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Full Length Article

Fibroblast responses and antibacterial activity of Cu and Zn co-doped TiO_2 for percutaneous implants

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ABSTRACT

In order to enhance skin integration and antibacterial activity of Ti percutaneous implants, microporous TiO_2 coatings co-doped with different doses of Cu^{2+} and Zn^{2+} were directly fabricated on Ti via micro-arc oxidation (MAO). The structures of coatings were investigated; the behaviors of fibroblasts (L-929) as well as the response of *Staphylococcus aureus* (*S. aureus*) were evaluated. During the MAO process, a large number of micro-arc discharges forming on Ti performed as penetrating channels; O^{2-}, Ca^{2+}, Zn^{2+}, Cu^{2+} and $PO_4{}^{3-}$ delivered via the channels, giving rise to the formation of doped TiO_2. Surface characteristics including phase component, topography, surface roughness and wettability were almost the same for different coatings, whereas, the amount of Cu doped in TiO_2 decreased with the increased Zn amount. Compared with Cu single-doped TiO_2 (0.77 Wt% Cu), the co-doped with appropriate amounts of Cu and Zn, for example, 0.55 Wt% Cu and 2.53 Wt% Zn, further improved proliferation of L-929, facilitated fibroblasts to switch to fibrotic phenotype, and enhanced synthesis of collagen I as well as the extracellular collagen secretion; the antibacterial properties including contact-killing and release-killing were also enhanced. By analyzing the relationship of Cu/Zn amount in TiO_2 and the behaviors of L-929 and *S. aureus*, it can be deduced that when the doped Zn is in a low dose (<1.79 Wt%), the behaviors of L-929 and *S. aureus* are sensitive to the reduced amount of Cu^{2+}, whereas, Zn^{2+} plays a key role in accelerating fibroblast functions and reducing *S. aureus* when its dose obviously increases from 2.63 to 6.47 Wt%.

1. Introduction

Osseointegrated percutaneous implants transferring the mechanical stress directly from skeleton to prosthesis are considered to be corresponding to biological mechanics [1]. Ti and its alloys with high mechanical properties, corrosion resistance and biocompatibility have been widely used as percutaneous implants (e.g. dental implant and intraosseous transcutaneous amputation prostheses). However, Ti is bioinert and can't form bio-integration with soft tissue like gingiva and skin. A low fixation strength of the implant/tissue interface as well as epithelial downgrowth should induce some failures of avulsion and marsupialization [2]. Moreover, Ti hasn't antibacterial activity and cannot protect against bacterial invasion. Bacteria colonize the implant surface, and the implant exit sites become the gateway to infection, which could lead to bacteria spreading internally and the failure of implant [1–3]. So, the soft tissue integration and antibacterial property play a key role in the performance life of an implant. Surface modification can give Ti biological multifunction. Hundreds of coatings containing various antibacterial agents including the inorganic and organic have been prepared to endow Ti or its alloys with antibacterial activity [3–7]. However, relatively fewer coatings which could promote the response of skin-forming cells (e.g. fibroblasts, keratinocyte) were reported, and they are titania, hydroxyapatite, fibronectin, laminin and keratin etc. [8–12]. Micro-arc oxidation (MAO) is a nonlinear process which can produce adhered and microporous TiO_2. It is known that during the MAO process, a gas envelope mainly composed of oxygen forms on Ti disc, resulting in forming an initially thin titanium oxide film. When the voltage increases, large numbers of micro-arc discharges break the oxide film, oxygen as well the target ions from electrolyte can penetrate into the inner region of coatings via these discharge channels, resulting in the formation of TiO_2 doped with biofunctional ions (e.g. Ca, Zn, Sr, Cu and P) [4,13,14]. Cu and Zn are essential trace elements for human beings. They can inhibit bacteria adhesion and reproduction, but not induce cytotoxicity in appropriate concentrations [15,16]. Their single effect on the behaviors of cells (e.g. fibroblasts, osteoblasts) and bacteria (e.g. *Staphylococcus aureus* (*S. aureus*)) have been widely reported. For example, the incorporation of Zn could promote bone marrow stem cells osteogenic differentiation, and inhibit the growth of both *S. aureus* and *Escherichia coli*, simultaneously [5].

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Table 1
Electrolyte contains for preparing different coatings.

Name	β-glycerophosphate (M)	calcium acetate (M)	copper acetate (M)	zinc acetate (M)
CA	0.02	0.002	0	0
Zn0	0.02	0.002	0.0013	0
Zn1	0.02	0.002	0.0013	0.005
Zn2	0.02	0.002	0.0013	0.01
Zn3	0.02	0.002	0.0013	0.02
Zn4	0.02	0.002	0.0013	0.04

In injured skin tissue, zinc took part in the complex regulation of the sequence of signal molecules and mediators such as cytokines and growth factors, enhancing the adhesion and proliferation of fibroblasts [14,17–19]. Bioactive glasses containing 2% mol Cu stimulated osteogenesis and angigenesis, and its ionic dissolution extract exhibited antibacterial effect against three different bacteria strains [20]. In our previous work, TiO_2 doped with a certain amount of Cu^{2+} had a good antibacterial activity and could accelerate the adhesion, proliferation, phenotype, differentiation and extracellular collagen secretion of fibroblasts [2]. Although it is reported that zinc combined with copper appeared more effective for wound healing in humans [14], the comprehensive effect of Cu and Zn on the behaviors of fibroblasts and bacteria has not been explored. In this paper, TiO_2 coatings co-doped with different amounts of Cu and Zn were prepared on Ti by MAO. The response of fibroblasts (L-929) including adhesion, proliferation and phenotype on the coatings were investigated to get the co-effect of Cu and Zn on skin regeneration potential. The antibacterial activity of the coatings against *S. aureus* was also examined.

2. Experimental methods

2.1. Preparation of Cu and Zn co-doped TiO_2 coatings

Commercial Ti plates (ϕ 14 mm × 2 mm, 99.99 Wt%) were gradually polished by 300, 800 and 1500 # SiC sandpapers and ultrasonically washed with alcohol. Then, Ti plates were MAOed for 2 min in by a pulsed DC power supply, with the applied voltage, pulse frequency and duty ratio of 480 V, 500 Hz and 7.5%, respectively. The aqueous electrolyte contained 0.02 M β-glycerophosphate, 0.2 M calcium acetate, 0.0013 M copper acetate and different concentrations of zinc acetate. After MAOed, samples were ultrasonically washed with ethyl alcohol, dried at room temperature and named according to Table 1.

2.2. Structural characterization of the coatings

The surface and cross-sectional morphologies of coatings were observed by a field emission scanning electron microscope (FESEM; SU6600, Hitachi, Japan). The element compositions of the coatings were estimated by an energy dispersive X-ray spectrometer (EDX; DX-4, Philips, The Netherlands). The phase compositions were measured by an x-ray diffractometer (X'Pert PRO, Netherland) in θ-θ geometry. The surface roughness measurements of the samples were performed using atomic force microscopy (AFM; SPM-9500J3, Japan), and presented by the arithmetic mean surface roughness (Ra). Three samples from each group were measured and two measurements were performed on each surface to obtain an average.

2.3. Evaluation of wettability, adhesion strength, and Cu^{2+} and Zn^{2+} released from the coatings

The hydrophilicity of the coating was measured using distilled water (2.5 μL) by a surface contact angle measurement machine (DSA30, Kruss, Germany), as described in detail elsewhere [4]. The adhesion strengths of coatings were measured by an auto scratch coating tester (Rockwell C diamond) and maintained at a constant speed over the surface under the same load. The critical load (Lc), was defined as the smallest load at which a recognizable failure occurred and determined from the load by SEM images. The critical

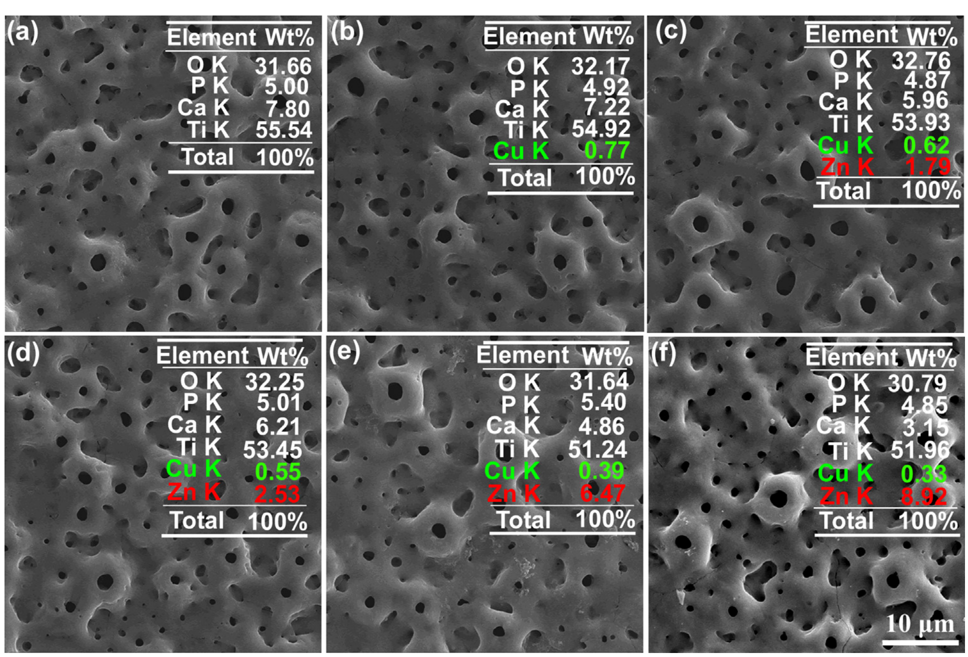

Fig. 1. Surface morphologies of different coatings: (a) CA, (b) Zn0, (c) Zn1, (d) Zn2, (e) Zn3, (f) Zn4; insets showing the corresponding element contents.