Surface & Coatings Technology 203 (2009) 2550–2553

Contents lists available at ScienceDirect

Surface & Coatings Technology

journal homepage: www.elsevier.com/locate/surfcoat

Biocompatibility of silver and copper plasma doped polyethylene

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ARTICLE INFO

Available online 4 March 2009

PACS:
82.35.-x
52.25.-b
87.15.-v

Keywords:
Plasma immersion ion implantation
Silver
Copper
Biocompatibility
Polyethylene

ABSTRACT

Plasma immersion ion implantation (PIII) is a viable technique to implant antibacterial metals into polymers to enhance the antibacterial properties. It has been shown that Ag and Cu PIII can produce excellent antibacterial results on polyethylene (PE). In the work described here, their biocompatibility is investigated and the effectiveness of Ag PIII and Cu PIII PE is experimentally compared. Our data reveal that the Ag elemental depth profiles are similar to those of Cu but there is a larger amount of surface Ag compared to Cu possibly due to the different charge states in the plasma. Moreover, Cu PIII induces more polar oxygen containing groups on the PE surface than Ag PIII, and more C=C bonds are observed on the Ag PIII PE surface. The different chemical states lead to better hydrophilicity on the Cu PIII PE. Based on cell assays, the Ag PIII PE and Cu PIII PE samples exhibit excellent biocompatibility for bone cells, demonstrating that Ag and Cu PIII not only enhances antibacterial properties but improves cell biocompatibility of PE as well. The biocompatibility is found to not greatly relate to the metal species but rather the chemical functional groups formed during the interactions between the plasma-implanted metals and molecules in the polymer.

1. Introduction

Medical polymers are important to modern medicine and play an important role in the replacement and repair of human organs. It is well known that the success and long-term durability of these implants are impacted by the presence of bacteria in the vicinity of the implants. Bacterial infection after implant placement can cause significant complications thereby increasing medical costs, morbidity, and patient dissatisfaction [1,2]. The ideal medical implant should possess both antibacterial function and excellent cell biocompatibility. Therefore, means to mitigate bacterial infection and enhance biocompatibility on polymeric surfaces has aroused interests [3]. Surface treatment techniques such as ultraviolet (UV) radiation, chemical and plasma grafting, ion implantation, and plasma immersion ion implantation and deposition (PIII&D) [2–7] have been proposed. Our previous experiments on plasma immersion ion implantation (PIII) reveal that it is possible to embed inorganic copper and silver into the near-surface region of organic polymers to improve the surface antibacterial properties [8,9]. These two elements have less toxicity compared to other heavy metals. From the antibacterial perspective, they can cause bacterial inactivation *in vitro* by binding to microbial DNA, preventing bacterial replication, and disrupting the sulfhydryl groups of metabolic enzymes in the bacterial electron transport chain [2,6,7]. In this work, the efficacy of Ag PIII and Cu PIII as well as the biocompatibility of the implanted materials is evaluated in order to fathom the enhancement mechanism of the antibacterial properties.

2. Experimental details

Medical-grade polyethylene (PE) samples (LDPE, 51215B) with dimensions of 2 cm × 2 cm × 0.2 cm produced by Beijing Haier Co., Ltd. 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,

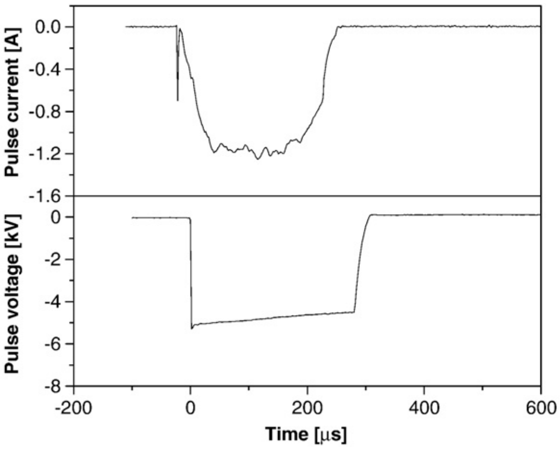

Fig. 1. Typical voltage and current waveforms during Cu PIII and Ag PIII of polyethylene.

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doi:10.1016/j.surfcoat.2009.02.073

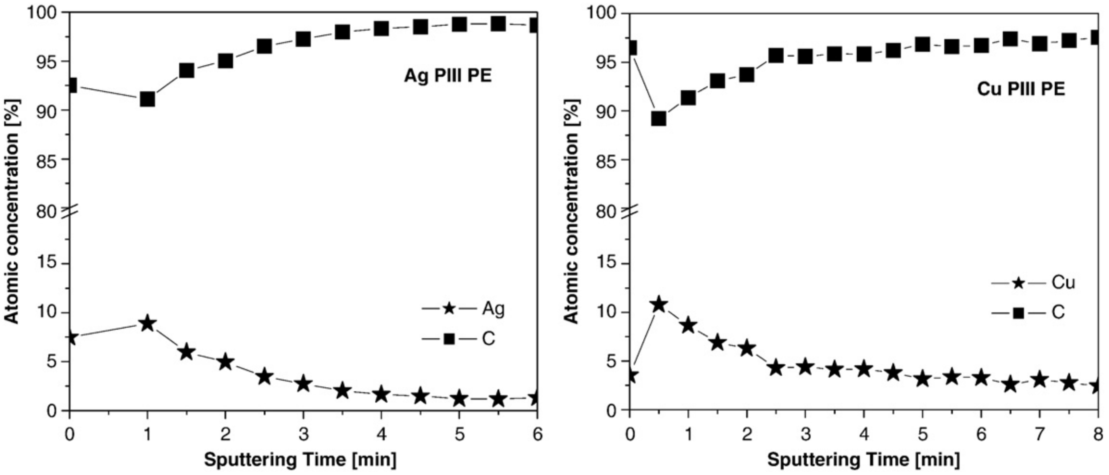

Fig. 2. Elemental depth profile in the Ag PIII PE and Cu PIII PE sample.

repetition rate of 30 Hz, and arc current of 1 A. The Ag plasma was guided into the vacuum chamber by an electromagnetic field via a curved magnetic 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 [8,9]. The typical pulse current and voltage waveforms are displayed in Fig. 1. The working pressure in the vacuum chamber was $1–2\times10^{-4}$ Torr and the implantation time was 10 min [10,11].

The elemental depth profiles and chemical states were determined by X-ray photoelectron spectroscopy (XPS) on a Physical Electronics PHI 5802 [12]. A monochromatic aluminum X-ray source was used and the elemental depth profiles were determined using argon ion sputtering. The sputtering rate of 1 nm/min was approximated based on that derived from silicon oxide under similar conditions. Static contact angle measurements using distilled water or glycerin as the media were performed by the sessile drop method on a Ramé-Hart (USA) instrument at ambient humidity and temperature. Contact mode atomic force microscopy (AFM) was conducted on a Park Scientific Instrument (PSI) Autoprobe Research System to evaluate the surface morphology across a scanned area of 15 µm × 15 µm.

Human fetal osteoblastic cells (hFOB, ATCC® Number: CRL-11372™) were cultured at 34 °C in a humidified atmosphere of 5% CO_2 in a 1:1 mixture of Ham's F12 medium (Invitrogen Cat no. 11765-047) and Dulbecco's modified Eagle's medium (D-MEM, Invitrogen Cat no. 11995-040) supplemented with 10% fetal bovine serum (Hyclone Cat no. SV30087.02). The culture medium was refreshed every 3 days. 5×10^5 cells/disc were plated onto the samples in 24-well tissue culture plates in a 100 µl aliquot medium prior to the addition of 0.9 ml of the growth medium. Before cell culturing, all the samples were sterilized with 70% alcohol overnight and rinsed with sterile phosphate-buffered saline (PBS) and then pre-treated by incubation in 0.9 ml growth medium for 2 h at 34 °C in a humidified atmosphere of 5% CO_2. After culturing for 6 days, the cells on the surface were fixed in a mixture of 10% acetic acid and 90% methanol for 20 min, stained by 10 µg/ml Acridine Orange 10-nonyl bromide in the PBS solution for 5 min, and then rinsed by the PBS solution. These samples were then inspected by fluorescence microscopy [13,14].

3. Results and discussion

After the PE sample was plasma-implanted with Ag or Cu using optimal conditions based on our previous experiments [8,9], XPS was conducted to obtain the elemental depth profiles. As shown in Fig. 2, Ag PIII and Cu PIII have been successfully conducted. Although some Ag or Cu atoms are deposited on the surface, most of them are located in the sub-surface region. The amount of implanted or embedded Ag and Cu is higher than that of deposited Ag or Cu. Our prior experiments demonstrate that the existence of both surface and embedded Ag and Cu is actually beneficial to the long-term antibacterial properties of the materials [8,9].

In order to further understand the differences between Cu and Ag PIII, the elemental depth profiles are compared. As shown in Fig. 3, there is a smaller amount of implanted Ag compared to Cu under similar PIII conditions. However, the amount of surface Ag is higher. This is believed to be due to the difference in the charge states in the

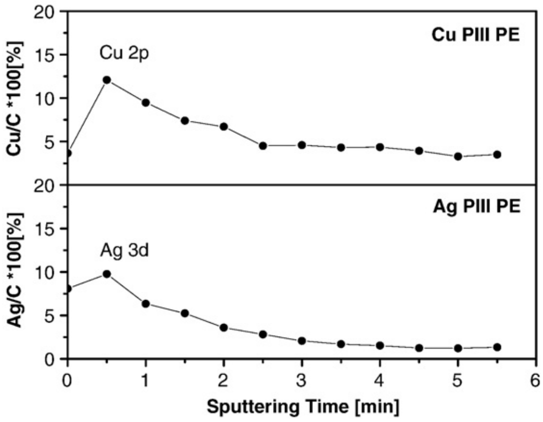

Fig. 3. Comparison of the Ag and Cu depth profiles obtained from the Ag PIII PE and Cu PIII PE under similar PIII conditions.

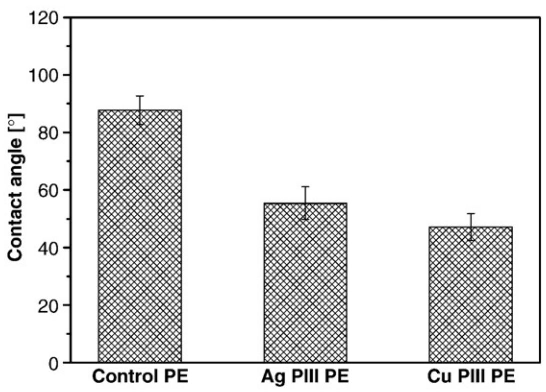

Fig. 4. Measured water contact angles on the control PE, Ag PIII PE, and Ag/N_2 PIII PE samples.

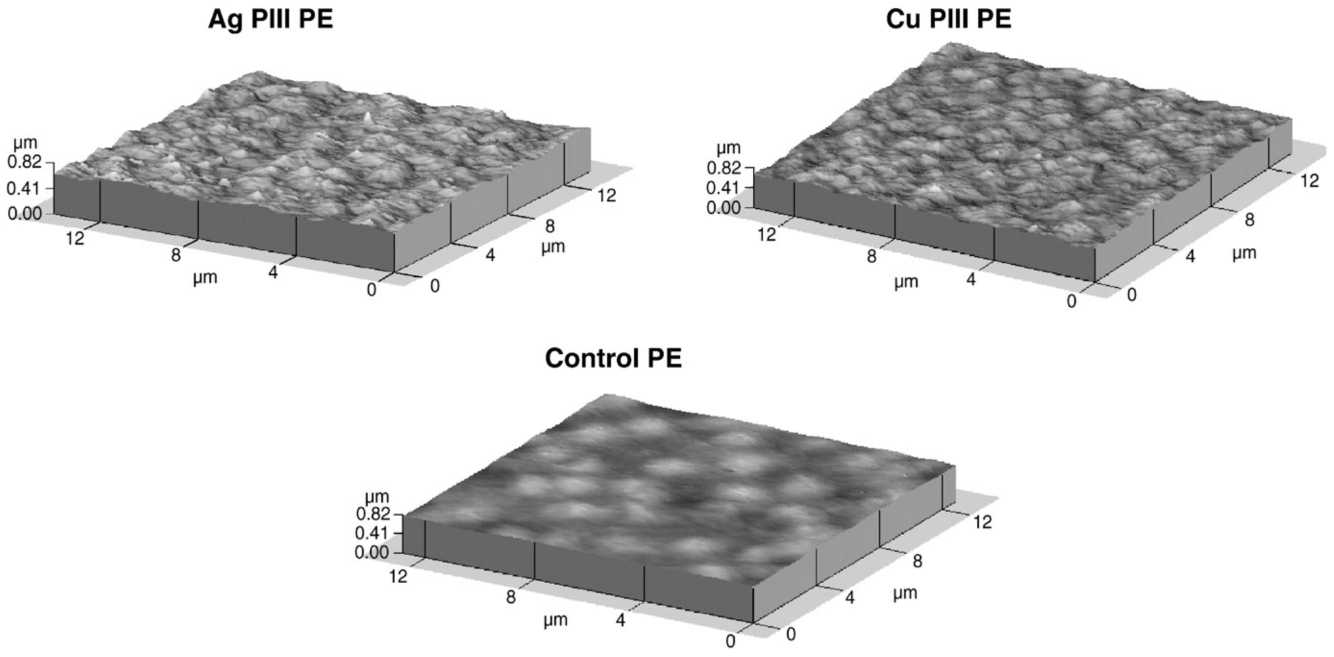

Fig. 5. AFM images of the control PE, Ag PIII PE, Cu PIII PE samples.

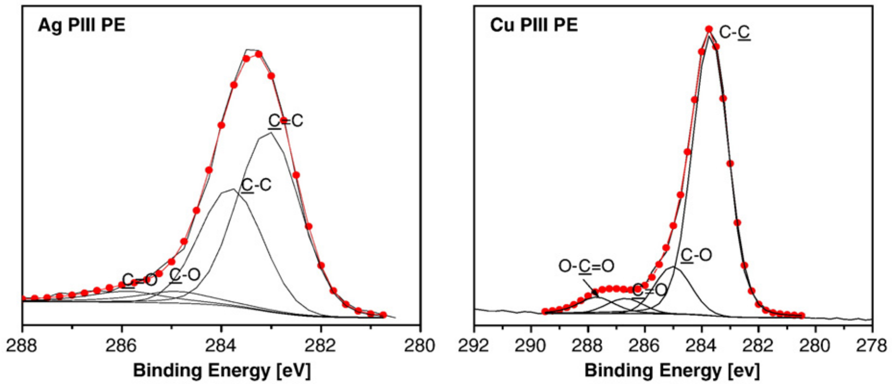

Fig. 6. C1s XPS spectra obtained from the Ag PIII PE and Cu PIII PE.

Ag and Cu plasmas [10,11]. A higher bias voltage is probably required to achieve a higher proportion of Ag implantation relative to deposition.

The surface contact angle plays an important role in the cell and bacterial behavior. Water is used in our experiments to evaluate the hydrophilicity of the control PE, Ag PIII PE, and Cu PIII PE. The results in Fig. 4 demonstrate that the contact angles are obviously reduced after both Ag and Cu PIII. That is to say, Ag PIII PE and Cu PIII PE yield better wetting properties compared to the control PE. This can be attributed to the change in the physical and chemical properties on the surface after PIII. Furthermore, the hydrophilicity affects the adhesion and growth of cells and bacteria on the surface.

The surface morphology is another important factor impacting cell and bacterial behavior, and thus AFM is utilized to determine the surface morphology. The AFM images acquired from the control PE, Ag PIII PE, and Cu PIII PE in Fig. 5 show that the surface roughness increases after PIII. This phenomenon may benefit cell and bacterial adhesion and may enhance the cell biocompatibility of PE surface.

XPS is conducted to characterize the C1s chemical state [12]. Fig. 6 shows that Ag PIII produces C=C double bonds in the surface region. In comparison, Cu PIII yields little C=C but many oxygen containing

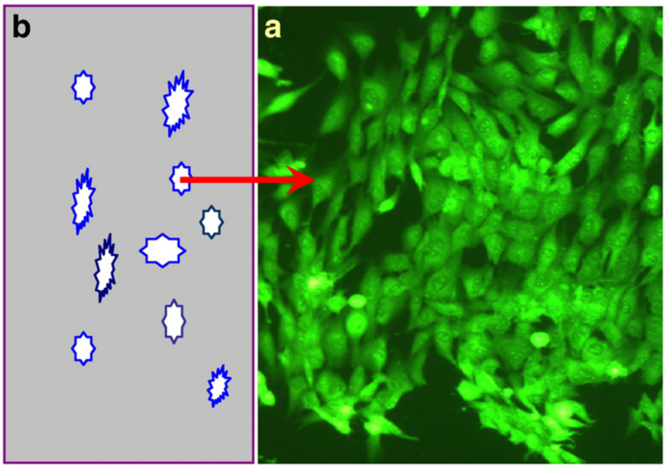

Fig. 7. hFOB cell growth behaviors on control PE: (a) image of hFOB cells on part of the surface and (b) distribution of hFOB cells on the entire surface.

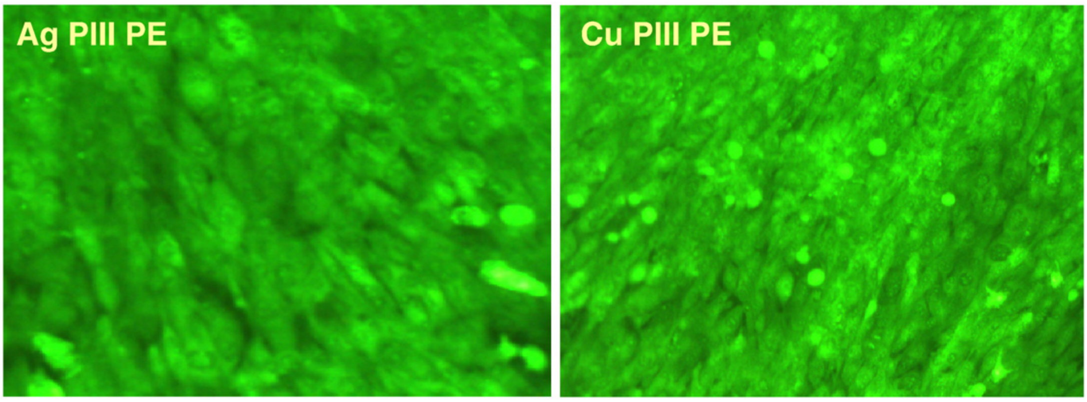

Fig. 8. hFOB cell growth behaviors after six day incubation.

groups such as C–O, C═O. The chemical states determined from the Ag PIII and Cu PIII samples can explain why Cu PIII PE has better wetting properties than Ag PIII PE due to the presence of more polar groups on Cu PIII PE.

To study the cell growth behavior on both modified PE samples, about 5×10^5 hFOB cells are put on the three samples (Ag PIII PE, Cu PIII PE and control samples) and subsequently incubated for 6 days. They are incubated 4 times, and then the samples are stained by Acridine Orange for fluorescence microscopy. The results indicate that cell growth is not uniform on the control PE surface (Fig. 7). Many cells aggregate in some areas (Fig. 7a) whereas very little cells are observed in other areas. The distribution of hFOB cells is illustrated in Fig. 7b. In contrast, as shown in Fig. 8, both the Ag PIII PE and Cu PIII PE samples are fully covered by bone cells. In comparison with the control PE, it is obvious that Ag and Cu PIII into PE yield excellent biocompatibility and bioactivity. It further demonstrates that the biocompatibility is not greatly related to the metal species but rather depends on the surface chemical functional groups formed [15,16]. As shown in Fig. 6, the C═C, C–O, and C═O groups on the surfaces favor cell growth.

4. Conclusion

We have investigated the effects of Ag and Cu plasma-implanted polyethylene and compared the biocompatibility of these two types of samples. PIII is an effective method to embed Ag or Cu in the near-surface region to enhance the surface antibacterial and biocompatible properties. The implanted Ag and Cu have similar elemental distribution and are located at a depth of several hundred nanometers under similar PIII conditions. In comparison with Cu PIII, there is a higher amount of surface Ag and less implanted Ag possibly due to the different charge states in the Cu and Ag plasmas. Cu PIII induces more polar oxygen containing groups on the PE surface than Ag PIII, and more C═C bonds occur on the Ag PIII PE surface. Bone cell assays demonstrate that the Ag PIII PE and Cu PIII PE samples exhibit excellent biocompatibility for bone cells. In summary, Ag and Cu PIII not only enhance the antibacterial properties but also improve the cell biocompatibility on PE.

Acknowledgement

This research was supported by the Hong Kong Research Grants Council (RGC) Competitive Earmarked Research Grant (CERG) No. CityU 112306.

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