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Featured Letter

Boron-containing micro/nano-structured TiO_2/bioceramics coatings with modulatory effects on SaOS-2 cell response

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ABSTRACT

In order to take the advantages of micro/nano-topography and benefits of boron (B), B-containing micro/nano-structured TiO_2/bioceramics coatings were developed on titanium (Ti) substrates for enhanced osteogenesis. The micro-arc oxidation was employed to fabricate a micro-topographical TiO_2 layer (referred as Micro-Ti). Then, the second layer of nano-rods was prepared on Micro-Ti surface *via* hydrothermal treatment (referred as Hier-Ti). These nano-rods transformed to nano-particles after a post heat treatment (referred as Hier-HT-Ti). The adhesion, proliferation and differentiation of SaOS-2 cells were inhibited on Hier-Ti, but promoted on Hier-HT-Ti. The results indicate that the development of B-containing micro/nano-topographical coatings on Ti substrates could lead to enhanced osteogenesis.

1. Introduction

The surface modification has been recognized as the best alternative to improve the biological performance of titanium (Ti) and its alloys [1]. Recently, hierarchical micro/nano-topographies on biomaterial surfaces have attracted extensive attention due to their bio-mimetic role on the structural complexity of natural bone tissue [2]. The micro/nano-structured coatings on Ti-based implant surfaces have been reported to enhance the *in vitro* osteogenic capacity of osteoblastic lineage cells and *in vivo* osteointegration [3,4]. In the clinical practice, one and two dimensional nano-structures such as tubes, rods and plates could be easily broken or peeled off due to the shearing force during implantation [5]. Thus, zero-dimensional nano-particles are considered to be better for avoiding denudation of the features. The incorporation of bio-active trace elements is another effective methodology to promote the biological performance of biomaterials [6]. Boron (B), as an important trace element in bone metabolism, has been regarded as an osteo-inductive agent recently [7]. The B ions supplemented to the culture medium or released from B-containing biomaterials have been reported to stimulate the osteogenic differentiation of mesenchymal stem cells (MSCs) and maturation of osteoblasts [8–10]. Considering the advantages of micro/nano-topographies and benefits of B, it is promising to develop novel B-containing micro/nano-structured coatings on Ti substrates for enhanced osteogenesis. In this study, the micro/nano-topographical coating was fabricated on Ti by micro-arc oxidation (MAO) and subsequent hydrothermal treatment. A post heat treatment was employed to modulate the morphology of the nano-structures. Finally, SaOS-2 cell response to various coatings was investigated.

2. Experimental procedure

2.1. Material preparation and characterization

Grade 2 commercially pure Ti discs were MAO treated at 250 V for 5 min in an electrolytic solution composed of 0.10 M $Na_2(EDTA)$, 0.10 M $Ca(CH_3COO)_2{\cdot}H_2O$, 0.25 M NaOH and 0.02 M $Na_2SiO_3{\cdot}9H_2O$ (referred as Micro-Ti) as previously described [11]. Then, the samples were hydrothermally treated in 0.02 M $Na_2B_4O_7{\cdot}10H_2O$ ammonia aqueous solution (pH = 11) at 200 °C for 4 h (referred as Hier-Ti). Finally, a post heat treatment was performed on Hier-Ti at 800 °C for 2 h (referred as Hier-HT-Ti). The surface topography and 3D profiling of the specimens were visualized by field emission scanning electron microscopy (FESEM; Merlin Compact, Zeiss, German) and 3D profiling system

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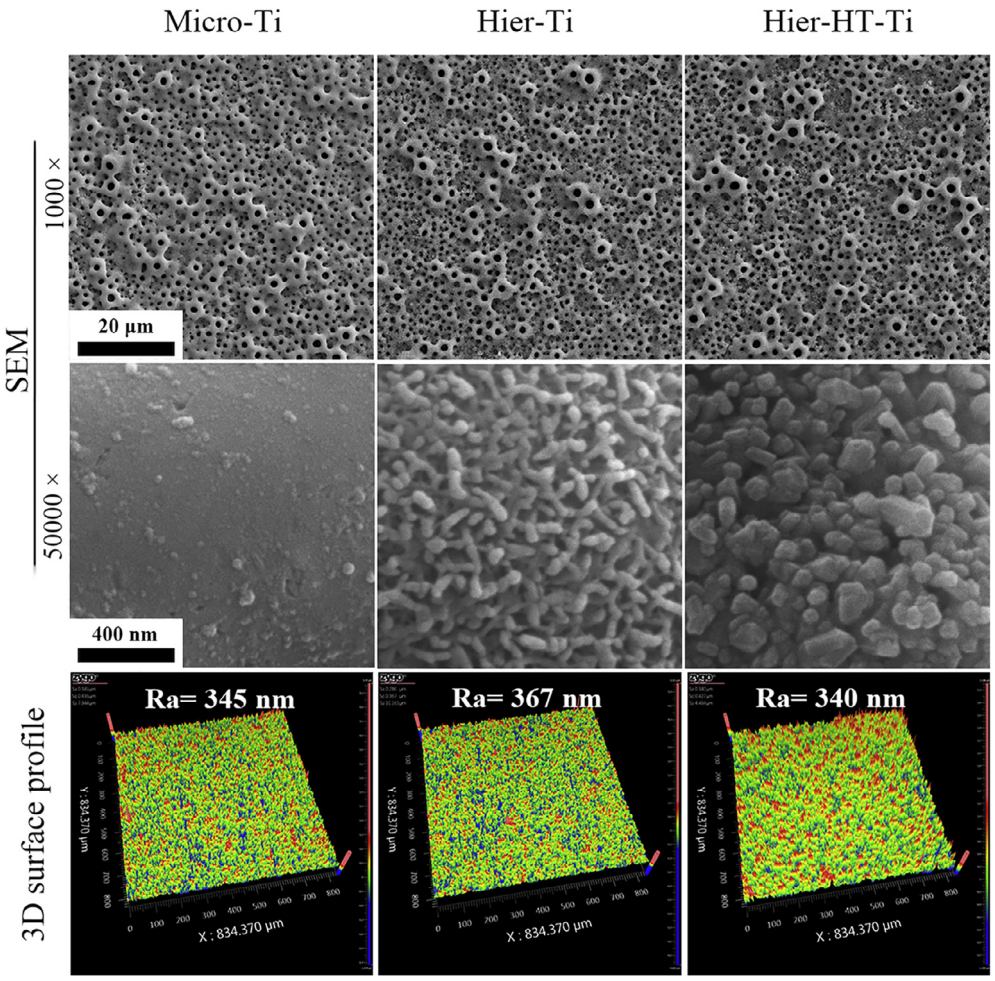

Fig. 1. The morphology and 3D profile of various coating surfaces.

(MicroXAM-3D Phase Shift, ADE Co., USA), respectively. The phase composition of the specimens was analyzed by X-ray diffraction (XRD; Rigaku, Tokyo, Japan). The element chemical state was evaluated by X-ray photoelectron spectrometer (XPS; Escalab 250Xi, Thermo Scientific, UK).

2.2. In vitro experiments

Human osteoblastic SaOS-2 cells were seeded onto coating surfaces at a density of 15,000 cells/cm^2 and cultured by complete culture medium consisted of McCoy's medium, 15% fetal bovine serum and 1% penicillin/streptomycin. At each defined time point, the cells were successively fixed, dehydrated, freeze dried, gold sputtered and observed using SEM as previously described [11]. The proliferation and ALP activity of SaOS-2 cells were evaluated by Cell Counting Kit-8 (CCK-8, Dojindo, Japan) and ALP kit (Jiancheng bio-engineering research institute of Nanjing, China), respectively. The concentrations of Ca, Si and B in the culture media after 48 h were by inductively coupled plasma atomic emission spectrometry (ICP-AES; Varian-Vista, Australia).

2.3. Statistical analysis

The data were expressed as mean ± standard deviation (SD). The statistical significance of differences in means was determined by one-way analysis of variance (ANOVA) followed by post hoc comparisons with least significant difference (LSD) method. A value of $p < 0.05$ was considered as statistically significant.

3. Results

As shown in Fig. 1, the coating surfaces were composed of numerous micro-sized crater-like protuberances, exhibiting similar micro-scale topographies at a low magnification (1000×). At a relatively higher magnification (50000×), a network of nano-rods (~44 nm in diameter, 100 nm in length and 80 nm in inter-rod spacing) was noticed on Hier-Ti surface. These nano-rods transformed to nano-particles (~65 nm in diameter) after post heat treatment. The surface roughness values of various surfaces were close in the sub-micron range.

As shown in Fig. 2a, the Micro-Ti and Hier-Ti were consisted of anatase on Ti substrates. However, the rutile and titanite (TiO_2-·CaO·SiO_2) phase were detected for Hier-HT-Ti. As shown in Fig. 2b and c, the Ca 2p and Si 2p peaks were corresponding to CaO, SiO_2 and $CaSiO_3$ for Micro-Ti, $6CaO·3SiO_2·H_2O$ for Hier-Ti and titanite for Hier-HT-Ti [11]. As shown in Fig. 2d, the B 1s peak was attributed to B_2O_3 for Hier-Ti [12]. However, this peak was not detected for Hier-HT-Ti, indicating that B diffused into the depths of coating matrix after heat treatment and became undetectable on the surface.

As shown in Fig. 3a, the attachment of SaOS-2 cells was found to be inhibited on Hier-Ti, but promoted on Hier-HT-Ti compared to the control. This was evidenced by the cell spreading area, which