence can be from $5e^{10}$ to $5e^{16}$ cm^{-2}. In detail, the Ag ions were produced by a IHC Bernas ion source. They were electrostatically accelerated to the designed high voltage. Together with a magnetic analyzer, ions can be selected according to their atomic mass. To ensure the uniformity, the beam was rastered over the sample. The PE films with area of 100 mm × 100 mm were implanted with different doses of silver ions. The beam energy was set to 190 keV, the temperature of PE films was kept less than 50°C, and the implantation angle was 7°. The injection doses were as follows, sample 0: none, sample 1: 5×10^{12} cm^{-2}, sample 2: 1×10^{13} cm^{-2}, sample 3: 5×10^{13} cm^{-2}, sample 4: 1×10^{14} cm^{-2}, sample 5: 5×10^{14} cm^{-2}, and sample 6: 1×10^{15} cm^{-2}.

2.3. Contact angle measurement

The water contact angles of PE film were measured using a Krüss DSA100 Drop Analysis System (Krüss GmbH, Hamburg, Germany) at room temperature (~20℃). 1 μL of DI water was added on the film surface. The contact angles were calculated using the DSA Registered Version software provided with the instrument. And the final result of water contact angle values was obtained by calculating the average of values in triplicate at different positions of the PE film after ion implantation.

2.4. Bacterial strains and bacterial attachment experiments

E. coli BL21, *S. aureus* 6538, and *P. aeruginosa* ATCC 9027 were inoculated into LB agar plates, and then transferred into LB broth with agitation at 37℃, 200 rpm for 12 h. The incubated cells were harvested in log phase, centrifuged (5000 g) for 15 min at 4℃, and washed twice with 0.08 M phosphate buffer saline (PBS, pH 7.4). The *E. coli* suspensions were diluted with PBS until the optical density at 600 nm equaled 0.6±0.05 (approximately 10^8 CFU/mL), and transferred into M63 medium with 1% (Friedlander et al., 2013). *S. aureus* and *P. aeruginosa* were added into TSB medium at the same concentration. PE films were placed at the bottom of a 6-well tissue culture plate at least in triplicate; M63 medium with *E. coli*, TSB medium with *S. aureus* or *P. aeruginosa* were poured into the 6-well tissue culture plate to cover the film. The plates were incubated at 37°C for 5 h.

2.5. Determination of resistance to bacterial growth

E. coli BL21, *S. aureus* 6538, and *P. aeruginosa* ATCC 9027 were inoculated into LB agar plates, and then transferred into LB broth with agitation at 37℃, 200

rpm for 12 h. The incubated cells were harvested in log phase. The three strains were separately diluted to make the concentration of the bacterial suspension 10^5-10^6 CFU/mL.

Refer to "Antibacterial products-Test for antimicrobial activity and Effects" (Japanese Industrial Standard JIS Z2801-2000). Each PE film after the silver ion implantation was cut into samples with a size of 1 cm×1 cm, washed with 70% ethanol, dried with nitrogen. 100 μL bacterial suspension was added on the surface of the film, and then it was covered with a sterile film.

The bacterial solution was evenly coated on the surface of the film, and the relative humidity of the incubator was adjusted to be more than 90%. After incubating in a 37℃ incubator for 24 h, the film was taken out and rinsed with sterile physiological saline. Then, the eluent was inoculated into LB solid medium to cultivate in a 37℃ incubator for 24 h. After cultivating, plate colony count was performed and recorded as A_t. Meanwhile, the number of plate colonies in the control group without silver ion implantation treatment was recorded as A_0. The calculation formula of antibacterial rate is as follows:

$$Antibacterial\ rate\ (\%)=(A_0-A_t)/A_0\times 100\%$$

2.6. Silver ion dissolution determination

After the silver ion implantation, each PE film was cut into samples with a size of 1 cm × 1 cm, washed with 70% ethanol and dried with nitrogen. Then we placed the material into 2 mL ultrapure water to soak it in the condition of 37℃ for 24 h. In the end, the water samples were collected and the content of silver ions was determined by atomic absorption spectrophotometer.

2.7. Confocal scanning laser microscopy (CLSM)

E. coli, *S. aureus* and *P. aeruginosa* grown on PE films were rinsed and fixed using the method of Friedlander et al. (Friedlander et al., 2013). Cells were permeabilized with 0.1% Triton X-100 in PBS for 15 min. Samples were then stained with Fluorescein isothiocyanate (FITC), 10 mg/mL for 30 min, and rinsed with PBS twice. PE films were placed face-down on a glass-bottomed sample dish (Shengyou Biotechnology Co., Ltd, Hangzhou, Zhejiang, China) and placed on the stage of the confocal laser scanning microscope (CLSM 710, Zeiss, Oberkochen, Germany). CLSM is equipped with an Ar laser at 488 nm. Images were obtained using the 63×oil immersion lens and processed using Image J software (NIH, Bethesda, MD, USA).

Three different fields of view were randomly chosen for analysis of the percentage of the images that show bacterial adhesion of the films.

2.8. Statistical analysis

Statistical analysis was conducted using SPSS Statistics 20.0, where $p < 0.05$ was used as the standard for significance. Origin 2018 and EXCEL 2016 were used to plot and analyze the data.

3. Results and discussion

3.1. Characterization of surface properties of PE film after silver ion implantation

In the condition of 190 keV and an angle of 7°, the stretched PE film was implanted with silver ions of different doses. According to the ion implantation simulation with SRIM (Stopping and Range of Ions in Matter), it can be seen that the silver element is mainly distributed at a depth of 75~250 nm, and the highest dose is at a depth of 170 nm, as shown in the inset of Figure 1. This result illustrates that the ion implantation only modifies the surface.

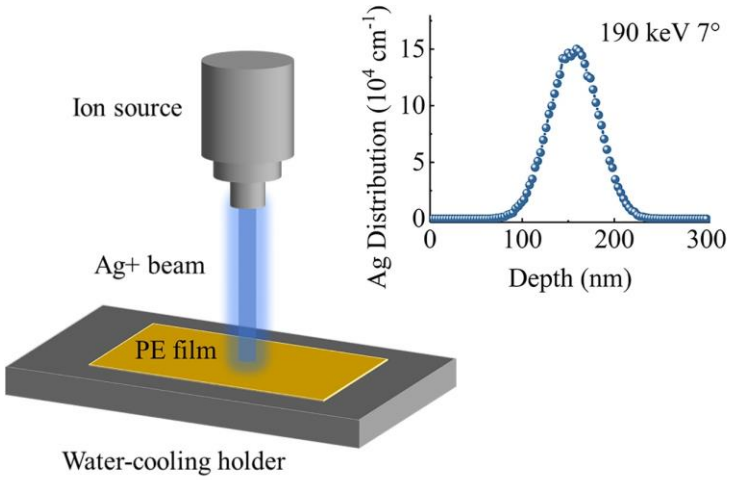

Fig. 1. The schematic of silver ion implantation on a PE film. Inset: Ag depth distribution on PE films after implantation.

The appearance of the stretched film which has been ion-implanted is shown in Figure 2(A). It can be found out that the color of the material was changed after the silver ion implantation. Samples 1-3 is similar to the control group (sample 0), which is still colorless and transparent. Sample 4 shows yellowish and sample 5 appears brown, but they are still translucent. Sample 6 has almost no transparency, appearing dark brown. In comparison with the control group, it can be found out that samples 5 and 6 become brittle and the toughness deteriorates largely. This phenomenon

indicates that different doses of silver ion implantation have different influence on the surface of the PE film.

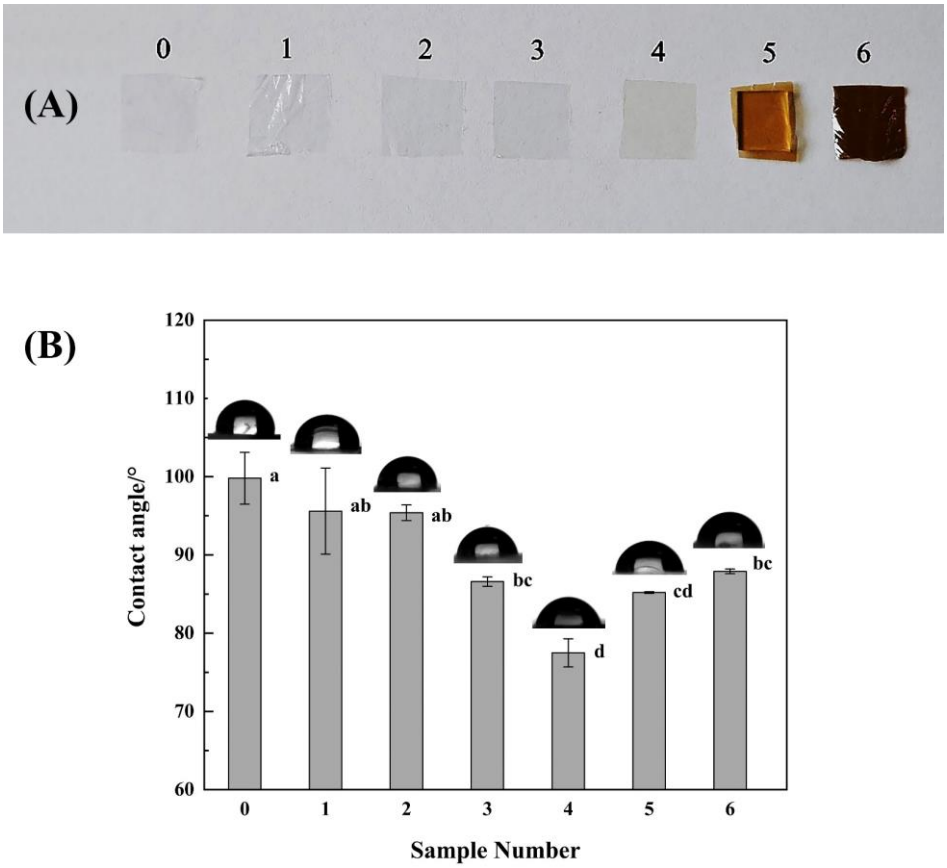

Fig. 2. The samples(A) and the contact angles(B) of PE films after ion implantation with different doses. 0: None, 1: 5×10^{12} cm^{-2}, 2: 1×10^{13} cm^{-2}, 3: 5×10^{13} cm^{-2}, 4: 1×10^{14} cm^{-2}, 5: 5×10^{14} cm^{-2}, 6: 1×10^{15} cm^{-2}, where $P < 0.05$.

The surface contact angle of samples 0 to 6 was measured in Figure 2(B). Compared with the control group, the contact angle of the PE film after silver ion implantation (sample 4) decreased by 24 from 100, indicating that silver ion implantation has improved the surface hydrophilicity of the material. The main reason is that silver ion implantation increases the surface roughness of the material. At the same time, the addition of silver ion itself changes the interaction force between water and the surface of the material. On the other hand, the contact angle increases for samples 5 and 6, suggesting that the surface roughness decreases. It can be understood as the excessive implantation doses damaged the internal molecular structure and the PE films were melted and reconstituted. Therefore, the samples 5 and 6 are not suitable for food packing materials or food contact materials because of their color

and change of structures. Based on these reasons, further measurements are only performed for samples 0-4.

3.2. Antibacterial adhesion of films

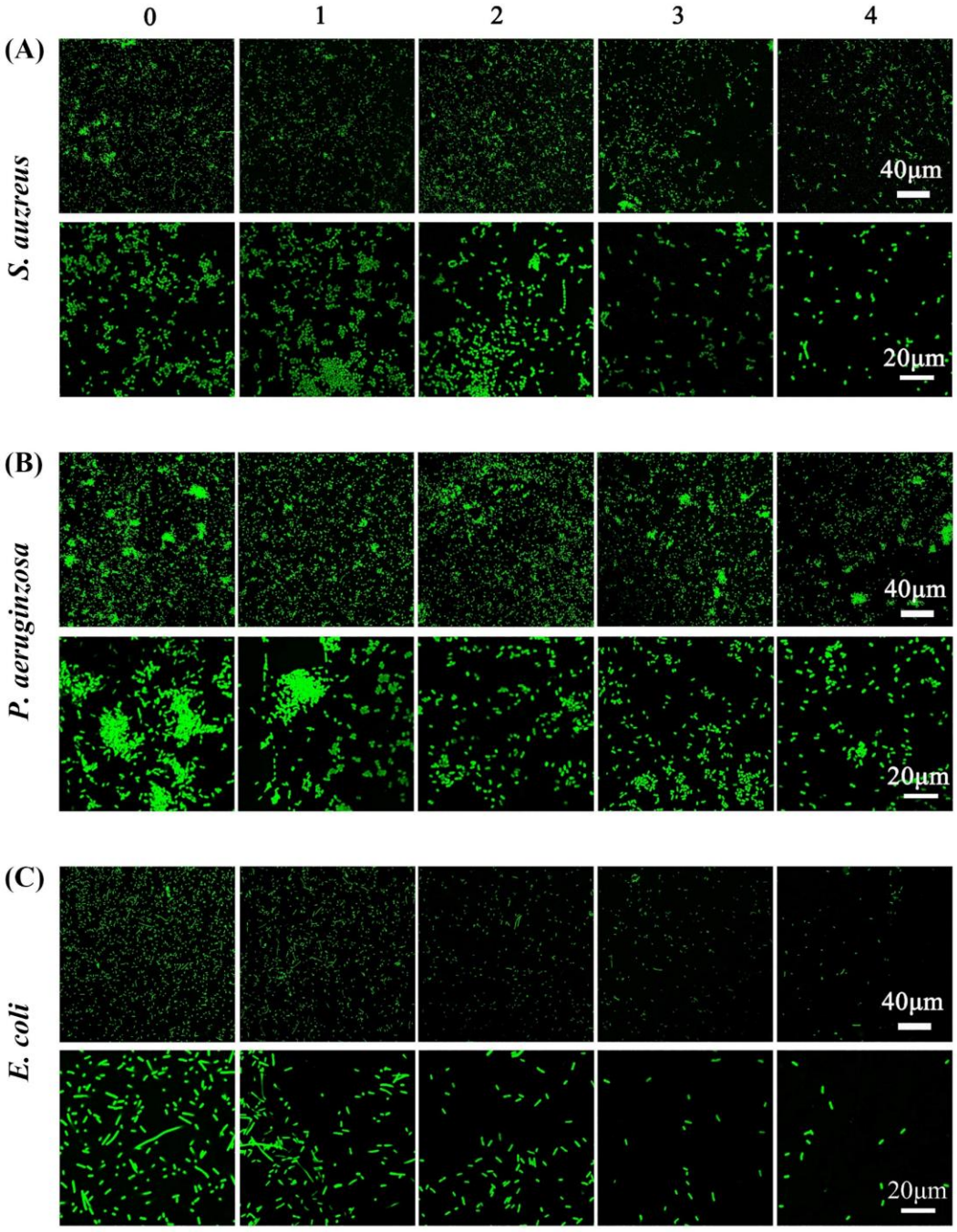

Fig. 3. CLSM images of *S. aureus*(A), *P. aeruginosa*(B) and *E. coli*(C) adhesion on PE films after ion implantation with different doses. 0: None, 1: 5×10^{12} cm^{-2}, 2: 1×10^{13} cm^{-2}, 3: 5×10^{13} cm^{-2}, 4: 1×10^{14} cm^{-2}.

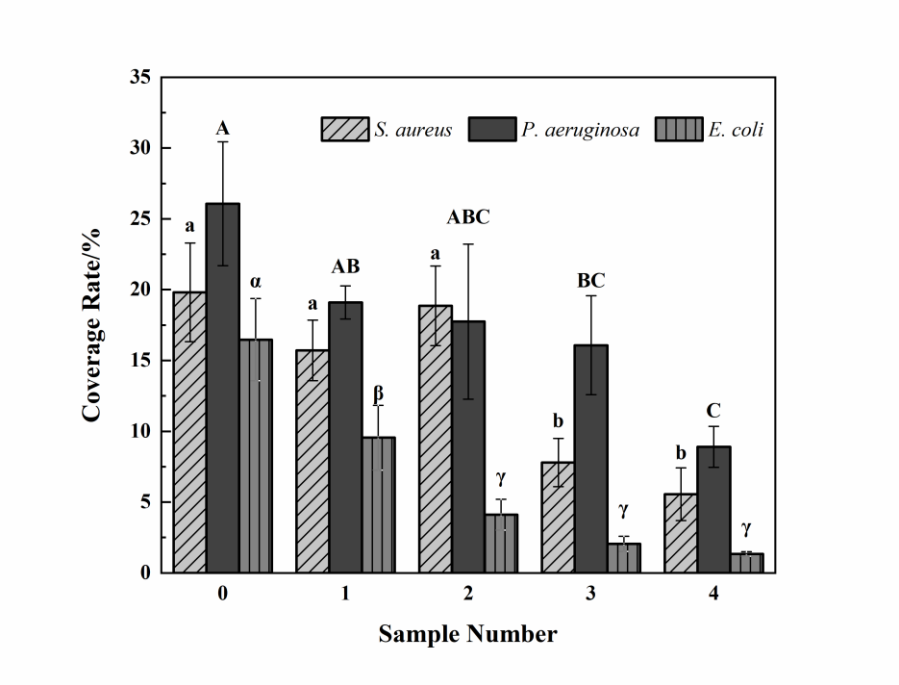

Fig. 4. The coverage rate of *S. aureus*, *P. aeruginosa* and *E. coli* counted base on the CLSM images. Histograms labeled with different letters (a-c, A-C, α-γ) represent significant differences.

Strong antibacterial adhesion can effectively hinder the formation of bacterial biofilm and slow down bacterial reproduction speed. The bacterial adhesion was identified by CLSM technology. The adhesion of *S. aureus*, *P. aeruginosa* and *E. coli* on the silver ion-implanted PE film is shown in Figure 3 and 4. For *S. aureus*, the adhesion of sample 2 was large, and samples 3 to 4 began to show a certain degree of inhibition. Sample 0-2 had a certain amount of bacterial cell agglomeration, which also indirectly illustrated that sample 3 and 4 have an inhibitory effect on the subsequent biofilm formation of *S. aureus*. For *P. aeruginosa*, samples 3 and 4 had inhibitory effects, and there were more single cell adhesion and no obvious bacterial aggregation, which indicated that the samples had a certain inhibitory effect on the subsequent biofilm formation. For *E. coli*, sample 2-4 all showed a good inhibitory effect. Comparing the adhesion of the three strains on the silver ion-implanted samples, it can be found out that, for different bacteria, the silver ion dose required to inhibit their adhesion is different. Among the three strains, the *E. coli* requires the lowest dose of silver ions, which is related to its poor adhesion ability (Lu et al., 2016); doses required against *S. aureus* and *P. aeruginosa* are similar.

The surface modification of PE film by silver ion implantation improves the ability of PE films to inhibit bacterial adhesion. According to the contact angle results, higher doses of silver ion implantation make a PE film more hydrophilic. And the

hydrophilic samples should have weak ability to inhibit bacterial adhesion (Lu et al., 2016). Therefore, the effect of silver ions on bacterial adhesion is greater than that of hydrophobicity improvement brought by ion implantation.

3.3. Antibacterial ability of the film

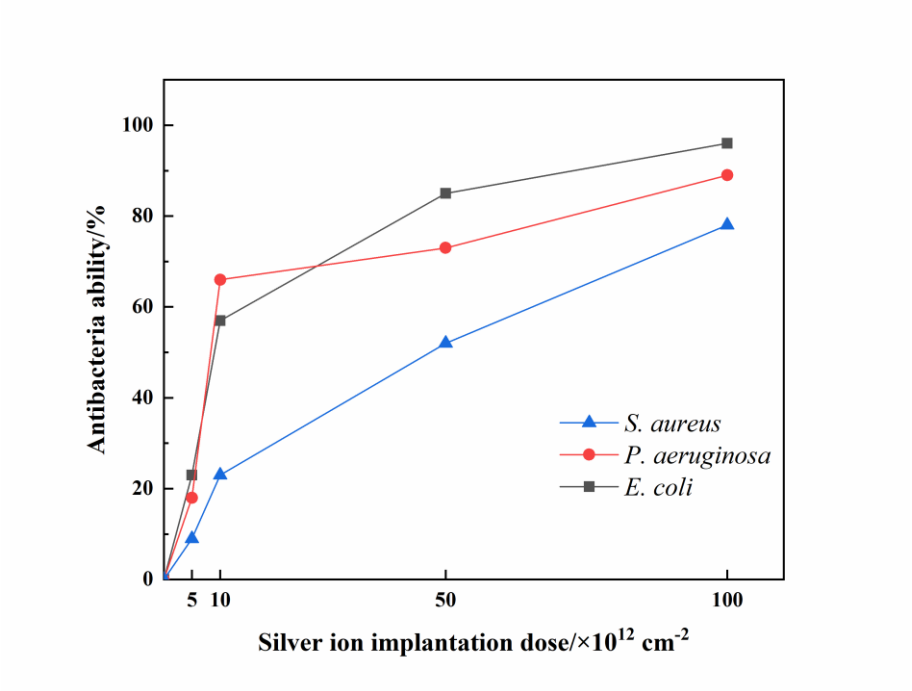

Fig. 5. Antibacterial ability of Ag ion-implanted PE films.

To investigate the antibacterial activity after the silver ions are implanted into the PE films, the quantitative experiments were conducted by the film adhesion method. As shown in Figure 5, after silver ion implantation, similar to the trend of the bacterial adhesion results, the antibacterial activity of sample 1 is low. When the silver ion dose increases, the antibacterial activity of the sample gradually increases. Different bacteria have different tolerances to silver ions. At a dose of 1×10^{13} cm^{-2}, more than half of *P. aeruginosa* and *E. coli* can be killed. In contrast, the same activity can be achieved at the dose of 5×10^{13} cm^{-2} for *S. aureus*. The activity difference comes from the different composition and structure of the cell walls of the bacteria. The peptidoglycan layer of the cell wall of gram-positive bacteria is thick and dense, with phosphorus acid embedded, less or no lipid, lipopolysaccharide, lipoprotein. Correspondingly, the cell wall of Gram-negative bacteria is thin and loose, and the outer membrane is composed by phospholipids, lipopolysaccharides, and proteins (Silhavy, Kahne, & Walker, 2010).

So far, Anh, D. H. et al. (Anh, Dumri, Anh, Punyodom, & Rachtanapun, 2016) have found that the PE/AgNP nanocomposites restricted common pathogenic bacteria

(*E. coli, Bacillus subtilis, Salmonella typhimurium*) in their early developmental stage. Aalaie, J. et al. (Aalaie, Mirali, Motamedi, & Khanli, 2011) have found that PE films with as little as 1 wt% nanosilver provided absolute antibacterial performance, while generally maintaining the mechanical properties. Marchetti, F. et al. (Marchetti et al., 2015) have embedded new silver (I) acylpyrazolonato derivatives with mononuclear, polynuclear or ionic properties in a PE matrix and found that most of the composite materials have better antibacterial effects. The data in this study further shows capability of the relevant materials to break the bacterial cell membrane. Brito, S. D. et al (Brito, Bresolin, Sivieri, & Ferreira, 2020) discovered that the packages incorporating silver nanoparticles inhibited the growth and reproduction of bacterial cells during the early stages. This is the same view as our study.

3.4. Determination of material safety

After immersing the samples in 2 mL ultrapure water for 24 h, the concentrations of silver ions in the solution were determined by atomic absorption spectroscopy. As shown in Table 1, the silver ion concentrations were all below the detection limit of the instrument, less than 0.01 mg/L. The silver ions after implantation are relatively stable on the surface of the material. This technique does not only guarantee the antibacterial activity of silver ions, but also greatly improves its safety. However, according to Commission Regulation (EU) No 10/2011 of 14 January 2011 on plastic materials and articles intended to come into contact with food, more research should be done before the material is applied directly to food and other fields.

Table 1 Dissolution of silver ions from different labels in water.

Label	Implantation dose of silver ions /cm^{-2}	Dissolution
0	0	Not detected
1	5×10^{12}	Not detected
2	1×10^{13}	Not detected
3	5×10^{13}	Not detected
4	1×10^{14}	Not detected
5	5×10^{14}	Not detected

4. Conclusions

We have explored the possibility of the Ag-implanted PE films as antibacterial food packing or food contact materials. When a dose is smaller than 1×10^{14} cm^{-2}, silver ion implantation will not color the PE films but can improve their surface

hydrophilicity. Meanwhile, the PE films show the ability to inhibit bacterial adhesion and have the bactericidal effect, both of which can be improved at higher doses. This method is relatively safe, because the silver ions are stable and their dissolution concentrations are all less than 0.01 mg/L. The results present the potential of ion implantation in the food packing or food contact materials.

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