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Ag and Ag/N_2 plasma modification of polyethylene for the enhancement of antibacterial properties and cell growth/proliferation

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Abstract

Polyethylene (PE) is one of the most common materials used for medical implants. However, it usually possesses low biocompatibility and insufficient antibacterial properties. In the work described here, plasma immersion ion implantation (PIII) is employed to implant silver into PE to enhance both its antibacterial properties and its biocompatibility. Our results show that Ag PIII can give rise to excellent antibacterial properties and induces the formation of functional groups such as C–O and C=C. These C–O and C=C groups on the modified surface can trigger the growth of the human fetal osteoblastic cell line (hFOB). Furthermore, combining N_2 and Ag PIII prolongs the antibacterial effects, but nitrogen-containing functional groups such as C–N and C=N created by N_2 co-PIII negatively impact proliferation of hFOB on the surface. According to our experimental investigation on cell proliferation, functional groups such as C–N and C=N created by nitrogen PIII are disadvantageous to cell growth whereas the C–O and C=C groups benefit cell growth. Both the antibacterial activity and biocompatibility of PE can be enhanced by means of the proper plasma surface treatment.

Keywords: Plasma immersion ion implantation; Silver; Polyethylene; Biocompatibility; Antibacterial

1. Introduction

Polymeric materials are widely used in biomedical implants and devices. However, it is difficult to find polymers that meet all the requirements, such as antibacterial ability, biocompatibility, bioactivity, hydrophilicity, roughness and mechanical properties [1]. Polyethylene (PE), one of the most common biomedical polymers possessing excellent mechanical properties, suffers from insufficient biocompatibility and bioactivity [1–7]. Moreover, the materials can be easily attacked by bacteria in vivo. One possible approach to achieving better disinfection and biocompatibility while retaining the favorable bulk properties is to modify the surface chemical composition and state [7].

Surface compatibility is usually investigated by monitoring cell adhesion and proliferation. A suitable material that favors cell adhesion generally also shows improved cell proliferation [7,8]. From an industrial prospective, suitable surface modification can transform inexpensive synthetic polymers into biomedical products with high added values [9,10]. Plasma immersion ion implantation (PIII) has been used to modify the surface of polymeric materials [11–14]. In this work, PE is plasma implanted with Ag in an attempt to enhance both the biocompatibility and the antibacterial properties. At the same time, nitrogen co-PIII is conducted to change the surface chemical states for better performance, and the effects of the surface chemical state on the behavior of bacteria and bone cells are studied and discussed [15,16].

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2. Experimental details

2.1. Sample preparation

Low-density polyethylene samples with dimensions of 2 cm × 2 cm × 0.2 cm were inserted into a plasma immersion ion implanter equipped with a silver cathodic arc plasma source [12,13]. The arc was ignited using a pulse duration of 300 μs, with a repetition rate of 30 Hz and an arc current of 1 A. The Ag PIII process was conducted by applying an in-phase bias voltage of −5 kV, with a repetition rate of 30 Hz and a pulse width of 300 μs, to the PE samples [15,16]. In the simultaneous Ag and N_2 PIII experiments, nitrogen gas was introduced into the vicinity of the silver arc discharge plume at a flow rate of 10 sccm (standard cubic centimeters). The dual PIII process was conducted by applying the same bias voltage as the Ag PIII treatment [15,16]. The working pressure in the vacuum chamber was 1–2 × 10^{-4} Torr.

2.2. Sample characterization

The elemental depth profiles and chemical states were determined by X-ray photoelectron spectroscopy (XPS) using a Physical Electronics PHI 5802 spectrometer [17]. A monochromatic aluminum X-ray source was used and elemental depth distributions were obtained using argon ion sputtering. The sputtering rate of 1 nm min^{-1} was approximated using that derived from silicon oxide under similar conditions. A cross-sectional transmission electron microscopy (TEM) image was acquired on a HITACHI H-800 microscope. After the plasma treatments were conducted on the PE samples, static contact angle measurements using distilled water as the medium were performed immediately by the sessile drop method on a Ramé-Hart (USA) instrument at ambient humidity and temperature [18]. Contact mode atomic force microscopy (AFM) was conducted on a Park Scientific Instrument Autoprobe Research System to evaluate the surface morphology on a scanned area of 15 μm × 15 μm [19].

2.3. Antibacterial assays

The antibacterial properties of plasma implanted samples were determined in vitro. Both the Ag PIII and Ag/N_2 PIII PE samples were immersed in 10 ml of simulated body fluid (SBF) at 37 ± 0.1 °C. The SBF has ionic concentrations similar to those of human blood plasma [20,21]. After immersion for 14 and 28 days, the samples were taken out and assayed for their antibacterial properties. The antibacterial performance against the Gram-negative *Escherichia coli* ATCC10536 was determined by the method of plate-counting. A 75% ethanol solution was used to sterilize the samples and then a 0.04 ml solution of bacteria (1–2 × 10^5 CFU ml^{-1}) was added onto the modified surface and covered by a PE film (15 mm × 15 mm). At a relative humidity 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 ± 0.2 To observe the active bacteria, 0.2 or 0.02 ml of the solution was put into 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:

$$R(\%) = ((B - C)/B) \times 100$$

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

2.4. Cell adhesion and proliferation [8,9,22]

Human fetal osteoblastic cells (hFOB 1.19 ATCC@number: CRL-11372) were used to study cell behavior on the control PE, Ag PIII PE and Ag/N_2 PIII PE samples. This cell line was maintained in the incubation liquid containing a mixture of 45% Dulbecco's modified Eagle's medium (Invitrogen Cat No. 11995-040), 45% F-12 (Invitrogen Cat No. 11765-047), and 10% fetal calf serum (Hyclone Cat No. SV30087.02). No antibiotic was added to the liquid. The control PE, Ag PIII PE, and Ag/N_2 PIII PE were sterilized by 75% ethanol for 5 h and then placed in a 24-well culture plate. To improve statistics, four measurements were taken for each type of sample. Drops of 1 ml of the incubation liquid containing the tested cells (hFOB cell line) were seeded on the sample surface. The number of tested cells is about 2 × 10^5 or 5 × 10^5. After the samples were incubated at 34 °C in 5% CO_2/air for 2–6 days, they were rinsed once with a phosphate-buffered solution (PBS) to remove weakly adherent cells. Afterwards, the cells on the surface were fixed in a mixture of 10% acetic acid and 90% methanol for 20 min, stained with 10 μg ml^{-1} acridine orange 10-nonyl bromide in PBS for 5 min, and rinsed with PBS. Finally, these samples were inspected under a fluorescence microscope.

3. Results

3.1. Chemical composition

XPS was used to obtain the elemental depth profiles from the Ag PIII PE and Ag/N_2 PIII PE [14,15]. As shown in Fig. 1a and b, the implant peak corresponds to about 9% Ag relative to C, implying that most of Ag is implanted into the surface region, although some (about 8% Ag relative to C) is deposited on the surface on both PE samples. N_2 plasma co-implantation has little effects on the distribution of Ag in the surface region. N_2 co-PIII has been observed to prolong the antibacterial characteristics, and its detailed role will be discussed later. The cross-sectional TEM image (Fig. 1c) of the Ag PIII PE sample reveals that

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