Biocompatibility of silver and copper plasma doped polyethylene

Wei Zhang\textsuperscript{a,b}, Yunjun Luo\textsuperscript{b}, Huaiyu Wang\textsuperscript{a}, Shihao Pu\textsuperscript{a}, Paul K. Chu\textsuperscript{a,⁎}

\textsuperscript{a} Department of Physics & Materials Science, City University of Hong Kong, Tat Chee Avenue, Kowloon, Hong Kong, China
\textsuperscript{b} Department of Chemical Engineering Polymer Division, School of Materials Science and Engineering, Beijing Institute of Technology, Beijing, China

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=O 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.

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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 \textit{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.
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
1–2×10^{-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\textsubscript{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\textsubscript{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

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

**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\textsubscript{2} PIII PE
samples.
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. 5. AFM images of the control PE, Ag PIII PE, Cu PIII PE samples.](image1)

![Fig. 6. C1s XPS spectra obtained from the Ag PIII PE and Cu PIII PE.](image2)

![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.](image3)
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 $5 \times 10^5$ 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.

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