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Surface modification of TiO_2 coatings by Zn ion implantation for improving antibacterial activities

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Abstract. **TiO_2 coating has been widely applied in orthopaedic and dental implants owing to its excellent mechanical and biological properties. However, one of the biggest complications of TiO_2 coating is implant-associated infections. The aim of this work is to improve the antibacterial activity of plasma-sprayed TiO_2 coatings by plasma immersion ion implantation (PIII) using zinc (Zn) ions. Results indicate that the as-sprayed TiO_2 coating is mainly composed of rutile phase. Zn-PIII modification does not change the phase compositions and the surface morphologies of TiO_2 coatings, while change their hydrophilicity. Zn-implanted TiO_2 coatings can inhibit the growth of *Staphylococcus aureus* (*S. aureus*) and *Escherichia coli* (*E. coli*), and the ability to inhibit *S. aureus* is greater than that to *E. coli.* Zn ion release and reactive oxygen species may be attributed to improving the antibacterial activity of TiO_2 coating. Therefore, Zn-PIII TiO_2 coatings on titanium suggest promising candidates for orthopaedic and dental implants.**

Keywords. **TiO_2; zinc; plasma spray; plasma immersion ion implantation; antibacterial.**

1. Introduction

Ti and/or TiO_2-based materials are successfully used as biomedical implants. Previous studies have presented that the TiO_2 layers improved the cellular behaviours of the osteoblast and the bone formation [1–4]. However, some failures caused by bacteria or microorganism infection occur after implantation [5]. Therefore, it is important to improve the antibacterial activities of implant surfaces and limit possible infection during and after surgery.

Various TiO_2-based materials containing metal atoms or ions such as silver (Ag), zinc (Zn) and copper (Cu) have been developed to improve the antibacterial characteristics of Ti implants [5–7]. Zn is an important trace element in human bone and plays an important role in improving the adhesion, proliferation and differentiation of bone cells [8]. Zn is also well known to exhibit inhibitory activity and antimicrobial effect on various bacteria [9–11]. In recent times, Zn-containing materials have been demonstrated owing to their excellent antibacterial activities and applications in dental and orthopaedic fields [12–14]. Furthermore, incorporation of Zn can enhance the antibacterial activity and biocompatibility of TiO_2 coatings [8].

Plasma spraying is a well-established coating technique which has been widely used in biomedical implants [15]. In our previous works, the production of TiO_2 coatings using the plasma spraying technique was succeeded. The TiO_2 coating was confirmed to have good mechanical properties, high bonding strength to substrates and excellent biocompatibility, suggesting a potential material for implants [16–18]. However, the as-prepared TiO_2 coating is lack of antibacterial activity. In order to address this aspect, Li *et al* [19] coated titanium alloy with Ag/TiO_2 and reported an enhanced antibacterial property of TiO_2 coating against *Escherichia coli* (*E. coli*). However, the antibacterial mechanism of Ag raises concerns about their potential cytotoxicity [20–22]. Plasma immersion ion implantation (PIII) as a suitable and versatile method without the line-of-sight process can introduce any elements into various substrates and biomedical components, suggesting it is a potential surface modification technology for biomedical implant materials [23]. In this work, plasma spraying and PIII technologies are combined to modify the surface of commercial pure Ti (cp-Ti) and the antibacterial activities of the Zn-PIII plasma sprayed TiO_2 coatings against *Staphylococcus aureus* (*S. aureus*) and *E. coli* are investigated.

2. Material and methods

2.1 *Coating fabrication*

TiO_2 nanopowders (P25, Degussa, Germany) were used as feedstocks. Coatings were deposited on cp-Ti with a dimension of 10 mm × 10 mm × 1 mm by an atmospheric plasma spraying system (9M, SulzerMetco, USA). Before plasma spraying, the cp-Ti substrates were ultrasonically cleaned in

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Table 1. Instrumental parameters of Zn-PIII process.

Samples	Voltage pulse duration (μs)		Pulsing frequency (Hz)		Ion implantation voltage (kV)	Ion implantation time (min)	Pressure (Pa)
	Target	Cathodic arc	Target	Cathodic arc			
Z0	450	450	6	6	30	0	2.5×10^{-3}
Z1						30	
Z2						90	
Z3						120	

absolute ethanol and sandblasted with brown corundum. The plasma spraying parameters are as follows: spraying power was 40 kW, Ar flow rate was 40 l min^{-1}, H_2 flow rate was 12 l min^{-1}, spraying distance was 100 mm and powder feed rate was 30 g min^{-1}.

2.2 *Zn-PIII treatment*

Zn was incorporated into the as-prepared TiO_2 coatings by PIII using a filtered cathodic arc plasma source. A magnetic duct with a curved shape was inserted between the plasma source and main chamber to remove macro-particles produced from the cathodic arc. The cathode rod of 10 mm in diameter was made of 99.99% pure metallic Zn. The Zn discharge was controlled by the main arc current between the cathode and anode. By applying a pulsed high voltage to the TiO_2 coatings, Zn ions were implanted and the implantation instrumental parameters are listed in table 1. Prior to PIII, the TiO_2 coatings were cleaned for 15 min with a radio frequency (RF) argon (Ar) plasma source at a bias of −500 V. During Zn-PIII, the sample stage was actively cooled by circulating water to keep the sample temperature at 25°C.

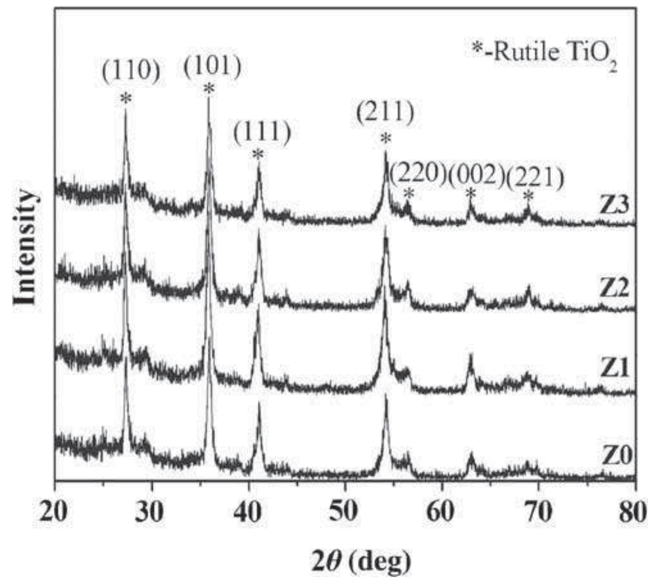

Figure 1. XRD patterns of the as-sprayed (Z0) and treated TiO_2 coatings by Zn-PIII for different times: (Z1) 30 min, (Z2) 90 min and (Z3) 120 min.

2.3 *Coating characterization*

The phase structures of the Zn-PIII-treated TiO_2 coatings were conducted by X-ray diffraction (XRD, D/max 2500PC, Rigaku, Japan) with Cu Kα radiation ($\lambda = 1.5418$ Å) in the range of 20–80° (2θ). The surface and cross-sectional morphologies of the coatings were observed by scanning electron microscopy (SEM, S-3400, Japan). The chemical composition of the coatings was determined by energy-dispersive X-ray spectrometry (EDS, Oxford).

The surface wettability of Zn-PIII-treated TiO_2 coatings was measured by contact angle measurement (Automatic Contact Angle Meter Model SL200B, Solon Information Technology Co., Ltd, China). Briefly, 1 μl of sessile distilled water droplet was dropped onto specimen surface, and then the cover of contact angle metre was shut down to ensure a dark space. Once the water droplet calmed down, the equipped camera system would capture the photography immediately. The room temperature was 20°C with atmospheric relative humidity of 30%. Contact angles of five drops were analysed on each sample and the experiment was repeated twice. Data were presented as means ± standard deviation (SD).

2.4 *Ion release test*

The samples were incubated in 10 ml saline water at 37°C without stirring for 7 and 14 days. The amounts of released ion were determined by analysing the resulting solutions by inductively coupled plasma optical emission spectrometry (ICP-OES).

2.5 *Antibacterial test*

The antibacterial activity on the TiO_2 coating and Zn-PIII TiO_2 coatings was evaluated by the bacterial counting method using *S. aureus* (Gram positive) (ATCC 25923) and *E. coli* (Gram negative) (ATCC 25922). The coatings were sterilized in 75% ethanol for 2 h. A suspension containing the bacteria at a concentration of 10^7cfu ml^{-1} was introduced onto the sample to a density of 0.06 ml cm^{-2}. The coatings with the bacterial solution were incubated at 37°C for 24 h. The dissociated bacterial solution was collected and inoculated into a standard agar culture medium. After incubation at 37°C for 24 h, the active bacteria were counted in accordance with the National Standard of China (GB/T 4789.2).

2.6 *Statistical analysis*

The statistical analysis was carried out using a GraphPad Prism 5 program and the data were expressed as mean ± standard deviation (SD). Statistically significant differences ($p < 0.05$) between the various groups were measured using one-way analysis of variance and Tukey's multiple comparison tests.

3. Results and discussion

Figure 1 presents the XRD patterns of the as-prepared and Zn-PIII-treated TiO_2 coatings. The TiO_2 coating is mainly composed of rutile phase (JCPDS no. 21-1276) corresponding to the planes of (110), (101), (111), (211), (220), (002) and (221), while incorporation of Zn by PIII into TiO_2 coating do not change the rutile phase structure, which may result from the amount of Zn is small.

SEM images and EDS spectrum of the coatings are displayed in figure 2. The as-sprayed TiO_2 coating presents a typical surface morphology of plasma spraying, which has some holes, lamellae, partially melted and unmelted particles [24]. From high-magnification images (figure 2b, d, f and h), many nanoparticles are formed on the surface of the coatings. No obvious differences on coating surface morphologies of Zn-PIII-treated TiO_2 coatings are observed compared to TiO_2 coating. Figure 2e inset image shows EDS spectrum

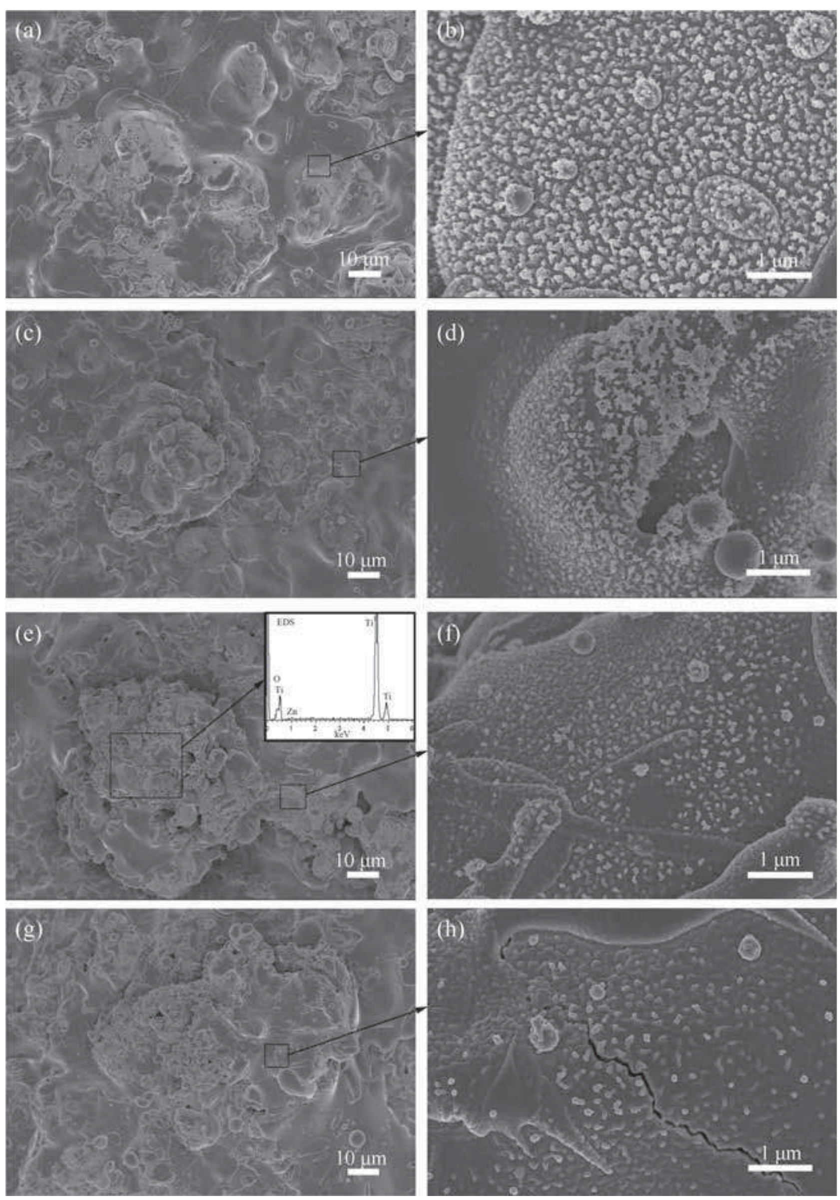

Figure 2. SEM images of the (**a**) as-sprayed and (**b**) treated TiO_2 coatings by Zn-PIII for different times: (**c** and **d**) 30 min, (**e** and **f**) 90 min and (**g** and **h**) 120 min (inset in **e** is related EDS result).

of the Zn-PIII-treated TiO_2 coating (Z3). It can been seen that the Zn-PIII-treated TiO_2 coating is mainly composed of Ti, O, and a small amount of Zn elements, which indicates Zn has been incorporated into TiO_2 coating.

Figure 3 shows the cross-sectional morphology of the as-prepared TiO_2 coating. The thickness of the TiO_2 coating is about 50 μm. Cross-section demonstrates that the TiO_2 coating is smooth and dense, and no gap is visible between the coating and cp-Ti substrate, indicating a good interfacial bonding between TiO_2 coating and Ti substrate. There are no obvious changes on cross-sectional morphologies after Zn-PIII treatment (not shown here). The interfacial bonding between the coating and the substrate plays an important role in the long-term stability of the implants and a strong bonding between the implant and the coating is a critical in avoiding coating delamination [25,26].

The water contact angles of the TiO_2- and Zn-PIII-treated TiO_2 coatings are displayed in figure 4. It can be observed that TiO_2 coating exhibits hydrophilicity. After Zn-PIII treatment, the surfaces of the coatings become hydrophobic. The water contact angles of Z0, Z1, Z2 and Z3 coatings are 76.6 $\pm$ 4.0°, 130.8 $\pm$ 0.5°, 128.9 $\pm$ 3.6° and 134.1 $\pm$ 1.0°, respectively. The surface topography and chemical composition of composite coatings are two dominant factors influencing wettability of materials [27,28]. The surface topographies of Z0, Z1, Z2 and Z3 coatings are the same with each other. There may be a ZnO thin film on the TiO_2 coating surface after Zn-PIII treated, and the ZnO thin film is hydrophobic [29].

The ion release kinetics is assessed by immersing the samples in 10 ml saline water for various time, and as shown in figure 5. Small amounts of Ti^{4+} released from the coatings and the concentrations are below 0.02 ppm, indicating Ti element is stable. The amounts of Zn^{2+} released increase with the elongation of PIII treatment and immersion time. At 7 days, the concentrations of Zn^{2+} released from Z1, Z2 and Z3 coatings are 0.08, 0.10 and 0.13 ppm, respectively. After immersion for 14 days, the concentrations of Zn^{2+} in saline water are 0.11, 0.13 and 0.18 ppm, respectively.

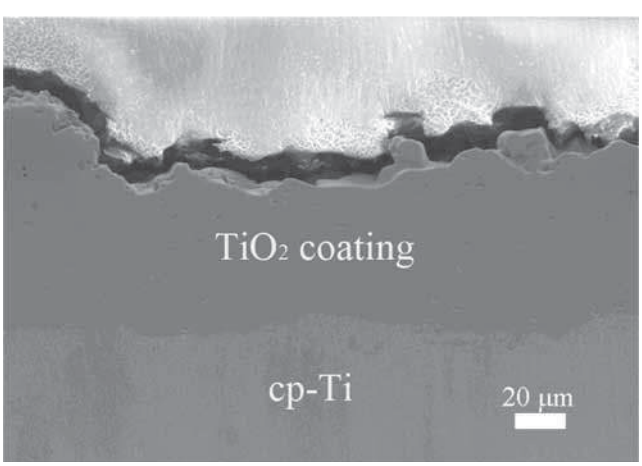

Figure 3. Cross-sectional image of the as-sprayed TiO_2 coating.

The morphologies of *E. coli* and *S. aureus* seeded on the surfaces of the as-sprayed and PIII-treated TiO_2 coatings are examined by SEM (figure 6). Figure 6a-1 shows the typical morphology of *E. coli* cultured on the surface of the as-sprayed coating (Z0). The bacterial cells are rod shaped and undamaged binary fission, exhibiting a corrugated morphology. With the increase of PIII treated time, there are substances secreted from *E. coli* bacterial cells, indicating that the growth of *E. coli* is inhibited (figure 6b-1–d-1). The morphology of *S. aureus* cultured on the surface of the as-sprayed coating is shown in figure 6a-2, displaying smooth and intact surfaces. On the surfaces of Zn-PIII-treated TiO_2 coatings, obvious bacterial cell debris are observed (figure 6b-2–d-2).

To further investigate the antibacterial activities of the coatings, the adhered bacteria on the coatings were re-cultivated on agar according to the bacteria counting method.

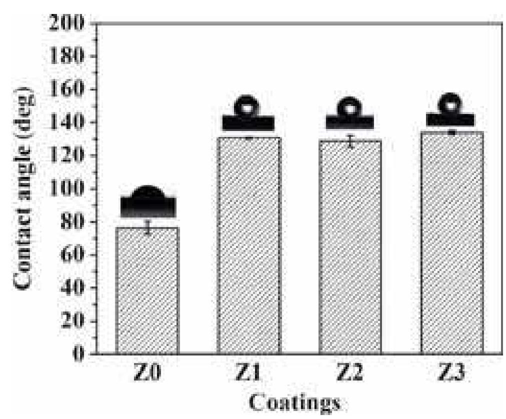

Figure 4. Water contact angles of the as-sprayed (Z0) and treated TiO_2 coatings by Zn-PIII for different times: (Z1) 30 min, (Z2) 90 min and (Z3) 120 min.

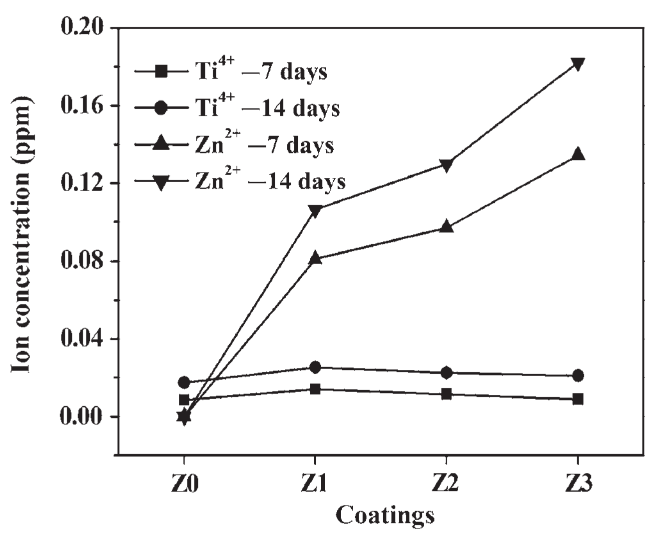

Figure 5. Zn ion concentrations of saline water with immersion for 7 and 14 days of the as-sprayed (Z0) and treated TiO_2 coatings by Zn-PIII for different times: (Z1) 30 min, (Z2) 90 min and (Z3) 120 min.

Figure 7 shows the number of bacterial colonies grown on the as-sprayed and PIII-treated TiO_2 coatings. Figure 8 displays the percentage reduction of *E. coli* and *S. aureus* colonies on the coatings. It can be seen that introduction of Zn ions is beneficial to improving the antibacterial activities for *S. aureus* and the effect is obvious and dose dependent. The percentage reduction of *S. aureus* seeded on Z2 coating can reach $93.4 \pm 4.1\%$ (figure 8b), indicating that introduction of a small amount of Zn can significantly enhance the antibacterial activity of TiO_2 coating. However, the number of *E. coli* colonies grown on the Z0, Z1, Z2, and Z3 coatings decreases slightly observed from figure 7a-1–d-1. The percentage reduction of *E. coli* seeded on Z0, Z1, Z2 and Z3 coatings are 24.2 ± 3.4, 25.6 ± 4.3, 31.2 ± 3.6 and $42.5 \pm 5.6\%$ (figure 8a), respectively, indicating a partly antibacterial effect. These results demonstrate that the PIII-treated TiO_2 coatings show enhanced bacterial activities to inhibit both *S. aureus* and *E. coli* bacteria, and *S. aureus* is more sensitive to the PIII-treated TiO_2 coatings than *E. coli*.

Surface chemical composition of TiO_2-based implants plays a crucial role in biological interaction because it is in direct contact with the biological environment. Zn is an important trace element for enhancing cell proliferation, ALP activity, collagen synthesis, and protein synthesis [8,30]. Zn can also stimulate osteogenesis, inhibit osteoclastogenes and induce bone formation [31,32]. Moreover, Zn ions dissolved from high concentrated medium or alkaline medium is responsible for the antibacterial activity. Cell lysis involves possibly two stages: (1) initial fast and passive process including physical adsorption or ion exchange at cell surfaces; (2) slower transport of metal ions into bacterial cells. The two processes may result in the leakage of cellular materials, thereby causing the damage of the bacterial cells and cell walls [14]. In this work, the mechanisms of enhanced antibacterial activities by introduction Zn ions into TiO_2 coatings may be two aspects. Firstly, the release of bacteriostatic Zn ions from PIII-treated TiO_2 coatings is important to improving their antibacterial activities [33]. However, the concentration of the released Zn ions is only 0.18 ppm (figure 5), which is beneficial to the cellular toxicity because it has been reported that the concentration of Zn within 3 ppm is noncytotoxic [34]. Zn ions at an appropriated

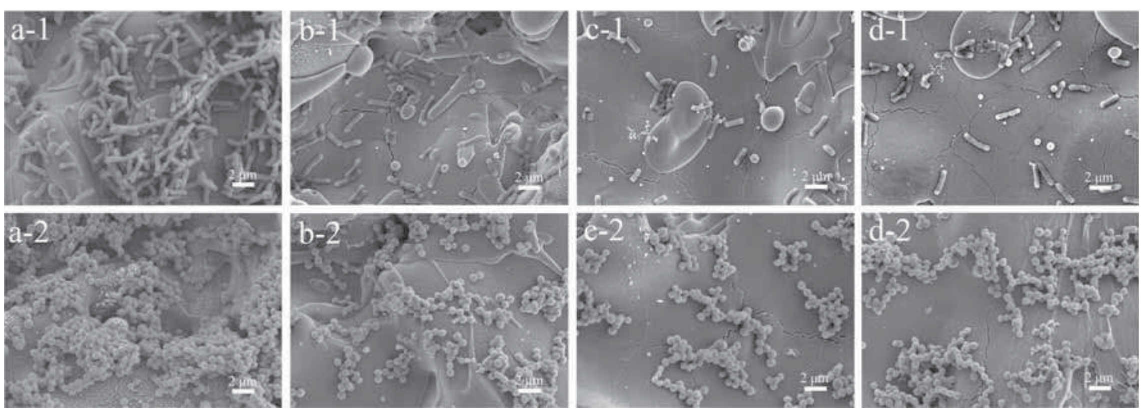

Figure 6. SEM morphologies of the *E. coli* (i-1) and *S. aureus* (i-2) species seeded on the various surfaces (i = a, b, c and d represent Z0, Z1, Z2 and Z3 coatings, respectively) (Z0: the as-sprayed, Z1: treated by Zn-PIII for 30 min, Z1: treated by Zn-PIII for 90 min and Z1: treated by Zn-PIII for 120 min).

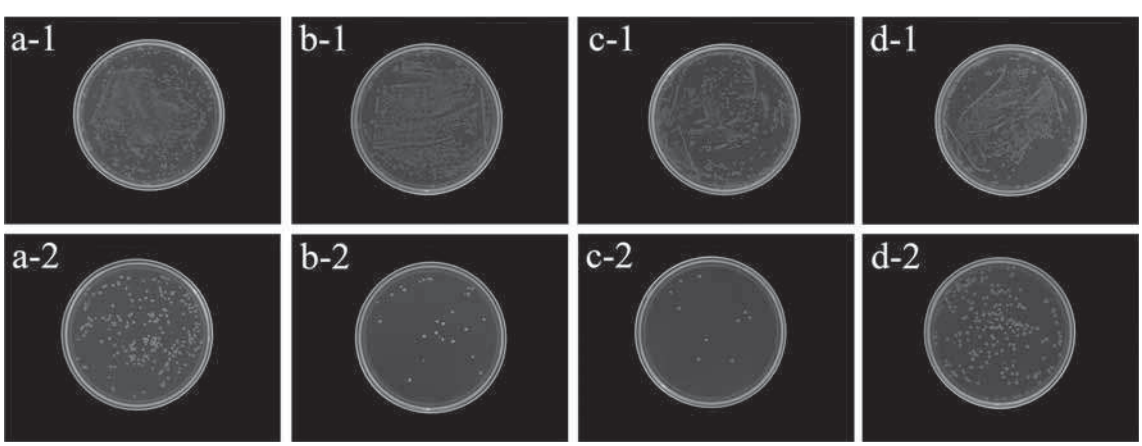

Figure 7. Re-cultivated bacterial colonies on agar: *E. coli* (i-1) and *S. aureus* (i-2) colonies are previously dissociated from (**a**) Z0, (**b**) Z1, (**c**) Z2 and (**d**) Z3 coatings after 24 h incubation (Z0: the as-sprayed, Z1: treated by Zn-PIII for 30 min, Z1: treated by Zn-PIII for 90 min and Z1: treated by Zn-PIII for 120 min).

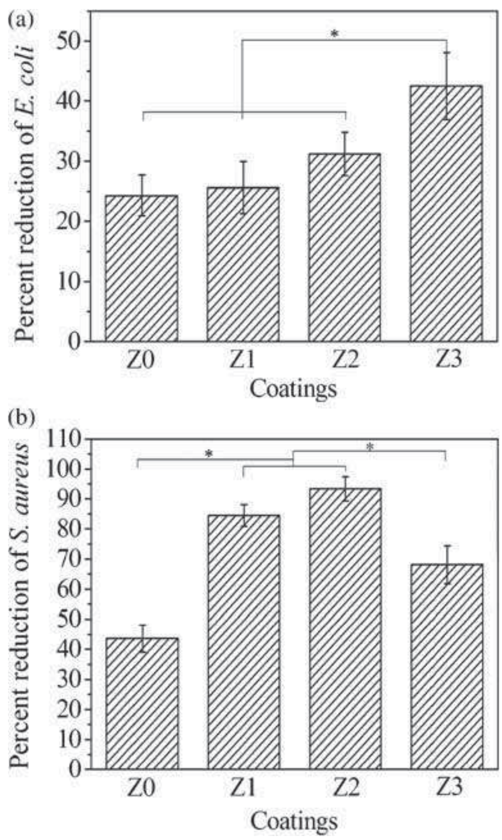

Figure 8. Percentage reductions of (**a**) *E. coli* and (**b**) *S. aureus* colonies re-cultivated on agar, which were previous dissociated from Z0, Z1, Z2 and Z3 coatings (Z0: the as-sprayed, Z1: treated by Zn-PIII for 30 min, Z1: treated by Zn-PIII for 90 min and Z1: treated by Zn-PIII for 120 min) ($*p < 0.05$).

concentration can promote osteoblast behaviour and have partly antibacterial effects on bacteria without introduction of undesired side effect [35]. Furthermore, the most likely antibacterial mechanism is the production of a larger amount of bacteriocidal reactive oxygen species (ROS) (including H_2O_2, OH^-, O_2^{2-}, etc.) by introducing Zn ions into TiO_2 coatings. It has been reported that Zn-implanted titanium surfaces could reduce the bacterial adhesion and inhibit the growth of bacteria by producing ROS [13,36]. In addition, the effects of Zn-PIII treatment on the antibacterial activities of TiO_2 coatings are different. Compared to *E. coli* (Gram-negative bacteria), *S. aureus* (Gram-positive bacteria) seem more susceptible. It has been reported that the higher susceptibility of Gram-positive bacteria than that of Gram-negative bacteria was related to the differences in cell wall structure, cell physiology, metabolism or degree of contact [8,37]. As a result, Zn-PIII-treated TiO_2 coatings exhibit a greater ability to inhibit bacteria for *S. aureus* than for *E. coli*.

4. Conclusion

TiO_2 coatings were deposited on cp-Ti substrates by plasma spraying and subsequently modified by the Zn-PIII technique. The structures and properties of the as-sprayed and Zn-PIII-treated TiO_2 coatings, including phase composition, surface morphologies, interfacial bonding state, Zn ion release and antibacterial (for *E. coli* and *S. aureus*) were investigated in correlation with the Zn-PIII treatment time. Zn-PIII treatment did not obviously change the crystalline structure, surface and cross-sectional morphologies of the TiO_2 coatings. The Zn-implanted TiO_2 coatings could partly improve antibacterial activities on both *E. coli* and *S. aureus*, but the effects were dose dependent. And the Zn-implanted TiO_2 coatings exhibited a greater ability to inhibit bacteria to *S. aureus* than to *E. coli*. This study indicates the potential application of TiO_2-based coatings in eliminating the infections of the implants.

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