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# Antibacterial properties of plasma-modified and triclosan or bronopol coated polyethylene

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**Abstract**

The antibacterial properties of medical polyethylene (PE) were enhanced by coating with triclosan or bronopol and plasma immersion ion implantation (PIII). $O_2$ plasma was first employed to produce a more hydrophilic surface on the PE, followed by argon or hydrogen plasma treatment to enhance the coating of triclosan or bronopol onto the surface. The modified surfaces were characterized by XPS, FTIR, SEM, and contact angle measurements. The antibacterial properties were evaluated utilizing the method of plate-counting of *Staphylococcus aureus* (Gram positive) and *Escherichia coli* (Gram negative). Our experimental results show that the plasma-modified PE with triclosan exhibits excellent antibacterial properties. Even after 6 weeks, the antibacterial effects against *E. coli* and *S. aureus* remain at high levels of 99.9 and 68.4%. The plasma-modified PE with bronopol has better antibacterial performances against *E. coli* and *S. aureus* in the beginning. Afterwards, the antibacterial effects degrade relatively rapidly. Our results reveal that non-reactive argon plasma was better than reactive hydrogen plasma in improving the antibacterial properties of PE. Bacterial adhesion on the modified samples was also investigated and the number of active adhered bacteria was observed to be always low.


*Keywords:* Plasma immersion ion implantation; Polyethylene; Antibacterial

## 1. Introduction

Infection of medical polymers is one of the major clinical complications causing a high rate of mortality and morbidity thereby significantly increasing health care costs [1–5]. According to statistical data from the American Disease Control and Prevention Center, 1.8 million of 35 million patients in America experienced infection from bacterial infection of medical polymer tubes [3]. Consequently, anti-infective properties are necessary for polymers in medical applications. Nowadays, approaches that can endow medical polymers with anti-infective properties mainly contain (a) mixing antibacterial reagents in bulk polymers; (b) copolymerization of antibacterial reagents with monomer for polymers; (c) surface modification of medical polymers [6–9]. Surface modification for anti-infection is to control the physicochemical interactions between the bacteria and polymer surface. It has some advantages, for example, no damage of the bulk properties of polymers, no release of antibacterial reagent from bulk polymers, and relatively simple and effective processes [10,11]. A number of surface modification techniques have been proposed to produce polymers with antibacterial surfaces. Although plasma modification may impose a higher cost, it has more advantages than other surface medication techniques [11–14].

We propose here to use plasma immersion ion implantation (PIII) to conduct surface modification. PIII is an excellent technique yielding good surface conformity and uniformity due to its non-line-of-sight and high energy characteristics [15,16]. Polyethylene (PE) is one of the common medical polymers [17,18]. Triclosan (2,4,4P-trichloro-2P-hydroxydiphenylether) and bronopol (2-bromo-2-nitropropane-1,3-diol) are two types of compounds that exhibit immediate, persistent, broad-spectrum antimicrobial effectiveness as well as little toxicity in clinical use. They also possess excellent biochemical and physical performances after plasma surface modification [19–21]. In this work, argon or hydrogen plasma was coupled with bronopol or triclosan deposition on PE to enhance the surface antibacterial properties. A comparison of

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the modified PE with modified PVC under similar conditions was also conducted from the perspective of anti-infection performances [13].

## 2. Experimental section

Medical-grade polyethylene (PE) samples (LDPE, 51215B Beijing Huaer Co., Ltd) with dimensions of 5 cm×5 cm×0.2 cm were laid on stainless-steel substrates and inserted into the plasma immersion ion implanter [22,23]. An initial $O_2$ plasma treatment was performed under the following optimal conditions based on trial experiments: Bias voltage = −12 kV, voltage pulse width = 20 μs, pulsing frequency = 30 Hz, gas flow = 20 sccm, RF power = 1000 W and treatment time = 30 min. After the initial plasma treatment, the samples were dipped into an antibacterial 20% reagent of triclosan or bronopol (Tian Jing Well-Real Chemical Technology, Co., Ltd) in alcohol and then taken out to uniformly coat the plasma-treated PE surface with a thin layer of the antibacterial reagent. After the alcohol had volatilized, the samples were reloaded into the plasma chamber and then underwent argon or hydrogen plasma ion implantation to ensure that the antibacterial reagent bonded well on the PE surface. The processing parameters were: bias voltage = −4 kV, RF power = 600 W, treatment time = 30 min and gas flow = 20 sccm. Again, these treatment conditions were based on trial experiments. Afterwards, the samples were washed three times using 70% ethanol to scour off loose triclosan or bronopol on the surface [19].

Several kinds of bronopol in crushed form were mixed with KBr and then pressed into pellets, and their Fourier transform infrared (FTIR) spectra were acquired using a Perkin–Elmer16 PC [24]. The surface chemical states were determined by X-ray photoelectron spectroscopy (XPS, PHI 5802) employing monochromatic Al Kα radiation at 14 kV and 350 W [25]. Scanning electron microscopy (SEM) was also used to study the surface of the samples. Static contact angles using distilled water as the medium were performed by the sessile drop method using a Ramé-Hart (USA) instrument at ambient humidity (80%) and temperature (25 °C). Each data point represents the average of five measurements conducted on different parts of the specimen for statistical accountability.

The antibacterial performances against *Staphylococcus aureus* ATCC6538 (*S. aureus*, Gram positive) and *Escherichia coli* ATCC10536 (*E. coli*, Gram negative) were determined by the method of plate-counting [14,15,26,27]. The samples were first washed with 70% ethanol to kill all the bacteria on the surface. After drying, a 0.2 ml solution of bacteria [2.0–5.0×$10^5$ CFU/ml] was added onto the modified surface and the surface was covered by a polyethylene (PE) film (4 cm×4 cm). At a relative humidity (RH) of higher than 90% and temperature of 37±1 °C, the samples were incubated for 24 h. Afterwards, they were thoroughly washed with 20 ml of a 0.87% NaCl solution containing Tween 80 with a pH of 7.0±2. For observation of active bacteria, 0.2 or 0.02 ml of the washing solution was added onto different dishes containing the nutrient agar. After 24 h of incubation under similar conditions, the active bacteria were counted and the antibacterial effect was calculated using the following relationship:

$$R(\%) = \left(\frac{B-C}{B}\right) \times 100 \quad (1)$$

where $R$ is antibacterial effect (%), $B$ is the mean number of the bacteria on the control sample (CFU/sample), and $C$ is the mean number of bacteria on the modified samples (CFU/sample).

In order to investigate bacteria adhesion on the modified surface of PE, bacterial adhesion experiments were conducted according to the following conditions [26–29]. All four kinds of samples were sterilized by 70% ethanol and cut into 16 pieces of approximately 2.0 $cm^2$. They were placed in four different flasks containing a cell suspension (2–5×$10^6$ CFU/ml) and kept at ambient conditions. Four samples were taken out sequentially from the bacteria suspension for a total time of 48 h and rinsed thrice with a 0.87% NaCl solution containing tween 80 with pH of 7.0±2. Thereafter, the adherent bacteria were detached from the samples in 10 ml of the same NaCl solution ultrasonically. The solution containing the bacteria was used to determine the viable counts.

Different types of processes were used for different samples. Sample 1 was the untreated control. Sample 2 underwent oxygen plasma treatment only. Sample 3 was treated with oxygen plasma, coated with triclosan, and then treated with argon plasma. Sample 4 was processed similarly as Sample 3 except that the reagent used was bronopol instead of triclosan.

## 3. Results and discussion

XPS is employed to determine the elemental composition of the modified PE surface and the results are shown in Table 1. Some oxygen and nitrogen are observed on the surface of control sample (namely, Sample 1). Similarly, oxygen and nitrogen are detected on the surface of Sample 2. The percentages of oxygen and nitrogen are 24.5 and 1.8%. This is because $O_2$ plasma bombardment produces functional groups containing oxygen. Moreover, upon exposure to air after the plasma treatment, nitrogen reacts with radicals on the surface. Six percent chlorine appears on the surface of Sample

Table 1
Atomic percentages and elemental ratios determined from Samples 1–4

| | C1s (%) | N 1s (%) | O 1s (%) | Cl 2p (%) | Br 3d (%) | N/C | O/C | Cl/C | Br/C |
|---|---|---|---|---|---|---|---|---|---|
| Sample 1 | 97.2 | 0.4 | 2.4 | – | – | 0.004 | 0.025 | – | – |
| Sample 2 | 79.2 | 1.4 | 19.4 | – | – | 0.018 | 0.245 | – | – |
| Sample 3 | 78.2 | 1.4 | 15.7 | 4.7 | – | 0.018 | 0.201 | 0.06 | – |
| Sample 4 | 73.3 | 4.6 | 20.5 | – | 1.6 | 0.063 | 0.280 | – | 0.022 |

3 suggesting that some chlorine containing triclosan is coupled onto the surface. Because the oxygen percentage in the molecule of triclosan is less than that of carbon after $O_2$ plasma, the ratio of oxygen to carbon on Sample 3 (20.1%) is reduced compared to that (24.5%) on Sample 2. However, the nitrogen content is the same on Sample 2 because they both come from nitrogen absorption and reaction with radical groups produced by the plasma treatment. All in all, our results show that triclosan combines with the surface of the sample and that the molecule of triclosan is not fully destroyed by the argon plasma. From Table 1, it is found that Sample 4 is similar to Sample 3 except that it is bronopol coated instead of triclosan coated. The appearance of bromine and increase in nitrogen on Sample 4 prove that bronopol can be incorporated onto the surface of PE by argon plasma.

In order to further evaluate the changes following the $O_2$ plasma treatment and triclosan/bronopol modification, the C1s high-resolution scans are fitted and evaluated. The full-width half-maximum (FWHM) is 1.3 eV. Fig. 1 depicts the typical C 1s high-resolution spectra used to identify the chemical states on the modified samples. The oxygen containing groups on the modified samples are C–O (284.3 eV), C=O (285.5 eV), O=C–O (286.7 eV), respectively, [25,26]. Compared to Samples 1 and 2, the amount of C–O group on Samples 3 and 4 is much higher after triclosan or bronopol modification. It is believed to be due to more C–O bonds.

The surface of medical-grade PE is hydrophobic. Triclosan and bronopol are easily crystallized and dissolved in alcohol, and the solutions are hydrophilic. Therefore, in order to more effectively coat the surface of the PE samples with these two antibacterial reagents, the sample surfaces are modified using oxygen plasma to enhance hydrophilic ability. The contact angles of distilled water in contact with the sample surfaces (namely, Samples 1 and 2) are reduced from 94.7 to 52.6° after the modification. The results indicate that the $O_2$-PIII PE surfaces are more hydrophilic, and so the modified sample can be coated better with the antibacterial reagents. At the same time, under these modified conditions, sample charging is not serious and no arcing is observed during the experiments. From the above chemical composition of Samples 1 and 2, the changes in the surface hydrophicity may be explained by that a large amount of oxygen is incorporated onto the surface, and the C–C and C–H groups are changed into the more polar C–O, C=O and O–C=O groups by oxygen plasma immersion ion implantation.

Fig. 2 shows the SEM micrographs obtained from Samples 1, 2, 3 and 4. No noticeable differences can be found between the surfaces of Samples 2 and 4 and Sample 1. Comparatively, the surface of Sample 3 changes significantly after modification. It is because triclosan can easily crystallize and some residues of the crystals remain on the surface of sample.

The antibacterial properties of Samples 3 and 4 are evaluated by plate-counting of *S. aureus* and *E. coli* which are the most representative bacteria and the results are shown in Table 2 [15]. After argon plasma modification, the antibacterial effects of Sample 3 (namely, triclosan modified PE) against

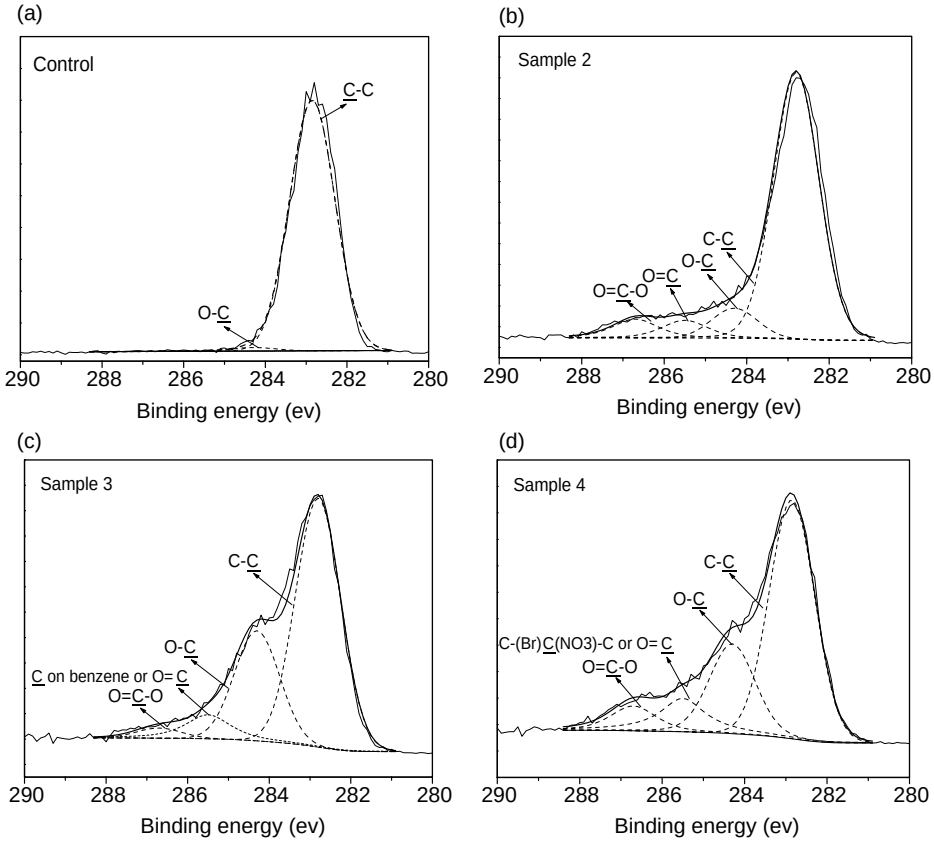


Fig. 1. Fitted C 1s high resolution scans of Samples 1–4.

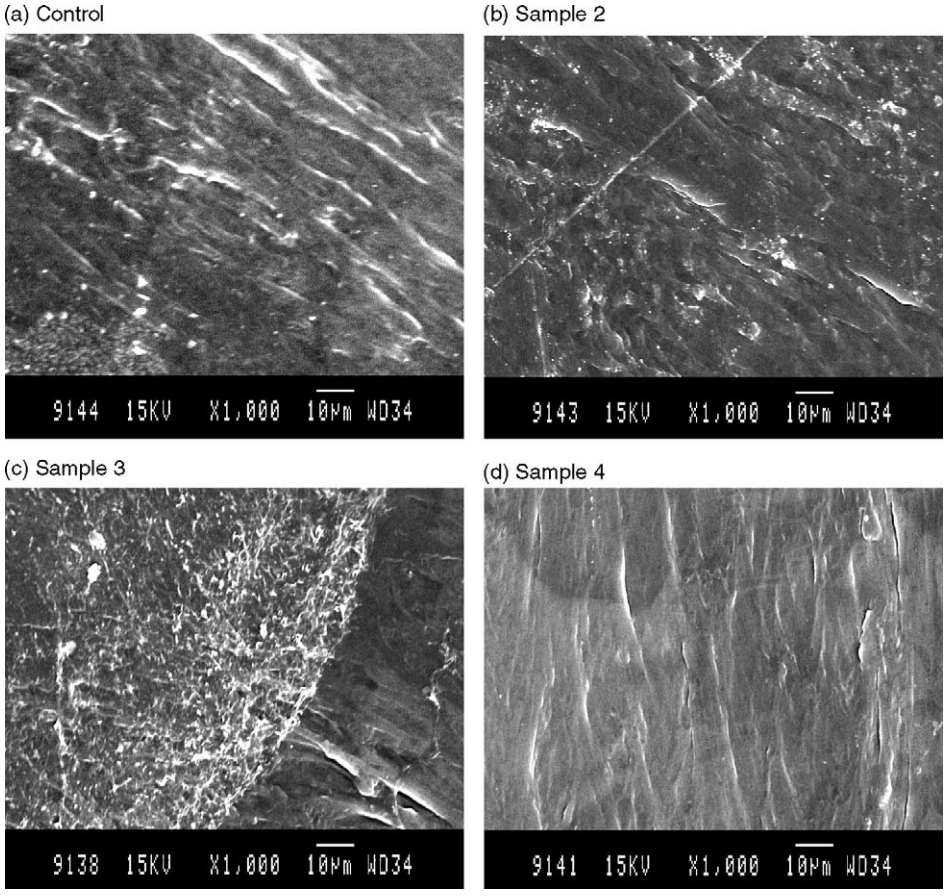


Fig. 2. SEM micrographs of Samples 1–4.

*S. aureus* and *E. coli* are 99.1 and 99.9%, respectively. This illustrates that after combining with the sample surface, triclosan still possesses excellent antibacterial properties. This phenomenon should be interpreted from the antibacterial mechanism of triclosan. Based on results reported recently [19,20], triclosan acts as a non-specific biocide by affecting the membrane structure and function of the bacteria. When it reacts with bacteria, triclosan forms a stable ternary complex by interacting with amino acid residues of the enzyme active site. At the same time, according to the aforementioned surface chemistry analysis of Sample 3, triclosan still has antibacterial effects for the C–Cl bond is not destroyed during the modification. However, when the molecule is fixed on PE, the activity and structure of the molecule are influenced by environmental changes. Hence, its antibacterial effect degrades to some extent. When hydrogen replaces argon as the plasma gas, the antibacterial effects of Sample 3 against *S. aureus* and *E. coli* are still 99.8 and 99.7%, respectively. This illustrates that after non-reactive argon or reactive hydrogen treatments, the triclosan modified PE samples have higher antibacterial efficiency.

Table 2 shows that when argon is the plasma gas and the conditions are the same as those of Sample 3, the antibacterial effects of Sample 4 against *S. aureus* and *E. coli* are better and 96.2 and 94.7%, respectively. There have been few reports about the antibacterial mechanism of bronopol, and the antibacterial activity of bronopol has not been reported in details. When hydrogen is employed instead of argon, the antibacterial effects of Sample 4 evidently decrease, 60.4 and 20.3%, respectively. It may be because hydrogen is a reactive gas that can react with bronopol under the plasma and change bronopol into smaller compounds. Therefore, the bronopol that is on the surface of PE has little antibacterial activity. As shown in the FTIR spectra of bronopol modified by argon or hydrogen plasma in Fig. 3, some peaks appear between 1100 and 1600 $cm^{-1}$ from the bronopol modified by argon and hydrogen plasma. Our results unequivocally illustrate that plasma modification changes the molecular structure of some bronopol molecules and this is also the reason why Sample 4 shows inferior antibacterial properties. As shown in Table 3, the antibacterial properties of the samples modified by triclosan and bronopol against *S. aureus* and *E. coli* are both better than that of modified poly vinyl chloride (PVC) under the same conditions [13]. These antibacterial properties of modified samples are believed to be related to the structure of

Table 2
Antibacterial effects of Samples 3 and 4 modified by Ar or $H_2$ plasma

| Gas plasma | Sample 3 (%) | | Sample 4 (%) | |
|---|---|---|---|---|
| | Ar | $H_2$ | Ar | $H_2$ |
| *S. aureus* | 99.1 | 99.8 | 96.2 | 60.4 |
| *E. coli* | 99.9 | 99.7 | 94.7 | 20.3 |

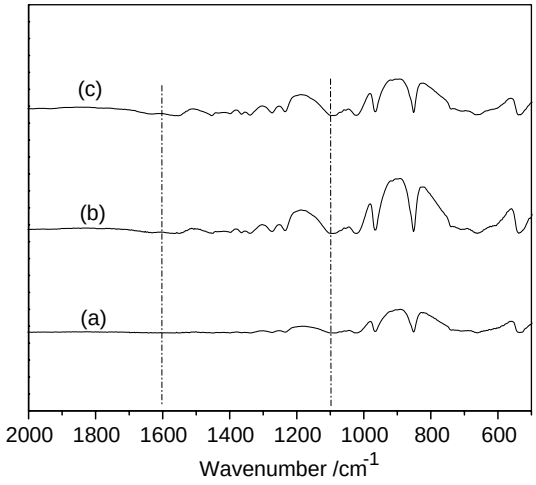


Fig. 3. FTIR spectra of three kinds of bronopol: (a) pure bronopol; (b) argon plasma modified bronopol; (c) hydrogen plasma modified bronopol.

Table 3
Comparison of antibacterial properties of modified PE and PVC[9]

| Sample | PE (%) | | PVC (%) | |
|---|---|---|---|---|
| | Sample 3 | Sample 4 | Sample 3 | Sample 4 |
| *S. aureus* | 99.1 | 96.2 | 82.2 | 98.0 |
| *E. coli* | 99.9 | 94.7 | 79.6 | 77.3 |

the macromolecules, antibacterial reagents, and plasma-modified conditions.

Since the macromolecule chain of polymers is apt to move, the plasma-treated surfaces tend to undergo changes with time. The modified samples were left under room temperature conditions at a relative humidity of about 80%, and then their antibacterial properties were determined at intervals of 3 weeks. The results shown in Table 4 indeed indicate that the antibacterial properties of them against *S. aureus* and *E. coli* degrade as time increases. The degradation rates for different samples are, however, different. Sample 3 exhibits smaller degradation in the antibacterial properties against *S. aureus* and *E. coli* than Sample 4. Sample 3 has high antibacterial effects against *E. coli*, almost 100%, after 6 weeks. Moreover, its antibacterial ability against *S. aureus* is just reduced from 99.1 to 68.4% after 6 weeks. On the other hand, the antibacterial effects of Sample 4 against *S. aureus* and *E. coli* degrade from 96.2 and 94.7% to 62.7 and 13.9%, respectively, after 6 weeks. It means that Sample 4 almost has no antibacterial ability against *E. coli* after 6 weeks. Comparing Samples 3 and 4,

Table 4
Antibacterial performances of modified samples against *S. aureus* and *E. coli* with time

| Sample | 0 Week | | 3 Weeks | | 6 Weeks | |
|---|---|---|---|---|---|---|
| | *S. aureus* | *E. coli* | *S. aureus* | *E. coli* | *S. aureus* | *E. coli* |
| Sample 3 (%) | 99.1 | 99.9 | 73.8 | 99.9 | 68.4 | 99.9 |
| Sample 4 (%) | 96.2 | 94.7 | 68.8 | 35.9 | 62.7 | 13.9 |

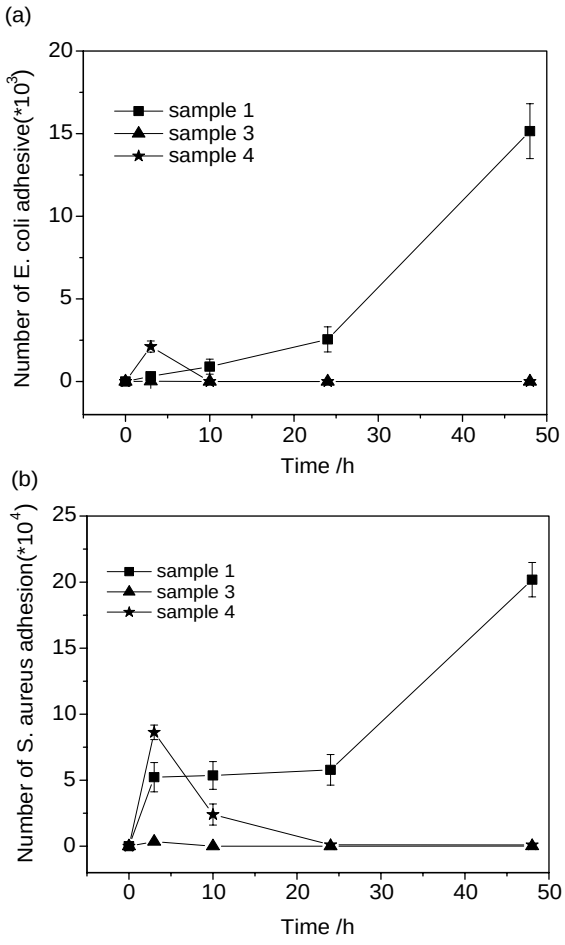


Fig. 4. Number of bacteria (*E. coli* (a) and *S. aureus* (b)) adherent onto Samples 1, 3 and 4 at different time.

argon plasma modification is an effective method to enhance the antibacterial ability of PE samples pre-coated with triclosan. Nevertheless, the mechanism of the degrading antibacterial ability for Sample 3 and 4 is not yet clear. More work is being conducted to comprehend the degradation mechanism and means to mitigate it.

By comparing Fig. 4(a) and (b) [29–31], it is not difficult to find the amount of *S. aureus* on all the samples (especially, Sample 1) is higher than that of *E. coli*. This is mainly due to the difference between the physicochemical characteristics of the bacteria and materials such as bacterial hydrophobicity, bacterial surface charge, material surface chemical composition, surface hydrophobic ability, and surface roughness. Nonetheless, the two kinds of bacteria exhibit a smaller degree of adherence on Samples 3 and 4 than Sample 1. In particular, bacteria adhesion on Samples 3 and 4 increases firstly and then decreases with time eventually reaching a low level. The modified samples exhibit higher antibacterial effects on *S. aureus* than *E. coli* in our antibacterial experiments by plate counting, and the antibacterial surface kills the adhered bacteria in a short time. Thus, there are fewer active bacteria on the surface of Samples 3 and 4.

## 4. Conclusion

Excellent antibacterial properties of medical polyethylene (PE) are achieved by plasma immersion ion implantation (PIII) followed by coating with triclosan or bronopol. The surfaces of the modified PE have good hydrophilic ability. Reagents including triclosan and bronopol can bond stably onto the plasma-treated surface. Utilizing the method of plate-counting, the antibacterial effects of triclosan modified PE against *S. aureus* (Gram positive) and *E. coli* (Gram negative) are 99.1 and 99.9%, respectively. Even after 6 weeks, the antibacterial effects remain at high levels. In comparison, the antibacterial properties of bronopol modified PE against *S. aureus* and *E. coli* are better in the beginning, but degrade with time and the samples exhibit no antibacterial properties after 6 weeks. Our results show that plasma immersion ion implantation (PIII) is a viable method to modify the surface of medical polyethylene to improve its anti-bacterial properties.

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