Ag and Ag/N$_2$ plasma modification of polyethylene for the enhancement of antibacterial properties and cell growth/proliferation

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Abstract

Polyethylene (PE) is one of the most common materials used for medical implants. However, it usually possesses low biocompatibility and insufficient antibacterial properties. In the work described here, plasma immersion ion implantation (PIII) is employed to implant silver into PE to enhance both its antibacterial properties and its biocompatibility. Our results show that Ag PIII can give rise to excellent antibacterial properties and induces the formation of functional groups such as C–O and C=C. These C–O and C=C groups on the modified surface can trigger the growth of the human fetal osteoblastic cell line (hFOB). Furthermore, combining N$_2$ and Ag PIII prolongs the antibacterial effects, but nitrogen-containing functional groups such as C–N and C=N created by N$_2$ co-PIII negatively impact proliferation of hFOB on the surface. According to our experimental investigation on cell proliferation, functional groups such as C–N and C=N created by nitrogen PIII are disadvantageous to cell growth whereas the C–O and C=C groups benefit cell growth. Both the antibacterial activity and biocompatibility of PE can be enhanced by means of the proper plasma surface treatment.

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1. Introduction

Polymeric materials are widely used in biomedical implants and devices. However, it is difficult to find polymers that meet all the requirements, such as antibacterial ability, biocompatibility, bioactivity, hydrophilicity, roughness and mechanical properties [1]. Polyethylene (PE), one of the most common biomedical polymers possessing excellent mechanical properties, suffers from insufficient biocompatibility and bioactivity [1–7]. Moreover, the materials can be easily attacked by bacteria in vivo. One possible approach to achieving better disinfection and biocompatibility while retaining the favorable bulk properties is to modify the surface chemical composition and state [7].

Surface compatibility is usually investigated by monitoring cell adhesion and proliferation. A suitable material that favors cell adhesion generally also shows improved cell proliferation [7,8]. From an industrial prospective, suitable surface modification can transform inexpensive synthetic polymers into biomedical products with high added values [9,10]. Plasma immersion ion implantation (PIII) has been used to modify the surface of polymeric materials [11–14]. In this work, PE is plasma implanted with Ag in an attempt to enhance both the biocompatibility and the antibacterial properties. At the same time, nitrogen co-PIII is conducted to change the surface chemical states for better performance, and the effects of the surface chemical state on the behavior of bacteria and bone cells are studied and discussed [15,16].

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2. Experimental details

2.1. Sample preparation

Low-density polyethylene samples with dimensions of 2 cm × 2 cm × 0.2 cm were inserted into a plasma immersion ion implanter equipped with a silver cathodic arc plasma source [12,13]. The arc was ignited using a pulse duration of 300 μs, with a repetition rate of 30 Hz and an arc current of 1 A. The Ag PIII process was conducted by applying an in-phase bias voltage of −5 kV, with a repetition rate of 30 Hz and a pulse width of 300 μs, to the PE samples [15,16]. In the simultaneous Ag and N2 PIII experiments, nitrogen gas was introduced into the vicinity of the silver arc discharge plume at a flow rate of 10 sccm (standard cubic centimeters). The dual PIII process was conducted by applying the same bias voltage as the Ag PIII treatment [15,16]. The working pressure in the vacuum chamber was 1–2 × 10−4 Torr.

2.2. Sample characterization

The elemental depth profiles and chemical states were determined by X-ray photoelectron spectroscopy (XPS) using a Physical Electronics PHI 5802 spectrometer [17]. A monochromatic aluminum X-ray source was used and elemental depth distributions were obtained using argon ion sputtering. The sputtering rate of 1 nm min−1 was approximated using that derived from silicon oxide under similar conditions. A cross-sectional transmission electron microscopy (TEM) image was acquired on a HITACHI H-800 microscope. After the plasma treatments were conducted on the PE samples, static contact angle measurements using distilled water as the medium were conducted immediately by the sessile drop method on a Ramé-Hart (USA) instrument at ambient humidity and temperature [18]. Contact mode atomic force microscopy (AFM) was conducted on a Park Scientific Instrument Autoprobe Research System to evaluate the surface morphology on a scanned area of 15 μm × 15 μm [19].

2.3. Antibacterial assays

The antibacterial properties of plasma implanted samples were determined in vitro. Both the Ag PIII and Ag/N2 PIII PE samples were immersed in 10 ml of simulated body fluid (SBF) at 37 ± 0.1 °C. The SBF has ionic concentrations similar to those of human blood plasma [20,21]. After immersion for 14 and 28 days, the samples were taken out and assayed for their antibacterial properties. The antibacterial performance against the Gram-negative Escherichia coli ATCC10536 was determined by the method of plate-counting. A 75% ethanol solution was used to sterilize the samples and then a 0.04 ml solution of bacteria (1–2 × 105 CFU ml−1) was added onto the modified surface and covered by a PE film (15 mm × 15 mm). At a relative humidity higher than 90% and temperature of 37 ± 1 °C, the bacteria on the samples were incubated for 24 h. Afterwards, they were thoroughly washed with 10 ml of 0.87% NaCl solution that contained Tween 80 with a pH of 7.0 ± 0.2 To observe the active bacteria, 0.2 or 0.02 ml of the solution was put into different dishes containing the nutrient agar. After 24 h of incubation under similar conditions, the active bacteria were counted and the antibacterial effect was quantitatively determined using the following relationship:

\[ R(\%) = \left( \frac{(B - C)}{B} \right) \times 100 \]

where \( R \) is the antibacterial effect (%), \( B \) is the mean number of bacteria on the control samples (colony-forming units (CFU) per sample) and \( C \) is the mean number of bacteria on the modified samples (CFU per sample).

2.4. Cell adhesion and proliferation [8,9,22]

Human fetal osteoblastic cells (hFOB 1.19 ATCC@number: CRL-11372) were used to study cell behavior on the control PE, Ag PIII PE and Ag/N2 PIII PE samples. This cell line was maintained in the incubation liquid containing a mixture of 45% Dulbecco’s modified Eagle’s medium (Invitrogen Cat No. 11995-040), 45% F-12 (Invitrogen Cat No. 11765-047), and 10% fetal calf serum (Hyclone Cat No. SV30087.02). No antibiotic was added to the liquid. The control PE, Ag PIII PE, and Ag/N2 PIII PE were sterilized by 75% ethanol for 5 h and then placed in a 24-well culture plate. To improve statistics, four measurements were taken for each type of sample. Drops of 1 ml of the incubation liquid containing the tested cells (hFOB cell line) were seeded on the sample surface. The number of tested cells is about 2 × 10^5 or 5 × 10^5. After the samples were incubated at 34 °C in 5% CO2/air for 2–6 days, they were rinsed once with a phosphate-buffered solution (PBS) to remove weakly adherent cells. Afterwards, the cells on the surface were fixed in a mixture of 10% acetic acid and 90% methanol for 20 min, stained with 10 μg ml−1 acridine orange 10-nonyl bromide in PBS for 5 min, and rinsed with PBS. Finally, these samples were inspected under a fluorescence microscope.

3. Results

3.1. Chemical composition

XPS was used to obtain the elemental depth profiles from the Ag PIII PE and Ag/N2 PIII PE [14,15]. As shown in Fig. 1a and b, the implant peak corresponds to about 9% Ag relative to C, implying that most of Ag is implanted into the surface region, although some (about 8% Ag relative to C) is deposited on the surface on both PE samples. N2 plasma co-implantation has little effects on the distribution of Ag in the surface region. N2 co-PIII has been observed to prolong the antibacterial characteristics, and its detailed role will be discussed later. The cross-sectional TEM image (Fig. 1c) of the Ag PIII PE sample reveals that
the implanted Ag segregates in the polymer matrix rather than distributes in the atom state. It is also known from this image that the implanted Ag is located several hundred nanometers from the surface.

### 3.2. Hydrophilicity

The surface contact angle plays an important role in the cell and bacteria behavior. Water was used to evaluate the hydrophilicity of the control PE, Ag PIII PE and Ag/N₂ PIII PE. The results disclose that the contact angles are reduced after Ag and N₂ PIII, from about 88° (control PE) to about 57° (both Ag PIII PE and Ag/N₂ PIII PE). That is to say, the Ag PIII PE and Ag/N₂ PIII PE yield better wetting properties compared to the control PE. This can be attributed to the change of the physical and chemical properties on the surface after PIII and the hydrophilicity affects the adhesion and growth of cells and bacteria on the surface [18,23].

### 3.3. Surface morphology

Surface roughness is another important factor impacting on cell and bacteria behavior, and thus AFM is utilized to determine the surface morphology. The AFM images acquired from the control PE, Ag PIII PE and Ag/N₂ PIII PE in Fig. 2 show that the surface roughness increases after PIII, and the root mean square roughness values calculated from the Ag PIII PE and Ag/N₂ PIII PE increase to 27.7 and 29.1 nm from 16.5 nm of the control PE, respectively. This phenomenon may benefit cell and bacteria adhesion [19,24].

### 3.4. Antibacterial properties

*E. coli* at a cell suspension concentration of $10^5$ CFU ml⁻¹ was used to determine the antibacterial effects of the Ag PIII PE and Ag/N₂ PIII PE samples. The results in Fig. 3 show that both the Ag PIII PE and Ag/N₂ PIII PE samples have excellent antibacterial performance. Their antimicrobial effects reach 99%. Only the incorporation of Ag by plasma implantation (Ag PIII PE) can endow PE with excellent antibacterial properties. Previous research conducted by our research group indicated that N₂ plasma implantation alone had relatively little effect on the antibacterial properties of the treated samples [14,15]. The results thus suggest that changes in the antibacterial properties mainly stem from the Ag plasma treatment and the deposited Ag on the sample surface, but not nitrogen functional groups. To investigate the variation of the antibacterial effects with time, the Ag PIII PE and Ag/N₂ PIII PE samples were immersed in 10 ml of SBF at 37 ± 0.1°C. It is found that good antibacterial properties can be retained after 14 days’ immersion in the SBF solution. As shown in
Fig. 3, the N₂ co-implantation treatment yields better antibacterial effects against \textit{E. coli} compared to the single Ag PIII process. This demonstrates that N₂ PIII helps to improve the long-term antibacterial performance. After further immersion in SBF for 28 days, the antibacterial efficacy of the Ag PIII PE is about 60%, but the antibacterial effect of the Ag/N₂ PIII PE is still above 80%, implying that the nitrogen-containing functional groups have an appreciable effect on bacteria killing or appear to be effective in adjusting the release rate of Ag.

### 3.5. Bone cell hFOB adhesion and growth

In order to evaluate the biocompatibility of the plasma implanted PE samples, hFOB was first employed to characterize cell adhesion and growth on the surface [25]. About \(2 \times 10^5\) hFOB cells were seeded onto the control PE, Ag PIII PE and Ag/N₂ PIII PE samples. Following incubation for 2 days, the cells were observed to proliferate by about 1.5 times, whence the samples were stained with acridine orange for fluorescence microscopy. The results are shown in Fig. 4. The hFOB cell line exhibited different adhesion and growth ability, although it adhered to all three sample surfaces after 2 days. Ag PIII induced more cell adhesion and growth compared to the control PE. The quantity of bone cells on the Ag/N₂ PIII PE was poorer than that on the Ag PIII PE, but still more than that on the control PE. Our experimental data reveal that some functional groups created by N₂ plasma impact the growth of bone cells negatively. This phenomenon illustrates the importance of the surface chemical structure on cell behavior.

To further study cell growth, more hFOB cells (about \(5 \times 10^5\)) were added to the three samples and subsequently incubated for 6 days. They proliferated fourfold, whence the samples were stained by acridine orange for fluorescence microscopy. The results indicate that cell growth was not uniform on the control PE surface. Many cells aggregated in some areas (Fig. 5a) whereas very few cells were observed in other areas. Fig. 5b shows the distribution of the hFOB cells on the entire sample based on fluorescence microscopy. In contrast, as shown in Fig. 6, the Ag PIII PE surface was fully covered by bone cells. By comparing the control PE with the Ag PIII PE, it is obvious that Ag implantation into PE yields better biocompatibility. In order to evaluate the difference between these two samples more unambiguously, only one part of the PE surface underwent Ag PIII while the other part was covered by aluminum foil. The resultant sample is displayed schematically in Fig. 7a. This sample was assayed after the growth of \(5 \times 10^5\) hFOB cells for 6 days. Although both parts of this sample were assayed under the same conditions, very few cells could be observed from the covered area, that is, the part which had not undergone Ag PIII (Fig. 7b), but many cells were observed from the Ag plasma
implanted portion of the sample (Fig. 7c). Fig. 7d shows that somewhat fewer cells were located at the boundary between the two areas. Our experiments unequivocally demonstrate that cells proliferate more easily on the Ag PIII PE surface. It is also observed in Fig. 8 that the Ag and N₂ plasma co-implanted sample had a similar number of cells on the surface compared to the Ag PIII PE, although few cells were observed on Ag/N₂ PIII PE when incubated with $2 \times 10^5$ of hFOB cells for 2 days.

4. Discussion

The elemental depth profiles in Fig. 1 show that the Ag PIII PE and Ag/N₂ PIII PE have new chemical and physical structures compared to the untreated control PE. The bone cell growth and antibacterial assays suggest that the new chemical structures favor bone cell adhesion and growth on the surface, while the deposited and embedded Ag simultaneously inhibit and kill bacteria. Fig. 9 illustrates the schematic of the preparation and actions of the Ag PIII PE and Ag/N₂ PIII PE.

Based on the hFOB cell results, the three types of samples show different degrees of cell proliferation. The hFOB cells adhere uniformly and grow on both the Ag PIII PE and Ag/N₂ PIII PE (both $2 \times 10^5$ and $5 \times 10^5$ seeded cells) after incubating for 2 and 6 days. In contrast, the hFOB cell distribution on the control PE is non-uniform after incubation for 6 days (Fig. 5). The results demonstrate that the Ag PIII and Ag/N₂ PIII have improved biocompatibil-
ity for bone cells. In order to observe the behavior of the hFOB cells from the beginning of incubation, the amount of hFOB cell seeded on both modified samples was reduced to $2 \times 10^5$ and the incubation time reduced to 2 days. It was found that the number of hFOB cells on both the Ag PIII PE and Ag/N$_2$ PIII was higher than on the control PE, but the quantity of cells on the Ag PIII PE was higher than that on the Ag/N$_2$ PIII. The data suggest that nitrogen-containing functional groups generated by N$_2$ co-PIII degrade cell adhesion and growth on the surface, although Ag PIII increases surface biocompatibility. Fig. 2 and contact angle results reveal that, in general, the plasma implanted surface exhibited better hydrophilicity and greater surface roughness, with the Ag PIII PE and Ag/N$_2$ PIII PE surfaces having similar hydrophilicity and roughness. Therefore, surface hydrophilicity and roughness do not appear to be important factors for the observed difference between the Ag PIII PE and Ag/N$_2$ PIII PE. The chemical changes introduced by PIII therefore appear to play a greater role in the improved cell behavior. In order to further evaluate the effects of the chemical structure, Fig. 10 depicts the C1s and N1s XPS high-resolution spectra acquired from the Ag PIII PE and Ag/N$_2$ PIII PE samples, and Fig. 11 shows the Ag 3d high-resolution spectra with depths [17].

Fig. 10a shows that Ag PIII produces C=C bonds in the surface region, and some oxygen-containing functional groups (mainly C–O) are also formed because the active groups on the surface react with O$_2$ from air after implantation. Fig. 10b and c indicates that N$_2$ co-PIII yields more N containing functional groups such as C–N and C–N in addition to a small amount of C–O. These polar groups result in better surface hydrophilicity [30]. Based on our experimental investigation on cell proliferation, N$_2$ co-PIII
results in a smaller amount of cells on the surface, although both the Ag/N₂ PIII PE and Ag PIII PE samples have similar hydrophilicity, roughness and Ag content. These results suggest that functional groups such as C–N, C≡N created by nitrogen co-PIII are disadvantageous to cell growth. Unger et al. [28] have also demonstrated that C–N and C≡N groups have a more negative effect on cell adhesion and proliferation compared to C–OH groups,
because the stronger electron donating ability of the C–N and C=O groups result in more positive charges on them during cell culturing. Better biocompatibility observed from the Ag PIII PE compared to the Ag/N2 co-PIII PE and control PE demonstrates that C–O and C=C groups created by Ag PIII benefit cell growth [28]. A detailed description of the effects of gas ion plasma implantation on the surface chemistry and cell behavior can be found elsewhere [29].

The antibacterial data shown in Fig. 3 suggest that Ag implantation is an excellent method to improve the antibacterial properties of PE. The antibacterial properties mainly stem from the reaction with Ag. The surface Ag shown in Fig. 1 provides immediate antibacterial properties and the embedded Ag that leaches out gradually provides more long-term effects. Fig. 11 shows that the implanted Ag atoms in the Ag PIII PE and Ag/N2 co-PIII PE samples have a zero valence state and do not bond chemically with other elements in the polymer. However, the polar functional groups created by N2 co-PIII can yield prolonged antibacterial properties. There is evidence that this is due to some interactions between these polar functional groups and implanted Ag, and more work is being conducted to study the mechanism in more detail. It should be noted that although this amount of Ag can inhibit and kill bacteria, the better growth of bone cells on the Ag PIII PE sample proves that implanted Ag does not negatively impact biocompatibility. These results are also consistent with other reports [14,26,27]. Therefore, both the antibacterial properties and biocompatibility of medical PE can be improved using the proper plasma surface treatment.

5. Conclusion

Our results demonstrate that better antibacterial properties can be achieved on polyethylene by proper silver plasma immersion ion implantation. Ag PIII induces the formation of a number of new functional groups, such as C–O and C=C. N2 co-PIII further creates nitrogen-containing functional groups, such as C–N and C=N, but has little effect on the distribution of Ag in the near surface region. Antibacterial tests reveal that N2 co-PIII can prolong the antibacterial effects. However, bone cell assays show that the Ag PIII PE surface containing C–O and C=C groups produces better adhesion and growth of bone cells (hFOB). The Ag/N2 co-PIII PE surface containing C–N and C=N groups exhibits reduced hFOB proliferation. Silver PIII treatment is an excellent approach to endow PE with antibacterial properties and can enhance both the biocompatibility and bioactivity of hFOB cells.

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