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Influence of implantation voltage on the biological properties of zinc-implanted titanium

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ABSTRACT

Titanium (Ti) has been widely used as orthopedic and dental implants, it is very important to improve the osteogenic activity and antibacterial ability of titanium implants. The objective of this work was to investigate the osteogenic and antibacterial ability of the zinc (Zn) ion implanted Ti at different implantation voltage. X-ray photoelectron spectroscopy results indicate that Zn was successfully implanted into titanium. Zn-implanted titanium surfaces exhibit enhanced cell proliferation ability, alkaline phosphatase (ALP) activity, and partial resistance to both *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*). The excellent osteogenic and antibacterial ability are highly related to the implantation voltage. The cell proliferation and antibacterial ability can be enhanced by increasing the implantation voltage from 15 kV to 30 kV, indicating that Zn implantation at 30 kV is more beneficial to both osteogenic and antibacterial ability.

1. Introduction

Titanium (Ti) and its alloys which possess excellent mechanical properties, good corrosion resistance and outstanding biocompatibility are widely used as standard orthopedic and dental implants [1]. Despite the fact that Ti implants have achieved a high success rate clinically, their osseointegration and antibacterial ability are inadequate, implant failures still occur owing to implant-associated bacterial infections and remain one of the intractable complications [2]. Therefore, surface modification of Ti implants which is effective and simple is urgently needed to improve both osteogenic and antibacterial ability simultaneously.

In recent years, Ag ion implantation has been considered as an important technology to endow biomaterials with specific antibacterial effect [3–5]. However, Ag is a toxic element, which may be restricted in the application of biomaterials, considering the stringent safety requirement for biomaterials. As an essential trace element, zinc (Zn) has been widely reported to have the ability to enhance osteogenetic function [6], promote cell proliferation, alkaline phosphatase (ALP) activity and osteoblast marker gene expressions [7,8]. In addition, Zn ions show antibacterial ability on various kinds of bacteria including both Gram negative and Gram positive bacteria at appropriate concentrations [9–11]. Our previous study demonstrated that titanium after Zn ion implantation for various times at the same implantation voltage can enhance the proliferation of osteoblasts, stimulate the initial adhesion and spreading activity, alkaline phosphate activity of rat bone mesenchymal stem cells (rBMSCs) [12]. It was also observed that Zn-implanted titanium exhibited partly antibacterial ability on both *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*).

Plasma immersion ion implantation (PIII) technology is a non-line-of-light process, which has been widely used for the surface modification of various biomaterials in recent years [1]. PIII technique has been demonstrated to enhance the osteogenic and antibacterial ability of various kinds of biomaterials [8,13]. In the present work, Zn was introduced into titanium surface using plasma immersion ion implantation (PIII) at different implantation voltage for 1.5 h to achieve both osteogenic and antibacterial ability. The influences of implantation voltage on the behaviors of osteoblasts, rBMSCs, and bacteria were investigated.

2. Experimental details

2.1. Plasma immersion ion implantation (PIII)

Titanium plates (10 mm × 10 mm × 1 mm) were ultrasonically cleaned in acetone, ethanol and deionized water successively, followed by 5 wt% oxalic acid etching at 100 °C for 2 h and ultrasonically cleaned in deionized water for 30 min. The titanium plates were cleaned by radio frequency (RF) argon ions for 15 min at a bias of −550 V before zinc ion implantation. The pulsed high voltage and arc current were synchronized at a pulsing frequency of 5 Hz. A zinc cathodic arc was utilized as the plasma source and zinc ions were implanted into titanium surface at 15 kV and 30 kV of implantation voltage for 1.5 h (Represented as Zn15 and Zn30, respectively). During ion implantation, the sample stage was cooled by circulating water in order to keep the specimens temperature at nearly 25 °C.

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2.2. Surface characterization

2.2.1. Surface structure and chemistry

The surface morphologies of titanium before and after Zn ion implantation were examined by scanning electron microscopy (SEM) (S-3400, Japan). The chemical states and zinc elemental depth profiles were measured by X-ray photoelectron spectroscopy (XPS, Physical electronics PHI 5802).

2.2.2. Dynamic potential polarization test

The dynamic potential polarization tests were acquired in 0.9% NaCl solution (at a pH of 7) using a CHI760C electrochemical workstation (CHI Instruments, Inc. Shanghai).

2.3. Cell proliferation assay

The proliferation ability of mouse osteoblastic MC3T3-E1 cells was determined using the alamarBlue™ assay (AbDSerotec Ltd., UK). Three samples were examined for each incubation period (1, 4, 7 days). After each incubation time, the culture medium was removed and 0.5 ml of fresh medium with 10% alamarBlue™ was added, the samples with the cells were incubated for another 4 h. Afterwards 100 μl of the medium were transferred to a 96-well plate (Nunc, USA). Accumulation of reduced alamarBlue™ in the medium was tested using an enzyme labeling instrument (BIO TEK, ELX 800), at extinction wavelengths of 570 nm and 600 nm. Calculation of proliferation rate followed the instruction of the alamarBlue™ assay.

After each incubation period, the specimens with the MC3T3-E1 cells were washed with PBS thrice and fixed with 2.5% glutaraldehyde. Before SEM examination, the specimens were dehydrated in gradient ethanol solutions (30, 50, 75, 90, 95 and 100 v/v %) for 10 min each sequentially, followed by drying in the hexamethyldisilazane ethanol solution series.

2.4. Alkaline phosphate activity assay

Rat BMSCs (Stem Cell Bank, Chinese Academy of Sciences, Shanghai, China) were seeded on the various surfaces to evaluate the ALP activity. After culturing for 7 and 14 days, the specimens with the cells were rinsed with PBS twice and fixed with 4% paraformaldehyde (PFA) solution (Sigma, USA), followed by incubating in a mixture of naphthol AS-MX phosphate and fast blue RR salt for ALP staining. For the quantitative analysis, the cells were incubated with *p*-nitrophenyl phosphate (*p*NPP) (Sigma, USA) at 37 °C for 0.5 h. The ALP activity was determined by measuring the absorbance at a wavelength of 405 nm, and the ALP levels were normalized to the total protein content, which was determined by the BCA protein assay.

2.5. Antibacterial assay

The antibacterial ability of the concerned specimens was evaluated using both *E. coli* (ATCC 25922) and *S. aureus* (ATCC 25923). The samples were sterilized in 75% ethanol for 2 h. 60 μl suspension with a bacterial concentration of 10^7 cfu/ml was dripped on each sample surface. After incubation at 37 °C for 24 h, the dissociated suspension was collected and introduced into an agar culture plate for further incubation for 24 h. The active bacteria were counted in accordance with the National Standard of China GB/T 4789.2 protocol and the antibacterial ratio was calculated as follows:

$$\frac{(A-B)}{A} \times 100\%,$$

where A is the number of bacteria on the control sample (cfu/sample) and B is the number of bacteria on the testing specimens (cfu/sample).

In the SEM test, a suspension with a concentration of 10^7 cfu/ml was dripped onto the various surfaces to a density of 60 μl/cm^2, after incubation at 37 °C for 24 h, fixed with 2.5% glutaraldehyde, and dehydrated according to the same procedures used in Section 2.3.

2.6. Statistical analysis

All the data were expressed as means ± standard deviations. Statistically significant differences (P) were determined using two-way analysis of variance, P values < 0.05 were considered statistically significant. Statistical analysis was done by a GraphPad Prism statistical software package.

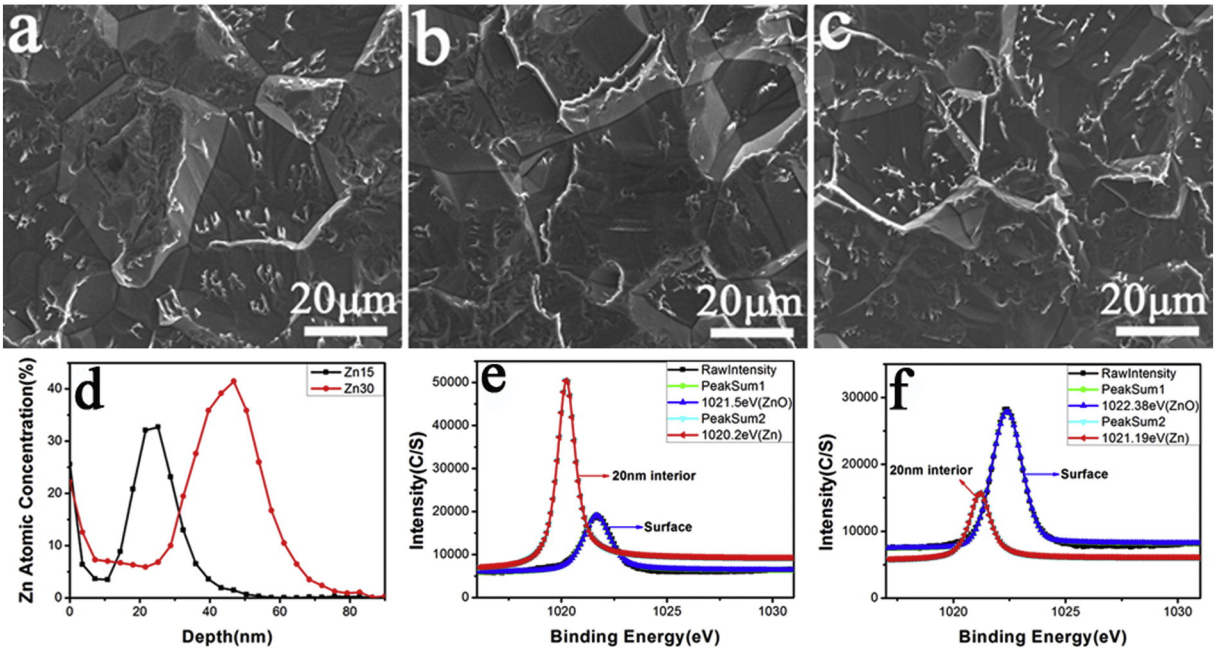

Fig. 1. Surface morphologies of the oxalic acid etched titanium before and after Zn ion implantation: (a) As-etched Ti, (b) Zn15, (c) Zn30; (d) Zn depth profiles of Zn15 and Zn30; (e) high resolution spectra obtained from the surface of Zn15; (f) high resolution spectra obtained from the surface of Zn30.

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