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Cellular responses to titanium successively treated by magnesium and silver PIII&D

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ABSTRACT

Surface modification of titanium based materials with balanced biological property, i.e. inhibit the adhesion of pathogenic bacteria but promote the positive functions of mammalian cells, is highly desired. Accordingly, titanium was successively treated with magnesium and silver plasma immersion ion implantation & deposition (Mg–Ag PIII&D). Scanning electron microscopy (SEM) observations revealed that nanoparticles were precipitated and bound to the titanium surface after Mg–Ag PIII&D. X-ray photoelectron spectroscopy (XPS) results indicated that these particles are metallic silver. Responses of both bacterial and mammalian cells were studied with *Escherichia coli* (*E. coli*) and osteoblast-like cell line MG63. The results indicated that the Mg–Ag PIII&D treated titanium inhibits the adhesion and proliferation of *E. coli*, but promotes the initial adhesion and alkaline phosphatase (ALP) expression of MG63 cells. It can be concluded that the antibacterial and osteogenic activities of titanium can be balanced by simultaneously taking advantage of the biological nature of magnesium and silver.

1. Introduction

Titanium and its alloys have been extensively used as materials for implantable biomedical devices because of their excellent properties in mechanics and corrosion resistance [1]. Although these devices have achieved high success rates in improving the life quality of aged or injured individuals, still two major complications may be encountered: the lack of osseointegration and the device associated infections [2,3]. Hence, surface modification of titanium based materials with balanced property, i.e. inhibit the adhesion of pathogenic bacteria but promote the positive functions of mammalian cells, is highly desired [4,5].

Silver nanoparticles (Ag NPs), known as a promising material for biomedical applications, are able to act strongly against a broad spectrum of bacterial and fungal species including antibiotic-resistant strains [6–9]. However, the ultra-small size and high mobility of Ag NPs also raise concerns about their potential cytotoxicity [10], e.g. diffusing Ag NPs may act as Trojan horses by entering mammalian cells and then releasing silver ions that damage their intracellular functions [11]. In order to balance the antibacterial activity and biocompatibility of silver nanoparticles, Ag NPs were immobilized onto titanium by a single step silver plasma immersion ion implantation & deposition (Ag PIII&D) [12]. Although these bound Ag NPs possess good compatibility to MG63 cells, the early stage spreading (within the first hours) of the cells on the Ag PIII treated surface, due to its hydrophobicity, is not as good as that on the bare titanium. On the other hand, magnesium (Mg) was suggested to interact with the integrins of mammalian cells, leading to enhanced cell adhesion and stability [13]. Therefore, introducing magnesium into the Ag PIII&D treated titanium may be a good strategy to improve the initial adhesion of MG63 cells, provided that the introduction does not deteriorate the antibacterial performance of the material.

Accordingly, pure titanium was successively treated by plasma immersion ion implantation & deposition (PIII&D) with ion sources from filtered cathodic vacuum arcs of magnesium and silver (designated Mg–Ag PIII&D), respectively. According to the results obtained, we conclude that the property of titanium can be balanced by simultaneously taking advantage of the biological nature of nutrient and toxic components, such as magnesium and silver.

2. Experimental

2.1. Plasma immersion ion implantation & deposition (PIII&D)

A silver and/or magnesium cathodic arc was used as the plasma source in plasma immersion ion implantation & deposition. Three different types of PIII&D samples can be fabricated, i.e. Mg PIII&D at 40 kV for 1.0 h, Ag PIII&D at 30 kV for 0.5 h, and Mg PIII&D at 40 kV for 1.0 h followed by Ag PIII&D at 30 kV for 0.5 h (designated Mg–Ag PIII&D). A schematic illustration of the PIII&D apparatus can be found in handbook [14]. A magnetic duct with a curved shape was inserted between the plasma source and the main chamber to remove macroparticles produced from the cathodic arc. The cathode rod (10 mm in diameter) was made of 99.99% pure metals. The discharge of the metals was controlled by the main arc current between the cathode and anode. By applying a pulsed high voltage to the samples (CpTi, in this work, supplied by BAOTIGROUP, P.R. China), metal ions were implanted.

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The main arc current and pulsed high voltage applied to the target were synchronized at a pulsing frequency of 7 Hz. Prior to PIII&D, the samples were cleaned for 15 min with a radio frequency (RF) argon (Ar) plasma source at a bias of −500 V. During PIII&D, the sample stage was actively cooled by circulating water to keep the sample temperature at about 25 °C.

2.2. Surface chemistry and structure characterization

The surface morphology of the samples was examined by scanning electron microscopy (SEM, JEOL JSM-6700F, Japan). The depth profiles and chemical states of specific element were determined by X-ray photoelectron spectroscopy (XPS) (Physical electronics PHI 5802, USA) with a Mg K_αX-ray source. The XPS depth profiles are plotted on a depth scale based on a sputtering rate calculated from a SiO_2 reference under similar conditions. The peak fit and deconvolution were performed on XPSpeak with a fixed 20% Gaussian–Lorentzian value.

2.3. Antibacterial assay

The antibacterial activity of the concerned samples was evaluated using *Escherichia coli* (ATCC 25922). The samples were sterilized in an autoclave at 121 °C for 40 min. A solution containing the bacteria at a concentration of about 1.0^6 cfu/ml was introduced onto the sample to a density of 0.06 ml/cm^2. The samples with the bacterial solution were incubated at 37 °C for 24 h. For morphology examinations by SEM, the specimens were dehydrated in a series of ethanol solutions (30, 50, 75, 90, 95, and 100 v/v%) for 10 min each sequentially, with the final dehydration conducted in absolute ethanol (twice) followed by drying in the hexamethyldisilazane (HMDS) ethanol solution series.

2.4. Cell viability assay

The viability of osteoblast-like MG63 cells was determined using the alamarBlue™ assay (Invitrogen) that measured the accumulative metabolic activity. Five samples were tested for each incubation period (1, 3, and 7 days). For each incubation period, the culture medium was removed and 1.0 ml of the fresh medium with 5% alamarBlue™ was added to each well. After incubation for 5 h, 100 μl of the culture medium was transferred to a 96-well plate for measurement. Accumulation of reduced alamarBlue™ in the culture medium was determined by an enzyme labeling instrument (Thermo Scientific) at extinction wavelengths of 570 nm and 600 nm. The operation procedures and calculation of cell viability of cells followed the instruction of the alamarBlue™ assay.

2.5. Adhesion and spreading assay

Osteoblast-like MG63 cells were seeded on the samples in 24-well plates at a density of 1.0×10^4 cell/ml (1 ml per well). After incubating for 1 h and 3 h, cells were fixed in 4% paraformaldehyde for 20 min at room temperature. And then, 0.1% Triton X-100 was added to the samples for 2 min before staining the actin cytoskeletons. Samples were incubating with FITC–Phalloidin (Enzo Life Sciences, UK) at room temperature in the dark for 60 min. Finally, the nuclei were stained with 40,6-diamidino-2-phenylindole dihydrochloride (DAPI) (Invitrogen). The cell morphology on different samples was observed using a fluorescence microscope (Olympus, GX71, Japan).

2.6. Alkaline phosphatase activity assay

For alkaline phosphatase (ALP) staining, the MG63 cells on different substrates, after being cultured for 3 days, were fixed with citrate buffered acetone for 30 s, incubated in a mixture of naphthol AS-MX phosphate and fast blue RR salt (Sigma-Aldrich) for 30 min, and immersed in Mayer's Hematoxylin solution for 10 min.

2.7. Statistical analysis

Statistically significant differences (p) were measured according to the one-way analysis of variance and Tukey's multiple comparison tests (GraphPad Prism 5 software package).

3. Results

3.1. Surface characteristics

Fig. 1 shows the microstructure evolution of the titanium surface after undergoing Mg PIII&D at 40 kV for 1.0 h, Ag PIII&D at 30 kV for 0.5 h, and Mg PIII&D at 40 kV for 1.0 h followed by Ag PIII&D at 30 kV for 0.5 h (designated Mg–Ag PIII&D). The original titanium surface was flat (Fig. 1a). No nanostructure was produced by Mg PIII&D

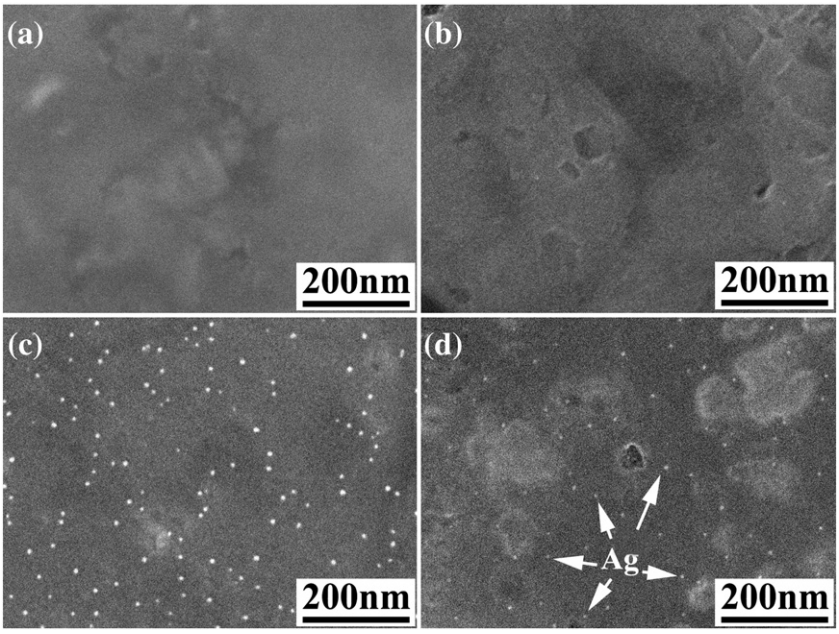

Fig. 1. SEM view of the titanium surfaces: (a) CpTi; (b) Mg PIII&D; (c) Ag PIII&D; (d) Mg–Ag PIII&D.