Effects of NH₃, O₂, and N₂ co-implantation on Cu out-diffusion and antimicrobial properties of copper plasma-implanted polyethylene

Wei Zhang a,b,d, Junhui Ji a, Yihe Zhang c, Qing Yan a, E.Z. Kurmaev e, A. Moewes f, Jun Zhao b, Paul K. Chu b,*

a Technical Institute of Physics and Chemistry, Chinese Academy of Sciences, Beijing 100080, China
b Department of Physics & Materials Science, City University of Hong Kong, Tat Chee Avenue, Kowloon, Hong Kong, China
c School of Materials Science and Technology, China University of Geosciences, Beijing 100083, China
d Graduate School of the Chinese Academy of Sciences, Beijing 100039, China
e Institute of Metal Physics, Russian Academy of Sciences-Ural Division, S. Kovalevskaya street 18, 620041 Yekaterinburg, Russia
f Department of Physics and Engineering Physics, University of Saskatchewan, Saskatoon, SK S7N 5E2, Canada

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Abstract

Metal antibacterial reagents are effective in the enhancement of the antimicrobial properties of medical polymers. However, incorporation of metal antibacterial reagents into polymers using conventional methods usually results in unstable antimicrobial effects. Our previous research demonstrates that plasma immersion ion implantation (PIII) can be used to effectively incorporate metal antibacterial reagents such as Cu into polyethylene (PE) in the near surface region up to several hundred nanometers without causing noticeable damage to the polymer matrix. In this work, various gases including NH₃, O₂, and N₂ were plasma-implanted in concert with Cu plasma immersion ion implantation to study the effects of these gas species on the release rate of Cu from the substrate. Our experimental results reveal that the copper depth profiles are not affected significantly by NH₃, O₂, or N₂ co-implantation and these gas elements have similar depth profiles as Cu. Chemical analyses demonstrate that polar functional C=O, C–O, C–N, C=N, and C≡N bonds formed in the substrate play an important role in regulating Cu out-diffusion. Among the three gas species, N₂ shows the best effects in regulating Cu out-diffusion and produces the best long term antibacterial properties. The Cu retention and out-diffusion mechanism in the ion-implanted polyethylene is described.

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1. Introduction

Polymers are commonly used as medical materials because of their superior properties such as easy processing, ductility, impact load damping, and excellent bio-comparability [1–6]. However, infection of medical polymers generated by microbes is one of the major clinical complications causing a high rate of mortality and morbidity [7–13]. Recent research activities have focused on the enhancement of the antimicrobial properties of medical polymers. The two conventional methods to enhance the surface anti-infection properties of medical polymers are the deposition of an antibacterial coating or introduction of an antibacterial reagent into the polymer matrix [14–17]. However, these techniques have many disadvantages involving short-lived antibacterial properties, unstable antibacterial properties, and rapid consumption of a large amount of antibacterial reagents. One of the ways to overcome these obstacles is to introduce an inorganic antibacterial reagent like Cu into the subsurface region of the polymer by plasma immersion ion implantation. The embedded Cu layer provides both instantaneous and long term antimicrobial effects [18–21]. In the work reported here, we investigate the influence of NH₃, O₂, or N₂ plasma co-implantation on the Cu release rate from polyethylene and the resulting antibacterial characteristics.
2. Experimental details

Low density polyethylene (PE) samples with dimensions of 2 × 2 × 0.2 cm were inserted into the plasma immersion ion implanter with a copper cathodic arc plasma source [18–24]. The arc was ignited using a pulse duration of 300 μs, repetition rate of 30 Hz, and arc current of 1 A. The copper plasma was guided into the vacuum chamber by an electromagnetic field [21–23]. NH₃, O₂ or N₂ was introduced into the vicinity of the copper arc discharge plume at a flow rate of 10 sccm (standard cubic centimeter) to conduct co-implantation. The dual PIII process 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. The working pressure in the vacuum chamber was 1–2 × 10⁻¹⁴ Torr and the implantation time was 10 min [20,22–24]. In this work, sample 1 refers to the sample plasma implanted with Cu only and samples 2, 3, and 4 underwent Cu/NH₃, Cu/O₂, and Cu/N₂ PIII, respectively.

The four different types of samples, namely control, Cu/NH₃, Cu/O₂, and Cu/N₂ PIII, with dimensions of 5 × 5 mm were immersed in 10 ml of simulated body fluids (SBF) at 37 ± 0.1 °C. The Cu concentration in the SBF was determined by inductively-coupled plasma mass spectrometry (ICPMS) after immersion times of 2, 7, 14, 21, and 28 days. The SBF was replenished after each sampling, and each data point is the average of four measurements.

The elemental depth profiles and chemical states were determined by X-ray photoelectron spectroscopy (XPS) employing a Physical Electronics PHI 5802 equipped with a monochromatic aluminum X-ray source [25]. The elemental depth profiles were acquired using argon ion sputtering. The sputtering rate of 1 nm/min was approximated using that of silicon oxide under similar conditions. X-ray emission spectra (XES) were obtained at the Advanced Light Source at Lawrence Berkeley National Laboratory. The non resonant Cu L₂,3 (3d⁴s → 2p transition) X-ray emission spectra were recorded at room temperature. All the measured spectra are normalized to the number of photons incident onto the sample monitored by a highly transparent gold mesh [26]. Attenuated total-reflection Fourier transforms infrared (ATR-FTIR) spectroscopy was conducted on a Perkin Elmer16 PC [27].

Escherichia coli ATCC10536 (E. coli) was used in our plate-counting test to determine the antibacterial characteristics [7,8]. Seventy percent ethanol was first employed to sterilize the samples. A 0.04 ml cell suspension (10⁶ CFU/ml) was added onto the modified surface and covered by a polyethylene (PE) film (15 × 15 mm) and then incubated at less than 90% relative humidity and 37 ± 1 °C 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 for 5 min. In order 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 calculated using a counting method, the details of which can be found elsewhere [21,24,29,30].

3. Results and discussion

3.1. Gas/metal plasmas co-implantation characteristics

The representative copper depth profiles of the four samples are shown in Fig. 1. Both copper and the implanted gas elements are located in the near surface region [20]. The Cu in-depth distributions in all four samples are quite similar.

![Fig. 1. Elemental depth profiles acquired by XPS from the four PE samples: sample 1 – control, sample 2 – Cu/NH₃ PIII, sample 3 – Cu/O₂ PIII, and sample 4 – Cu/N₂ PIII.](image-url)
indicating that they are not affected significantly by NH$_3$, O$_2$, or N$_2$ co-implantation. Moreover, the elements (N, O) have similar in-depth profiles as Cu and their close proximity allows possible physical or chemical interactions with Cu. The peak Cu concentration is about 11% Cu (by comparing the ratio of copper to carbon) and the surface Cu concentration is about 3% which arises from some surface deposition during PIII [18]. The presence of some surface Cu can provide immediate antibacterial effects [8].

3.2. Copper out-diffusion characteristics

Fig. 2 shows that a relatively large amount of Cu leaches into the SBF from all four samples after the first two days, primarily arising from the surface Cu. Fig. 2 shows the cumulative amounts of leached Cu, and so a slower increase implies a lower degree of out-diffusion. Afterwards, the Cu release rate diminishes. Our results indicate that different gases (NH$_3$, O$_2$, and N$_2$) have different impact on the Cu release rates. Compared to the Cu PIII PE sample (sample 1), the O$_2$ and N$_2$ plasma co-implanted samples (samples 3 and 4) show reduced Cu leaching rates. The Cu release from the NH$_3$ PIII sample (sample 2) is faster than samples 3 and 4 initially (up to the 5th day) and less afterwards. Although both NH$_3$ and N$_2$ contain N, their effects on the Cu release rates are different, and it can probably be explained by the different chemical structure in their substrates after PIII [18,19]. These effects will be discussed further in a latter section. Our results indicate that both N$_2$ and O$_2$ PIII can regulate Cu out-diffusion and give rise to steadier release rates.

3.3. Chemical structures

In order to investigate the chemical states in the substrate, high-resolution C1s XPS spectra were acquired after sputter cleaning the surface for 0.5 min. The spectra in Fig. 3 also show the individual deconvoluted subpeaks [25]. The presence of C=C bonds in all four spectra suggests dehydrogenation induced by plasma implantation [20]. In addition to C=C, different chemical bonds are observed from the NH$_3$, O$_2$, and N$_2$ PIII samples. For example, C=N and C=N bonds are.

Fig. 2. Cumulative amounts of Cu released into the SBF from the four samples after immersion times of 2, 7, 14, 21, and 28 days. The SBF was replenished after each sampling.

Fig. 3. Fitted C 1s high resolution spectra obtained from the 4 samples.
observed from sample 2, C–O and C=O bonds from sample 3, C–N, C=N and C≡N bonds are observed from sample 4. At the same time, the soft Cu L_{2,3} X-ray emission spectra (3d4s → 2p_{1/2,3/2} transition) in Fig. 4 show that based on the I(L_2)/I(L_3) intensity ratio of copper, the element exists in the metallic state rather than Cu oxides.

ATR-FTIR and Raman spectra are used to further determine the chemical state of the four samples. The ATR-FTIR spectra are depicted in Fig. 5. The peaks at 2921 and 2841 cm\(^{-1}\) that are the stretching vibration of C-H exhibit reduced intensity after plasma implantation [27]. It further corroborates dehydrogenation of the PE. The Raman spectra in Fig. 6 reveal different scattering yields, especially for the O_2 and NH_3 PIII polyethylene. This phenomenon can be ascribed to defects caused by ion implantation [27]. According to diffusion theory [30], increased defects generally enhance copper diffusion into the SBF solution. However, the opposite results observed in our experiments suggest that defects may not be the main factor affecting copper out-diffusion. The appearance of the peak at 1598.5 cm\(^{-1}\) in the Raman spectra of samples 2 and 3 also indicate dehydrogenation and conversion of sp\(^3\) into sp\(^2\) C along the polyethylene chain. However, the peak at 1598.5 cm\(^{-1}\) is not observed in the Raman spectrum of sample 4, suggesting that less dehydrogenation and formation of C–C and C≡N bonds occur after conversion of sp\(^3\) C to sp\(^2\) C in the polymer chains. C=C and C≡N bonds are more polar and thirsty for electrons and believed to detain Cu atoms more effectively. Consequently, a smaller release rate of Cu is observed during the first two days of immersion, and the release rate is steadier subsequently. Although both the NH_3 and N_2 plasmas introduce N into the PE samples, the effects are different. It may be because –C–NH_3 and –C–NH groups are formed in the substrate due to the scission of NH_3 according to the following possible reaction:

\[
\begin{align*}
\text{NH}_3 \xrightarrow{\text{scission}} \text{H} + \text{NH}_2
\end{align*}
\]

The C-NH_3 groups have saturated bonds and are ineffective in holding onto Cu and are believed to be the reason why the Cu diffusion rate in sample 2 is higher than those in samples 3 and 4 in the beginning. Our results indicate that the altered chemistry and polymeric structures play an important role in the Cu diffusion characteristics.

3.4. Antimicrobial properties

*E. coli*, which is one main source of nosocomial infection [7], was employed to assess the antimicrobial properties of the
plasma implanted samples for various immersion times of 0, 14, and 28 days [28,29]. Fig. 7 discloses that all four samples have excellent antibacterial effects (less than 95%) against E. coli with a cell concentration of 10^6 CFU/ml initially (day 0). Cu PIII can thus provide immediate antimicrobial effects. The antibacterial effectiveness of all the samples diminishes with immersion times. In comparison, samples 3 and 4 have better antibacterial performance than samples 1 and 2 and the results are consistent with the Cu release characteristics shown in Fig. 2. Our results indicate that the Cu/N2 PIII sample has the best long term antibacterial properties.

4. Conclusion

Copper plasma immersion ion implantation (PIII) is an excellent method to produce antibacterial effects on medical polymers such as polyethylene. Gas co-implantation in concert with Cu PIII can regulate the copper release rate from the substrates and enhance the long term effects. Our experimental results show that the NH₃ plasma does the least in regulating the Cu leaching rate whereas the N₂ plasma produces the best results. Chemical state analyses demonstrate that plasma implantation leads to dehydrogenation and produces C=O, –C=O, –C=N, and –C≡N bonds. These unsaturated bonds are believed to play important roles in the slower release of Cu from the substrate. In particular, more C≡N and –C≡N bonds in the Cu/N₂ PIII PE are the most effective. Plate counting antibacterial tests show that all the plasma-treated samples have excellent antibacterial performance against E. coli. However, as the immersion time increases, the Cu/N₂ PIII sample shows the best and steadiest antibacterial properties which are consistent with the Cu release data.

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