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Zinc-ion implanted and deposited titanium surfaces reduce adhesion of *Streptococccus mutans*

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ABSTRACT

While titanium (Ti) is a commonly used dental implant material with advantageous biocompatible and mechanical properties, native Ti surfaces do not have the ability to prevent bacterial colonization. The objective of this study was to evaluate the chemical composition and bacterial adhesive properties of zinc (Zn) ion implanted and deposited Ti surfaces (Zn–PIIID–Ti) as potential dental implant materials. Surfaces of pure Ti (cp–Ti) were modified with increasing concentrations of Zn using plasma immersion ion implantation and deposition (PIIID), and elemental surface compositions were characterized by X-ray photoelectron spectrometry (XPS). To evaluate bacterial responses, *Streptococcus mutans* were seeded onto the modifiedTi surfaces for 48 h and subsequently observed by scanning electron microscopy. Relative numbers of bacteria on each surface were assessed by collecting the adhered bacteria, reculturing and counting colony forming units after 48 h on bacterial grade plates. Ti, oxygen and carbon elements were detected on all surfaces by XPS. Increased Zn signals were detected on Zn–PIIID–Ti surfaces, correlating with an increase of Zn-deposition time. Substantial numbers of *S. mutans* adhered to cp–Ti samples, whereas bacterial adhesion on Zn–PIIID–Ti surfaces signficantly decreased as the Zn concentration increased ($p < 0.01$). In conclusion, PIIID can successfully introduce Zn onto a Ti surface, forming a modified surface layer bearing Zn ions that consequently deter adhesion of *S. mutans*, a common bacterium in the oral environment.

1. Introduction

Dental implants are commonly used as a conventional treatment for restoring dentition defects and anodontia. Pure titanium (Ti) and Ti alloys have been recognized as standard dental materials because of their excellent mechanical properties, biocompatibility, corrosion resistance and machinability. However, an ideal dental implant material used in the oral bacterium environment not only should integrate with the host tissue, but should also exhibit anti-bacterial properties; the latter of which is not prevalent in Ti.

Studies indicate that plaque accumulation occurs around a Ti implant (especially at the neck of an artificial tooth) soon after dental implantation and restoration, which can lead to peri-implantitis and a subsequent loss of osseointegration at the dental implant interface [1,2]. Consequently, the implant loosens or even displaces completely [3,4]. Thus, it is essential that supra- and sub-gingival surfaces of dental implants remain plaque-free.

Presently, some studies aim to improve the anti-bacterial properties of dental implant materials, primarily by the incorporation of Ag or F^- ions into the biomaterial [5,8]. It is also known that high concentrations of Zn exhibit anti-bacterial properties [9–11]; indeed, Petrini et al. demonstrated that Ti surfaces chemically modified with ZnO significantly reduced the viability of five streptococcus bacterial strains in vitro [12].

While there are many techniques to modify implant material surfaces, plasma immersion ion implantation and deposition (PIIID) has attracted wide attention for biomedical applications [13,14], particularly because surface wear resistance, corrosion resistance and anti-fatigue properties of metal materials treated with PIIID have been shown to significantly improve [15]. Previous shortcomings of poor coating adhesion on Ti surfaces (easy exfoliation) have been overcome by using PIIID [16]. Additionally, PIIID has demonstrated little effect on the mechanical properties and surface roughness of biomaterials [17]. Using PIIID to incorporate anti-bacterial ions onto Ti surfaces, Yoshinari et al. found that

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modifications performed with F^- ion implantation significantly inhibited the growth of both *P. gingivalis* and *A. actinomycetemcomitans*, as opposed to the bacterial growth on polished Ti [5]. Similarly, Zhang et al. reported enhanced anti-bacterial properties of polyethylene that were achieved by the incorporation of Ag–PIIID [8]. Recently, PIIID was used to deposit Zn onto Mg–Ca alloys to evaluate the effect of Zn implantation on corrosion resistance; however, the anti-bacterial properties were not investigated [18].

Here, we aimed to introduce Zn onto a pure Ti surface using PIIID to potentially enhance the anti-bacterial properties of the modified Ti surface. We evaluated the chemical composition of the modified Ti surfaces and observed the effect of the surface modification on the adhesion and relative growth of *S. mutans*, a common bacterium in the oral environment.

2. Materials and methods

2.1. Preparation and surface characterization of samples

Commercial class-4 pure Ti (cp–Ti, Baoji, Shanxi Province, China, TA3 purity 99.99%) was fabricated into disc-shaped samples measuring 10 mm in diameter and 1 mm in thickness, and discs were then ground and polished on a grinding machine to an average surface roughness (Ra) of 0.4 μm. The PIIID process was conducted on a G4 PIIID device developed by the State Key Laboratory of Advanced Welding Production Technology (Harbin Institute of Technology, Heilongjiang Province, China). Briefly, the Ti body material underwent repeated ultrasonic cleaning with pure acetone followed by absolute ethanol before placement in a vacuum chamber. The body material surface was then Ar^+ sputter-cleaned for 10 min prior to PIIID. The implantation source included a pulsed cathodic arc plasma with the following parameters: 20 kV of voltage (V), 300 μs of implantation pulse width (τ), 300 μs of Zn cathodic arc pulse width and a 1×10^{-1} Pa working gas pressure (P). Four groups of modified discs were fabricated by increasing the Zn-ion implantation and deposition time by 20 min intervals: Zn–Ti-20, Zn–Ti-40, Zn–Ti-60 and Zn–Ti-80 (12 discs per group). A control group consisted of cp–Ti discs without Zn-ion implantation or deposition.

The X-ray photoelectron spectrometry (XPS) analysis was conducted using a VG Scienta ESCALab 220i-XL photoelectron spectrometer, and the excitation source was an Al Kα X-ray with approximately 300 W of power. The basic vacuum degree for the analysis was 3×10^{-7} Pa. Electron bonding energy was adjusted by the carbon pollution peak (C1s = 284.8 eV).

The XPS elemental percentage values (including C content) were calculated based on an average of 12 discs per group, and were determined using the built-in Elemental Relative Quantitative Formula in the XPS software that analyzes peak intensity and relative element sensitivity factors.

2.2. Bacterial culture and seeding

S. mutans type strains UA159 (reference strains from the bacteria library of the Research Institute of Beijing Stomatological Hospital) were used in the experiments. Freeze-dried strains of the experimental bacteria were recovered and seeded on mannitol salt agar (MSA) plates and cultivated for 48 h in a micro-aerobic environment (37 °C) created by an anaerobic cultivation system (Anoxomat Mark II, MART Microbiology Co., Ltd., Holland). After the culture was confirmed as pollution-free by a Gram stain, the experimental bacteria were seeded on MSA plates for an additional 48 h at 37 °C. The strains were then suspended with phosphate buffer saline (PBS, pH 7.2, 0.2 mol/L) at a density of 1×10^9 CFU/mL. Sterilized Ti discs were placed in a 24-well plate and 100 μL of UA159 bacterial suspension was plated on each Ti disc surface. MSA liquid medium (900 μL) was gently added into the well from the side wall, and the samples were incubated at 37 °C in a micro-aerobic environment for 48 h according to previous protocols [19].

2.3. Qualitative assessment of bacterial adhesion on disc surfaces

Four discs from each group were removed from the 24-well tissue culture plate, and surfaces were gently cleaned with PBS, fixed in glutaraldehyde (2.5%) for 24 h, washed with PBS and dehydrated with a series of ethanol. The discs were exposed to a critical drying point and a conductive gold coating and then observed with a Hitachi S-520 scanning electron microscope to evaluate the adhesion of *S. mutans* on the sample surfaces.

2.4. Quantitative assessment of bacterial adhesion

The eight remaining discs from each group were gently rinsed to remove unbound bacteria. Subsequently, 1 mL of sterile PBS was added to each disc and samples were exposed to ultrasonic vibrations for 1 min to release bound bacteria. The bacterial liquid was diluted according to a 10-fold serial dilution of 1:100,000. Next, 100 μL of the diluted bacterial liquid was collected from each group and smeared over MSA plates evenly. After 48 h of micro-aerobic

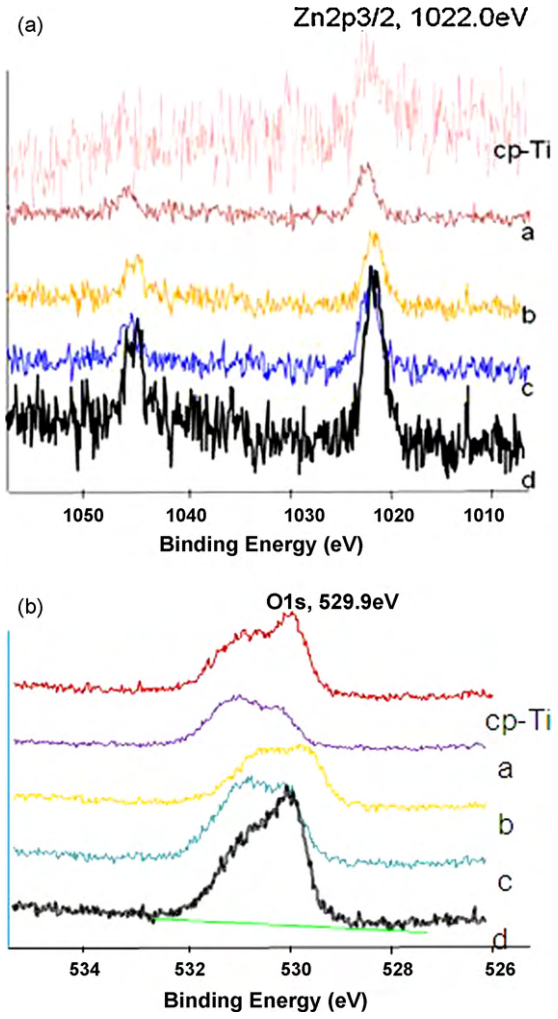

Fig. 1. XPS spectra of the non-implanted titanium surface, cp–Ti, and the Zn–PIIID–Ti surfaces. (a, b, c, d) represent 20, 40, 60 and 80 min implantation/deposition time periods, respectively.