Enhancement of antibacterial properties and biocompatibility of polyethylene by silver and copper plasma immersion ion implantation

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1. Introduction

In recent years, medical polymers have attracted increasing attention in bone tissue engineering as they allow new tissues to take over various biological functions without potential chronic problems associated with the presence of biostable implants [1,2]. However, it is difficult to find polymers that meet all the requirements including antibacterial ability, biocompatibility, bioactivity, hydrophilicity, roughness, and mechanical properties [2–4]. Polyethylene (PE), one of the most common biomedical polymers possessing excellent mechanical properties, suffers from insufficient biocompatibility and bioactivity. Moreover, the materials can be easily attacked by bacteria in vivo [2]. Surface treatment is a viable approach and techniques such as UV radiation, chemical and plasma grafting, ion implantation, and plasma immersion ion implantation and deposition (PIII&D) [5–8] have been proposed. Our previous experiments on plasma immersion ion implantation (PIII) have revealed that it is possible to embed copper and silver into the near surface region of medical polymers to improve the surface antibacterial properties and biocompatibility [9,10]. It has been reported that silver and copper can cause bacterial inactivation in vitro by binding to microbial DNA, preventing bacterial replication, and disrupting the sulphydryl groups of metabolic enzymes in the bacterial electron transport chain [2,4,8]. In this work, we compare the properties of Cu PIII and Ag PIII polymers systematically. The associated antibacterial properties and the growth behavior of different cell lines on the modified surfaces are determined. The differences between Ag PIII and Cu PIII are also studied in order to better understand the role and efficacy of metal PIII.

2. Experimental details

2.1. Sample preparation

Medical-grade polyethylene (PE) samples (LDPE, 51215B) with dimensions of 2 cm×2 cm×0.2 cm were laid on stainless steel substrates and inserted into the plasma immersion ion implanter equipped with a silver or copper cathodic arc plasma source. The arc was ignited using a pulse duration of 300 μs, repetition rate of 30 Hz, and arc current of 1 A. The Ag or Cu plasma was guided into the vacuum chamber by an electromagnetic filter to eliminate deleterious macro-particles. Ag PIII or Cu PIII was conducted by applying an in-phase bias voltage of −5 kV with a repetition rate of 30 Hz and pulse width of 300 μs to the PE samples [9–12]. The typical pulse current and voltage waveforms are displayed in Fig. 1. The working pressure in the vacuum chamber was 1–2×10^{-4} Torr and the implantation time was 10 min [11,12].

2.2. Chemical structure determination

The elemental depth profiles and chemical states were determined by X-ray photoelectron spectroscopy (XPS) using a Physical Electronics PHI 5802. A monochromatic aluminum X-ray source was used and the elemental depth distributions were obtained using argon ion...
sputtering. The sputtering rate of 1 nm/min was approximated using that derived from silicon oxide under similar conditions [13].

2.3. Antibacterial assays

The antibacterial performance against Escherichia coli ATCC10536 (E. coli, gram-negative) was determined by the method of plate-counting. 70% ethanol was first employed to sterilize the samples and then a 0.04 ml solution of bacteria (10^5 CFU/ml) was added onto the modified surface and covered by a polyethylene (PE) film. At a relative humidity (RH) of 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±2. To observe the active bacteria, 0.2 or 0.02 ml of the washing solution was added into the 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 [2,9]:

$$R(\%) = \frac{(B - C)}{B} \times 100$$

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

2.4. Cell adhesion and proliferation

In the cell assay conducted on the control PE, Ag PIII PE and Cu PIII PE, two types of cell lines, namely the Chinese hamster Ovary (CHO-K1 ATCC@number: CRL-9618) cells CHO and human fetal osteoblastic cell (hFOB 1.19 ATCC@number: CRL-11372), were used. They were maintained in the incubation liquid consisting of a mixture of 45% Dulbecco’s Modified Eagle Medium (D-MEM) (Invitrogen Cat no. 11995-040), 45% F-12 (Invitrogen Cat no. 11765-047), and 10% Fetal Calf Serum (FCS, Hyclone Cat no. SV30087.02) without antibiotics. The control PE, Cu PIII PE, and Ag PIII PE were sterilized by 75% ethanol for 5 h and then placed in the 24-well culture plate. There were four samples of each kind for better statistics. One ml of the incubation liquid containing the tested cells (CHO and hFOB) were seeded onto the sample surface. The amounts of tested cells were 20,000 and 50,000, respectively. After the samples had been incubated at 37 °C for CHO cells and 34 °C for hFOB in 5% CO2/air for 2 and 6 days, the culture medium was removed and the cells were subsequently rinsed once with a phosphate buffered solution (PBS) to remove weakly adherent cells. Subsequently, the cells on the surfaces were fixed in a mixture of 10% acetic acid and 90% methanol for 20 min, and the air-dried cells were stained by 10 μg/ml Acridine Orange 10-nonyl bromide in the PBS solution for 5 min and then rinsed by the PBS solution. Finally, these samples were studied by means of fluorescence microscopy [10,14–16].

3. Results and discussion

3.1. Chemical state and structure

After the PE samples were plasma-implanted with Cu or Ag using optimal conditions based on our previous experiments [9,10], XPS was conducted to obtain the Cu2p and Ag 3d depth profiles. Our previous results reveal the desirable effects of Cu PIII and Ag PIII with regard to the antibacterial properties [13]. In order to understand the differences between Cu and Ag PIII, the elemental depth profiles are compared and displayed in Fig. 2. It is found that Cu penetrates deeper into the PE substrate and the amount of surface Ag is higher. It is believed to be due to the smaller mass of Cu and also that the Cu plasma mainly contains Cu2+ whereas the Ag plasma is primarily composed of Ag+ [12]. A higher bias voltage is thus required to achieve a deeper penetration of Ag.
The Raman spectra acquired from both implanted samples shown in Fig. 3 exhibit the similar characteristic peaks of polyethylene at 1060, 1127, 1293, 1440, 2727, 2853, and 2881 cm$^{-1}$. Moreover, photoluminescence on the Raman spectrum is enhanced after Cu and Ag implantation. It may correspond to an increase in the amount of defects in the polyethylene crystal caused by implantation of energetic particles. The experimental evidence suggests that dehydrogenation takes place and the chemical structure of PE is changed after PIII [9].

3.2. Antibacterial properties

$E. coli$ with a cell suspension concentration of $10^5$ CFU/ml is used to determine the antibacterial effects of the Cu PIII PE and Ag PIII PE samples and the results are shown in Fig. 4. Both Cu PIII and Ag PIII can improve the antibacterial performance of PE. It is also found that Ag PIII yields better antibacterial properties than Cu PIII despite similar implantation conditions. The Ag PIII PE shows almost 100% antibacterial ability and it is believed to be due to a larger amount of Ag on the outermost surface and the intrinsically better antibacterial performance of Ag compared to Cu.

3.3. Osteoblastic cell growth behavior

In order to evaluate the biocompatibility of Cu PIII PE and Ag PIII PE samples, the human fetal osteoblastic cell line (hFOB) is first employed to characterize cell adhesion and growth on the surfaces. About $5 \times 10^5$ hFOB cells are seeded on the control PE, Cu PIII PE, and Ag PIII PE samples and subsequently incubated for 6 days. After that, the resulting samples are stained by Acridine Orange for fluorescence microscopy. The results in Fig. 5 indicate that cell growth is not uniform on the control PE surface. This mainly stems from the low hydrophilicity and non-biocompatible chemical states on the surface. Many cells aggregate in some areas (Fig. 5-a1) whereas very few cells are observed in other areas. Fig. 5-a2 shows the distribution of the hFOB cells on the entire sample using fluorescence microscopy. In contrast, as shown in Fig. 5b and c, the Cu PIII PE and Ag PIII PE surfaces are fully covered by bone cells. There is unequivocal evidence that both Cu and Ag PIII enhance the biocompatibility of PE. Previous research demonstrates that modified chemical state results in good biocompatibility [10]. Our date further disclose that Cu and Ag have very little impact on the growth behavior of bone cells as they can proliferate more easily on the Cu and Ag PIII PE surfaces.

3.4. Chinese Hamster Ovary cells (CHO) cells assays

In order to further understand the influence of Cu and Ag PIII on the PE surface with regard to cell growth behavior, about 50,000 of Chinese Hamster Ovary cells (CHO cells) are employed in another test
to characterize the adhesion and growth on the control PE, Cu PIII PE, and Ag PIII PE. After they have been incubated for 2 days, 4 times proliferation is observed. The samples are stained by Acridine Orange for fluorescence microscopy observation. The results depicted in Fig. 6 show non-uniform CHO cells distribution on the control PE, which are similar to those observed for bone cells. However, as shown in Fig. 6b and c, the CHO cells also display non-uniform distributions on the Cu PIII PE and Ag PIII PE surfaces although bone cell growth is enhanced on both the Cu and Ag PIII PE samples. It demonstrates that the Cu or Ag PIII PE surfaces do not differ much from the control PE for CHO cells and the behavior of CHO cells deviates from that of hFOB cell proliferation. The CHO cell has little spreading abilities on all three types of samples and thus the modified surfaces have little adhesion and biocompatibility for CHO cells. Our results reveal an important observation that different cells behave differently and surface treatments that are appropriate for certain applications, for example, orthopedics, may not be suitable for others.

4. Conclusion

Plasma immersion ion implantation is an effective method to incorporate antibacterial Ag and Cu into the near surface of medical polyethylene. The ability of Cu PIII and Ag PIII to enhance the antibacterial performance on PE is studied and compared. Our results show that there is a higher amount of surface Ag but Cu penetrates deeper on account of the mass and charge state differences. According to the Raman spectra, photoluminescence is enhanced after Cu and Ag implantation indicating structural change in the polymer such as dehydrogenation. The antibacterial tests against E. coli show that the Ag PIII PE samples possess better antibacterial properties compared to the Cu PIII ones. Cell assays employing bone cells reveal that both Ag PIII and Cu PIII improve the surface biocompatibility. However, CHO cells show non-uniform distributions on the control, Cu PIII PE, and Ag PIII PE surfaces disclosing that the modified surfaces have little adhesion and biocompatibility for CHO cells. The different cell behavior suggests surface treatment that bodes well for certain applications such as orthopedics may not be suitable for others.

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References


Fig. 6. CHO cell growth behaviors on (a) Control PE, a1: Image of CHO cells on part of the surface, a2: Schematic distribution of CHO cells on the entire surface, (b) Cu PIII PE and (c) Ag PIII PE in 2 day incubating time.