



The synergistic effect of hierarchical micro/nano-topography and bioactive ions for enhanced osseointegration

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ABSTRACT

Both surface chemistry and topography have significant influence on good and fast osseointegration of biomedical implants; the main goals in orthopedic, dental and maxillofacial surgeries. A surface modification strategy encompassing the use of bioactive trace elements together with surface micron/nano-topographical modifications was employed in this study in an attempt to enhance the osseointegration of Ti alloy (Ti-6Al-4V), a commonly used implant. Briefly, we developed strontium-substituted hardyssonite (Sr-HT) ceramic coating with a hierarchical topography where the nanosized grains were superimposed in the micron-rough coating structure. Its ability to induce new bone formation was evaluated by an *in vivo* animal model (beagle dogs). Hardyssonite (HT), classic hydroxyapatite (HAp) coated and uncoated Ti-alloy implants were parallelly investigated for comparison. In addition, we investigated the effects of surface topography and the dissolution products from the coatings on the *in vitro* bioactivity using canine bone marrow mesenchymal stem cells (BMMSCs) cultured on the implant surface as well as using extracts of the coated implants. Micro-CT evaluation, histological observations, biomechanical test (push-out test) and sequential fluorescent labeling and histomorphometrical analysis consistently demonstrated that our developed Sr-HT-coated Ti-alloy implants have the highest osseointegration, while the uncoated implants had the lowest. The osseointegration ability of HAp-coated Ti alloy was inferior to that seen for HT- and Sr-HT-coated Ti alloy. We demonstrated that the dissolution products, particularly strontium (Sr) from the Sr-HT-coated implants, enhanced the ALP activity and *in vitro* mineralization ability, while the micro/nano-topography was more related to the promotion of cell adhesion. Those results suggest that our developed Sr-HT coatings have the potential for future use as coatings for orthopedic/dental and maxillofacial devices.

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1. Introduction

Successful integration of orthopedic implants with host bone, not only needs initial stability supported by enough bone stock but

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rapid osseointegration. The term “osseointegration” was first introduced by Branemark to describe the modality for stable fixation of titanium (Ti) to bone tissue [1,3]. Ti alloy is well-established biomaterial in dental and orthopedic applications due to its good mechanical properties, biocompatibility and anti-corrosion property. However, Ti alloy cannot achieve sufficient osseointegration due to its suboptimal surface osteoconductivity, thus resulting in the increased aseptic loosening and subsequent premature failure of the implants. In order to enhance the osseointegration and increase the successful rate of Ti-alloy implants, various surface modification methods have been utilized. And the incorporation of bioactive trace elements such as calcium (Ca), silicon (Si), zinc (Zn)

and strontium (Sr) is the most commonly used and useful approach [4,5].

Ca and Si ions, both of which are essential elements for human body, have been proved to promote osteoblast proliferation and differentiation [6,7]. They have been utilized to chemically modify biomaterials for enhanced bioactivity. For example, Ca ion-implantation can alter the surface chemistry of Ti and in turn modulate the progression of bone cell cycle and enhance the response of bone cells to implants [8,9]; Si ions have been widely used to modify hydroxyapatite (HAp), one of the most popular biomaterials for bone replacement and regeneration. More rapid remodeling of bone was observed surrounding the Si incorporated HAp (Si-HAp) granules compared to pure HAp granules [10]. Calcium silicates including CaSiO_3 and Ca_2SiO_4 are the most typical ceramic materials capable of releasing Ca and Si ions and their potential for use in bone replacement and regeneration applications have been demonstrated *in vitro* and *in vivo* [11–13]. However, high degradation rate of CaSiO_3 can result in high pH value in the surrounding environment which is detrimental to cell viability [14]. Moreover, if used as coatings for orthopedic applications, the fast degradation rate of the CaSiO_3 coatings will increase the failure rate of the implants, where high chemical stability is required, leading to loosening of the interface. These drawbacks were addressed by incorporating ZnO into CaSiO_3 resulting in the development of hardystonite ceramic ($\text{Ca}_2\text{ZnSi}_2\text{O}_7$, HT) with better chemical stability and bioactivity [15,16].

Using plasma spray techniques, we recently coated nanostructured HT ceramics onto Ti-6Al-4V and demonstrated enhanced bioactivity of the coated Ti-6Al-4V [17]. Zn ion was found to inhibit osteoclastic activity and promote bone mineralization [18]. Its incorporation endowed the new nanostructured ceramic coatings (HT) with an ability to release Zn ions into the surrounding environment while simultaneously enhancing the chemical stability of the coating due to the decreased release rate of Ca, Si from the coating. Sr is another important trace element found in human bone and its beneficial effect on bone formation has been well documented [19]. Both *in vitro* and *in vivo* studies have shown that Sr ions stimulate bone formation and decrease bone resorption [20,21]. We previously demonstrated that the incorporation of Sr into CaSiO_3 ceramics improved the physical and biological properties of the pure CaSiO_3 ceramics [22,23] and induced *in vitro* bone formation with enhanced bioactivity and osseointegration properties *in vivo* [23]. In this study, we used $(\text{Ca}_{0.8}\text{Sr}_{0.2})_2\text{ZnSi}_2\text{O}_7$ (Sr-HT) ceramic to develop new coatings for commonly used orthopedic/dental implants by substitution of Sr for 20% Ca in HT, with a hypothesis that Sr incorporation would further enhance the osteoconductivity of our previously developed HT coatings.

Topographical modification is another important approach to improve biological performance of dental and orthopedic implants. The beneficial effects of microscale roughness on the *in vitro* osteoblast activity and *in vivo* osteoconductivity have been proved by many studies [24]. Cells *in vivo* live in a three-dimensional (3D) extracellular matrix (ECM) which is composed of not only microscale topographical features but also various nanoscale ones. Others in the field have demonstrated the effects of nanosized topographic features on promotion of osteoblast adhesion [25,26] as well as the synergistic effects of micro and nanoscale hybrid structures on osteoblast activity [27–29], implying the promise of dual-length scale topographical modification of the implants. Considering the significant importance of both micro and nano topographical features, our developed Sr-HT ceramic coating as well as the HT coating was designed to have a hybrid micro/nanoscale structure where nanosized grains (less than 100 nm in size) were superimposed in microscale rough structures using plasma spraying technique.

In this study, the *in vivo* osseointegration of Sr-HT- and HT-coated Ti-6Al-4V implants was evaluated using a canine femur implantation model. Pure Ti alloy and HAp-coated Ti-6Al-4V implants, currently used in orthopedic applications, were used as controls for comparison. Twelve weeks after implant insertion, new bone formation around implants in the bone marrow cavity was evaluated using Micro-CT, histological and push-out test. In addition, sequential fluorescent labeling was used to compare the new bone formation rate of each group. To validate the beneficial effects of hybrid micro/nanoscale structures and bioactive trace elements, *in vitro* experiments were also performed using canine BMMSCs.

2. Materials and methods

2.1. Material synthesis and coating preparation

Sr-HT and HT powders were synthesized by high temperature solid reaction. Briefly, reagent grade CaCO_3 , ZnO and SiO_2 powders were mixed in a 2:1:2 m proportions for synthesizing HT ceramic powders; for synthesis of Sr-HT powders, SrCO_3 was used to replace 20% (m) CaCO_3 . The mixed powders were wet-ground in absolute ethanol for 6 h, and dried at 100 °C, followed by sintering at 1200 °C for 3 h. Then, the as-sintered powders were ground and sieved, powders passing through a sieve with a mesh size of 75 μm were reconstituted using PVA solution to make powders more flowable for plasma spraying. The procedure for reconstituting powders can be found in our previous paper [17]. The reconstituted powders were finally sieved using 80-mesh sieves. Those below 80-mesh (<177 μm) were used for plasma spraying.

An atmospheric plasma spray system (APS, Sulzer Metco, Switzerland) was applied to deposit HT and SP coatings on commercial Ti alloy (Ti-6Al-4V) plates (Dia.: 15 mm; Thickness: 2 mm) and rods (Dia.: 3 mm; Length: 10 mm) (Baoji Junhang Metal Material Co., Ltd.) for *in vitro* and *in vivo* studies, respectively. Clinically used HAp coatings used as a control group in this study were provided by Key Laboratory of Inorganic Coating Materials (Shanghai Institute of Ceramics, Chinese Academy of Sciences, China). Those coatings were produced using vacuum plasma spray (VPS, Sulzer Metco, Switzerland) with F4-VB spray gun, according to a procedure for fabricating clinic-used HAp coatings. The main spraying parameters are listed in Table 1.

2.2. Characterization of coatings

The surface morphology of the coatings was examined by field emission scanning electron microscopy (Zeiss Ultra Plus FE-SEM). The thickness of the coatings was measured using the SEM images taken on the cross-sectional coating samples. Chemical composition of the coatings was analyzed using energy dispersive spectroscopy (EDS) attached to the SEM instrument. The phase composition of the coatings was analyzed using X-ray diffraction (XRD, Siemens D6000, Germany) with a Cu K α 1 radiation at a step size of 0.02°. Data were obtained from 20 to 50° 2 θ at a scanning rate of 2° min⁻¹.

The surface roughness (R_a) was measured by surface profilometry. Three samples for each type of coatings and five different tracks on each sample were measured. The R_a values were expressed as means \pm standard deviations (SD) ($n = 15$).

The tensile bonding strength between coating and substrate was measured in accordance with ASTM C-633-79. The testing procedure can be found in our previous work [17]. Five samples were tested independently for each type of coatings and the results were reported as mean \pm standard deviation (SD).

2.3. Canine BMMSCs culture

Canine BMMSCs were isolated and cultured as we previously described [30]. After general anesthesia using ketamine (10 mg/kg) and xylazine (4 mg/kg), about 4 ml of bone marrow were harvested via needle aspiration from the iliac crest. The bone marrow was mixed with 10 ml phosphate buffered saline (PBS), and then centrifuged at 1000 rpm for 15 min. Nucleated cells were resuspended in Dulbecco's modified Eagle's medium (DMEM; Gibco, USA) containing 10% (v/v) fetal bovine serum (FBS; Gibco, USA). Cell suspension was incubated at 37 °C in a humidified atmosphere of 95% air and 5% CO₂. After 5 days culture, non-adherent cells were rinsed away and fresh medium was added. The culture medium was then changed

Table 1
Plasma spraying parameters for fabrication of HAp, HT and Sr-HT coatings.

Coatings	Ar (slpm)	H ₂ (slpm)	Spraying distance (mm)	I (A)	U (V)	Vacuum (mbar)
HAp	40	8	280	550	57	100
HT	40	12	100	570	75	N/A
Sr-HT	40	12	100	570	75	N/A

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