Antimicrobial polyethylene with controlled copper release

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Abstract: Good antiinfection properties of medical polymers, especially those used in artificial organs, are crucial to the minimization of microbial attack in nosocomial treatments. However, medical polymers fabricated by conventional methods usually have unstable and short-lived antimicrobial effects because of unsteady out-diffusion of the antibacterial species from the organic matrix. Here, we introduce a dual plasma implantation process to enhance the properties. An inorganic antibacterial element, copper, is introduced into a medical polymer, polyethylene (PE), by means of copper plasma immersion ion implantation (PIII) and a subsequent nitrogen PIII process is used to regulate the release of the implanted Cu. X-ray photoelectron spectroscopy and transmission electron microscopy reveal that a relatively large amount of copper of about 11% is implanted into PE to a depth of several hundred nanometers. Chemical analyses confirm that the implanted Cu does not bond with the polymer matrix. However, the N2 plasma treatment produces various functional bonds such as C=≡N and C≡≡N which exert appreciable influence on regulating the out-diffusion rate of copper. The large amount of embedded Cu, coupled with controlled release of the element to the surface, gives rise to excellent and long-lasting surface antibacterial properties of the plasma-treated polymer. The capability of controlling the release and storing the antibacterial reagent in a buried layer leads to better antimicrobial polymeric materials for medicine. © 2007 Wiley Periodicals, Inc. J Biomed Mater Res 83A: 838–844, 2007

Key words: antimicrobial; plasma immersion ion implantation; copper; polyethylene

INTRODUCTION

Medical polymers are widely used in the treatment of diseases and biomedical implants because of their excellent mechanical properties and biological properties.1–3 However, when medical polymers are implanted inside the human body, they can become places for microbes to adhere and breed, and thus infection of medical polymers is one of the major clinical complications.4–7 Nowadays, there is an increasing interest in the development of antinfective medical polymers by the biomedical industry. Antimicrobial properties on medical polymers can be achieved by two main approaches.8–12 The first technique is the deposition of antibacterial reagents directly onto the surface of the polymers by means of vapor deposition, sputter coating, or ion beam assisted surface modification and deposition. The second method is the direct incorporation of antibacterial regents into the polymers. Unfortunately, these approaches have common drawbacks such as compromised bulk properties, chemical pollution, and unstable antimicrobial properties. Such ineffectiveness can lead to a high rate of mortality and morbidity thereby significantly increasing health care costs.

In our previous work, we found that by means of plasma immersion ion implantation (PIII), Cu could be incorporated into the surface region of polyethylene (PE) to promote the antimicrobial properties.13 Low-energy (several keVs) Cu PIII can introduce a large amount of Cu into the polymer without causing too much damage to the polymer surface.13–19 In this work, medical-grade PE specimens were implanted with Cu in a plasma immersion ion implanter equipped with a copper cathodic arc plasma source and nitrogen PIII was performed to change the structure of the implanted region of the substrate.14–17 Compared with the single Cu PIII process, this dual
plasma implantation process (Cu/N₂ PIII) can better regulate the copper release rate and improve the long-term antibacterial properties of the PE samples. This process thus creates a buried (stored) layer of the antimicrobial reagent with the ability to control the release of Cu by means of N₂ PIII. The antibacterial properties of the treated PE can thus be significantly enhanced particularly with respect to the long-term effects.

**MATERIALS AND METHODS**

**Sample preparation**

Medical-grade PE samples (LDPE, 51215B Beijing Huaer) 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 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 copper plasma was guided into the vacuum chamber by an electromagnetic field. The Cu 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.13–15 Another PE sample underwent N₂ plasma implantation at the same time. The nitrogen gas was bled into the vicinity of the copper arc discharge plume at a flow rate of 10 sccm (standard cubic centimeter) with the other processing conditions being similar to those of Cu PIII.18 The working pressure in the vacuum chamber was 1–2 × 10⁻⁴ Torr and the implantation time was 10 min.

**Copper release rate determination**

Four samples of each type of samples (Cu PIII PE and Cu/N₂ PIII PE) with dimensions of 5 mm × 5 mm were immersed in 10 mL of simulated body fluids (SBFs) at 37 ± 0.1°C.20 The SBF has ionic concentrations similar to those of human blood plasma. Samples were taken out after 2, 7, 14, 21, and 28 days to determine the amount of leached copper after different immersion durations. Inductively-coupled plasma mass spectrometry (ICPMS) was employed to determine the Cu concentrations in the resulting SBF. The SBF was replenished each time and each data point represents the average of four measurements.

**Chemical and physical structure determination**

The elemental depth profiles and chemical states were determined by X-ray photoelectron spectroscopy (XPS; Physical Electronics PHI 5802).21 A monochromatic aluminum X-ray source was used and the elemental depth distributions were acquired using argon ion sputtering. The sputtering rate of 1 nm/min was approximated using that of silicon oxide under similar conditions. The cross-sectional transmission electron microscopy (TEM) image was acquired on a HITACHI H-800, and attenuated total-reflection Fourier transform infrared (ATR-FTIR) spectroscopy was conducted on a Perkin Elmer 16 PC 26.22

**Antimicrobial assays**

The antibacterial performance against *Escherichia coli* ATCC10536 was determined by the method of plate-counting.13,17,23 Seventy percent ethanol was used to sterilize the samples (2 cm × 2 cm × 0.2 cm) and then 0.04 mL solution of bacteria (10⁶ CFU/mL) was added onto the modified surface and covered by a PE film (1.5 cm × 1.5 cm). 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 (pH 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:

\[
R(\%) = \left( \frac{(B - C)}{B} \right) \times 100. \hspace{1cm} (1)
\]

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).

**RESULTS AND DISCUSSION**

XPS was used to characterize the in-depth copper profiles in the Cu PIII PE and Cu/N₂ PIII PE.13,21 Figure 1(a) shows that the implanted copper is located in the near surface region as a result of the low implantation energy. The amount of implanted copper is about 11% at the peak (by comparing the ratio of copper to carbon), and the surface Cu concentration is about 3% which stems from some surface deposition during PIII. Such surface Cu concentration offers immediate and direct killing of bacteria or inhibition of cells that are in contact with the materials surface.4,24 Based on the metal ion antimicrobial mechanism,2,7 Cu ions are consumed during the antibacterial reactions, and so the effects of surface Cu can be short-lived. Furthermore, if the surface Cu concentration is too high, there may be side effects on cells directly in contact with the materials surface. Therefore, our sample which has a relatively low amount of surface Cu and larger amount of embedded Cu has many advantages. Most importantly, the buried Cu serves as a continuous supply of the antibacterial reagent to the surface to produce longer lasting antimicrobial effects. As shown in the result acquired from the Cu/N₂ PIII PE sample [Fig. 1(b)], the in-depth copper profile is not affected significantly by N₂ plasma coimplantation. The nitrogen distribution is also similar to
that of copper and so chemical and physical interactions between the implanted N and Cu or polymer matrix in the implanted region can occur (to be discussed later in this paper). The cross-sectional TEM image (Fig. 2) of the Cu PIII PE sample reveals that the implanted Cu is segregated in the polymer matrix. In addition, no diffraction patterns can be obtained from the sample further confirming that the implanted Cu does not form crystalline structures in the polymer matrix. This segregated and unbonded Cu state is believed to facilitate effective out-diffusion.

The copper leaching rate to the surface directly impacts the surface antibacterial effects. Therefore, ICPMS is conducted to evaluate the release rate of the implanted Cu from the substrates. Figure 3(a) indicates high released quantities of Cu into the SBFs from the Cu PIII PE and Cu/N2 PIII PE after 2 days. It has been reported that this amount of Cu does not raise health concerns. Afterwards, Cu out-diffusion from the Cu PIII PE sample diminishes. Figure 3(a) shows the cumulative amounts of leached Cu, and so a slower increase implies a lower degree of out-diffusion. In comparison, the Cu leaching rate from the Cu/N2 PIII PE sample is steadier and approximately 10 ppb/day/cm². This suggests that the N2 plasma treatment is effective in regulating Cu out-diffusion and prolonging the surface antibacterial performance.

An *E. coli* suspension with a concentration of 10⁶ CFU/mL is employed to assess and compare the antimicrobial properties of the Cu PIII PE and Cu/N2 PIII PE samples at immersion times of 0, 14, 28 days [Fig. 3(b)]. Before they are immersed in SBF (that is day 0), both Cu PIII PE and Cu/N2 PIII PE have excellent antibacterial effects against *E. coli*, 96.2% and 95.5%, respectively. This mainly stems from the surface-deposited Cu that can deliver immediate antimicrobial effects. After immersion in SBF for 14 days, the Cu PIII PE and Cu/N2 PIII PE still possess good antibacterial performances against such a high cell suspension in spite of the reduced Cu release rates. The antimicrobial effects against *E. coli* are 70.6% and 84.3% respectively. It should be noted that the Cu/N2 PIII PE sample exhibits better antibacterial effects. The difference is even more evident after immersion for 28 days. Our results thus unequivocally demonstrate the excellent antibacterial effect of the dual Cu/N2 PIII treatment. The process allows for the continuous release of buried Cu to retain the surface antibacterial ability for a longer period of time. In separate experiments, we stored the prepared samples under room temperature in air for 6 weeks and compared their antimicrobial effects with those of freshly prepared samples. The antimicrobial effects against *E. coli* were found to hardly change indicating excellent long-lasting effects during normal storage.

It is well known that the antimicrobial properties are primarily related to the release of the antibacterial reagent, Cu. In the absence of guttering effects, Cu out-diffusion from the substrate is believed to follow Fick’s first law of diffusion:

\[
\frac{dN}{dt} = -DS \frac{dC}{dx},
\]

Figure 1. Elemental depth profiles acquired by XPS from: (a) Cu PIII PE and (b) Cu/N2 PIII PE. The argon ion sputtering rate of 1 nm/min is approximated using that of silicon oxide under similar conditions.

Figure 2. Cross-sectional TEM image of Cu PIII PE.
where $N$ is the amount of copper, $dC/dx$ is the Cu concentration gradient with distance, $t$ is the time, $S$ is the surface area of the samples, and $D$ is the diffusion coefficient. Here, $S$ is the same and $dC/dx$ is more or less the same because the same Cu PIII conditions are used for both samples. Consequently, the Cu diffusion rate, $dN/dt$, depends mainly on the diffusion coefficient, $D$. We propose a diffusion process from the substrate consisting of two zones, as schematically described in Figure 4. Diffusion in zone B is described by the above equation whereas in zone A also depends on the chemistry and guttering effects between Cu, N, and the PE matrix.

The Raman spectra of both the modified samples shown in Figure 5 exhibit the characteristic peaks of PE at 1060, 1127, 1293, and 1440 cm$^{-1}$. After implantation, it is found that the effect of photoluminescence on the Raman spectrum is enhanced. It may correspond to an increase in the amount of defects in the PE crystal caused by implantation of energetic particles. The experimental evidence suggests that dehydrogenation takes place and the chemical structure of PE is changed after plasma implantation.$^{19}$

![Graph](image1)

**Figure 3.** (a) Cumulative leached amounts of Cu from Cu PIII PE and Cu/N$_2$ PIII PE after various immersion times and (b) corresponding antimicrobial effects against *E. coli*.

![Diagram](image2)

**Figure 4.** Schematic diagram illustrating two zones in the copper implanted PE.

![Graph](image3)

**Figure 5.** Raman spectra acquired from Cu PIII PE and Cu/N$_2$ PIII PE.
A series of the high-resolution C 1s, N 1s, and Cu 2p XPS spectra were acquired from the two samples to further clarify the structural characteristics in zones A and B. The montages (Figs. 6–8) illustrate the change in the XPS peak shape and position at different stages of the depth profiling analysis. After sputter cleaning the surface for 0.5 min, the high-resolution C 1s and N 1s XPS spectra (S-2) at a depth with the maximum Cu concentration in zone A are deconvoluted by standard XPS software. The similarity in the high-resolution Cu 2p XPS spectra between the Cu PIII PE and Cu/N₂ PIII PE [Figs. 6(a,b)] samples suggests that N₂ PIII does not result in apparent changes in the chemical states of the implanted Cu in both cases. The S-1 spectra of both samples show that the surface Cu has the divalent state due to surface oxidation after exposure to air, whereas the S-2–10 spectra suggest that the embedded Cu atoms, mostly implanted, have the zero valence state. Hence, the implanted Cu atoms do not appear to bond with other atoms in the PE matrix.

Different chemical states are observed in the C 1s spectra acquired from the Cu PIII PE and Cu/N₂ PIII PE [Figs. 7(a,b)]. The S-2 spectrum of Cu PIII PE [Fig. 7(a)] corroborates the formation of $-\text{C} = \text{C}-$ bond, implying that dehydrogenation occurs during the plasma implantation. The S-2 spectrum of Cu/N₂ PIII PE [Fig. 7(b)] explicitly illustrates that Cu/N₂ PIII not only leads to the formation of $-\text{C} = \text{C}-$ bond but also produces a large amount of $-\text{C} \equiv \text{N}-$ and $-\text{C} = \text{N}-$ bonds resulting from chemical reactions between the nitrogen atoms introduced from the plasma and the polymer matrix. On the other hand, there is no change in the N 1s XPS spectra throughout the implanted depth. The fitted S-2 spectrum further shows that there are mainly $-\text{C} \equiv \text{N}-$ and $-\text{C} = \text{N}-$ bonds formed in the surface region after N₂ plasma coimplantation, and this result is con-
consistent with the C 1s in-depth spectra. These \(-\text{C}=\text{N}-\) and \(-\text{C}==\text{N}-\) bonds are more polar and thirsty for electrons than the \(-\text{C}=\text{C}-\) bond thereby increasing the interactions between Cu and the modified polymer matrix. These polar functional groups are believed to be primarily responsible for the retarded out-diffusion of Cu and regulate the leaching of copper in our immersion test.

CONCLUSION

PIII is an effective method to introduce a large quantity of metal inorganic antimicrobials like Cu into organic medical polymers such as PE up to a depth of several hundred nanometers without causing appreciable damage to the polymer matrix. The use of N$_2$ PIII in concert with Cu PIII produces new polar unsaturated functional groups such as \(\text{C}==\text{N}\) and \(-\text{C}==\text{N}\) in the near surface of the polymer. They play an important role in regulating the out-diffusion rate of Cu and prolonging the antibacterial effects significantly. Our work demonstrates that an inorganic antimicrobial agent can be effectively incorporated into an organic biomedical polymer and by using a nitrogen plasma treatment, the release of Cu to the surface can be regulated. Consequently, the antimicrobial ability of the treated polymer can be prolonged significantly to increase its usefulness in medicine.

References