Applied Surface Science xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Applied Surface Science

journal homepage: www.elsevier.com/locate/apsusc

Full Length Article

Antibacterial effect of zinc oxide/hydroxyapatite coatings prepared by chemical solution deposition

Naofumi Ohtsu*, Yuko Kakuchi, Tsubasa Ohtsuki

School of Earth, Energy and Environmental Engineering, Kitami Institute of Technology, 165 Koen-cho, Kitami, Hokkaido 090-8507, Japan

ARTICLE INFO

Article history:
Received 15 July 2017
Received in revised form 31 August 2017
Accepted 13 September 2017
Available online xxx

Keywords:
Hydroxyapatite coating
Zinc oxide
Bacterial contact killing
Zn ion release

ABSTRACT

In the present study, we introduce a newly designed antibacterial hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$, HAp) coating that exploits the contact killing capabilities of ZnO. The HAp coating, incorporating ZnO precipitates on its topmost surface layer, was prepared on a Ti substrate using chemical solution deposition followed by heating at 650 °C. The amount of ZnO precipitates could be controlled by changing the ZnO concentration in the deposition solution; furthermore, the Zn release rate from the surface could be controlled by varying the ZnO amount. The ZnO/HAp coating showed excellent antibacterial efficacy against *Escherichia coli* and *Staphylococcus epidermidis* strains; however, no correlation was observed between the degree of efficacy and Zn release rate. The antibacterial efficacy of the ZnO/HAp coating likely originates from the contact killing effect of the ZnO precipitates. In summary, the coatings introduced in this work are promising candidates for the surface modification of Ti implants, with a potential ability to combine the prevention of infectious diseases with osteogenic activity.

1. Introduction

Combining the prevention of infectious diseases with osteogenesis induction is a key strategy to improve the outcome of surgical procedures based on titanium (Ti) implants. Incorporating antibacterial agents in hydroxyapatite (HAp, $Ca(PO_4)_6(OH)_2$) coatings is one of the most effective approaches to accomplish this task. Silver (Ag) is often considered as an adequate antibacterial agent for these applications, owing to its strong antibacterial efficacy and broad-spectrum antibiotic activity [1–8]. However, Ag is not an essential element in biological systems, and its release from an implant surface does not provide specific benefits to the human body; furthermore, released amounts of Ag ions exceeding a certain limit are known to be cytotoxic [9,10]. Accordingly, in terms of the long-term safety of patients, essential elements possessing antibacterial properties are preferable as additive agents.

Zinc is an essential mineral for biological processes such as DNA synthesis, enzyme activity, and cellular metabolism [11]. Furthermore, the incorporation of Zn in an implant material promotes the proliferation and differentiation of osteoblast cells, leading to enhanced osteogenesis [12,13]. Based on these properties, the addition of Zn to HAp coatings is expected to be beneficial for enhancing bone formation on a medical implant. In addition to the functions described above, Zn ions also exhibit antibacterial efficacy, although their activity is rather low compared with Ag ions. Several studies have thus investigated the antibacterial efficacy and bioactivity of HAp materials containing Zn [14–20]. For instance, Stanić et al. synthesized Zn-doped HAp nanocrystals and confirmed their antibacterial efficacy against three bacterial strains in a buffer solution [15]. Thian et al. also prepared Zn-substituted HAp powders containing 1.6 wt.% Zn, and confirmed their antibacterial efficacy [17]. Although these previous studies confirmed the excellent antibacterial efficacy of Zn-containing HAp powder, a reduced efficacy was observed when Zn-containing HAp was used as a coating material. For instance, although Samani et al. reported the reduction of *methicillin-resistant Staphylococcus aureus* (MRSA) on HAp coatings containing 2.5 wt.% Zn, they also observed MRSA reduction on Zn-free HAp coatings [20], which suggested that the antibacterial effect of Zn incorporation was negligible. The reduced antibacterial efficacy observed for the coatings was probably due to the lower amounts of Zn released from the surface, even though a higher release of Zn is also associated with an increased risk of cytotoxicity. New approaches are thus required to prepare Zn-containing HAp coatings to combine antibacterial efficacy and biocompatibility.

In the present study, we investigate a newly designed antibacterial HAp coating exploiting the so-called “contact killing” effect

* Corresponding author at: Instrumental Analysis Center, Kitami Institute of Technology, 165 Koen–cho, Kitami, Hokkaido 090–8507, Japan.
E-mail address: nohtsu@mail.kitami-it.ac.jp (N. Ohtsu).

http://dx.doi.org/10.1016/j.apsusc.2017.09.101
0169-4332/

of ZnO, which is derived from the reactive oxygen species generated by the reaction of ZnO with water without light illumination [21,22]. HAp coatings incorporated with ZnO precipitates in the topmost surface layer were prepared using chemical solution deposition and subsequent heating. The antibacterial efficacy against gram-positive and gram-negative bacterial strains and the Zn release behavior of the ZnO/HAp coatings were subsequently investigated to assess their potential as antibacterial biomaterials.

2. Materials and methods

2.1. Preparation and characterization of coatings

The precursor solution for the HAp coatings was prepared by mixing a 3.34 M ethanolic solution of $Ca(NO_3)_2$ with an equivalent amount of 2 M aqueous solution of H_3PO_4. Subsequently, a 0.02, 0.2, or 1.2 M ZnO aqueous solution was added to an equivalent amount of HAp solution in order to prepare the Zn-containing HAp solution. The Zn to Ca atomic ratio ([Zn/Ca]) changed from 0.012 to 0.12 and thereafter to 1.2 with the increase in Zn concentration, whereas the Ca to P ratio ([Ca]/[P]) was set to the constant value of 1.67.

Ti plates ($20 \times 20 \times 1$ mm) with 99.9% purity (Furuuchi Chem. Co.) were used as substrates. The surface of the substrate was ground with 1000-grade SiC paper and thereafter ultrasonically washed in ethanol. Ultraviolet light with a wavelength of 254 nm was subsequently irradiated on the surface for 30 min to obtain a hydrophilic surface. The ZnO/HAp coating was deposited on the substrate using the spin coating technique. After dropping 80 μL of Zn-containing HAp solution on the substrate surface, the substrate was rotated at 2000 rpm for 10 s and 5000 rpm for 3 s, in sequence. For comparison, a Zn-free HAp coating was prepared following the same procedure. The coatings were dried at 125 °C for 10 min and subsequently heated at 650 °C for 15 min to improve the crystallinity and to form ZnO precipitates. The coating process was repeated three times to obtain a coating with sufficient thickness.

The surface morphology of the specimens was inspected using scanning electron microscopy (SEM, JCM-5000, JEOL) with an acceleration voltage of 10 kV. X-ray diffraction (XRD, New D8 Advance, Bruker AXS) patterns were obtained using a Bragg-Brentano geometry with Cu K_α radiation. The elemental depth profiles and chemical states were analyzed using X-ray photoelectron spectroscopy (XPS, PHI 5000 Versa Probe, Ulvac-Phi) with monochromatized Al K_α radiation ($h\nu = 1486.6$ eV), with an X-ray probe of diameter approximately 100 μm. The photoelectron take-off angle was set at 45°. An Ar etching system with an acceleration voltage of 3 kV was used for obtaining the elemental depth profiles. The etching rate estimated from the SiO_2 layer was 14.3 nm min^{-1}. The chemical composition and thickness of the coatings were determined using wavelength dispersive X-ray fluorescence (XRF, S8 Tiger, Bruker AXS) analysis, using the fundamental parameter algorithm for thin films preinstalled in the instrument. The thickness of the coating was calculated by assuming its density as 3.19 g cm^{-3}.

2.2. Zn release

We measured the amount of Zn ions released into a physiological solution (phosphate-buffered saline, PBS) to analyze the Zn release behavior of the coatings. The ZnO/HAp-coated Ti plates were soaked in 30 mL of PBS in a polypropylene centrifuge tube, and the tube was subsequently incubated at 37 °C for 4 h in an incubator shaken at 65 rpm. Thereafter, the Zn concentration in the PBS solution was determined using flame atomic absorption spectrometry (FAAS, Z-2010, Hitachi, Japan) according to the manufacturer's instructions.

Table 1
Labels used to denote the prepared ZnO/HAp coatings and corresponding thickness, [Zn]/[Ca], and [Zn]/[Ca] ratios determined using XRF.

Symbol	Thickness (nm)	[Zn]/[Ca]	[Ca]/[P]
ZnHAp1	274	0.021	1.57
ZnHAp2	219	0.11	1.55
ZnHAp3	295	1.0	1.86

2.3. Antibacterial activity

The antibacterial efficacy of the coatings was examined using *Escherichia coli* (*E. coli*) (ATCC 25922) and *Staphylococcus epidermidis* (*S. epidermidis*) (ATCC 14990) strains. Prior to the antibacterial test, both bacteria were cultured on nutrient agar plates at 37 °C. A 2×10^6 colony-forming unit (CFU) mL^{-1} bacterial suspension was prepared using a 1:500 diluted nutrient broth (NB). The ZnO/HAp- and HAp-coated substrates were sterilized using autoclaving for 30 min at 121 °C. A 23 μL aliquot of the bacterial suspension was dispensed into the sterilized specimens and covered with a 15×15 mm cover glass. The specimens were subsequently placed into an incubator set at 37 °C with 95% humidity and incubated for 4 h. The bacterial suspension covering the specimen was subsequently washed in a broth of soybean-casein digest with lecithin and polysorbate 80 (SCDLP), which has growth characteristics and performs neutralizing action. The SCDLP broth serially diluted with 1:500-diluted NB broth was subsequently cultivated on the surface of a nutrient agar plate, and the colonies formed on the plate were counted.

The antibacterial tests were performed with $n = 3$ and repeated at least two times. Statistical analyses were performed using analysis of variation combined with a Student-Newman-Keuls post-hoc test to identify the levels of significance of the data ($^*p < 0.05$ or $^{**}p < 0.01$).

3. Results and discussion

3.1. Properties of the ZnO/HAp coating

Table 1 lists the labels used to denote the prepared ZnO/HAp coatings, together with the corresponding [Zn]/[Ca] and [Ca]/[P] ratios determined using XRF. The [Zn]/[Ca] ratios of the coatings almost exactly match the corresponding nominal ratios used in the coating solution, whereas the [Ca]/[P] ratios are close to the stoichiometric ratio of HAp ([Ca]/[P] = 1.67), even though the ratio of the ZnHAp3 coating was slightly higher than that of the others. We attribute this difference to the error in the structural model used for the calculation of the XRF fundamental parameter; in particular, the quantitative analysis was based on the assumption that all the constituent elements were distributed homogeneously, but Zn was concentrated in the top surface layer of the coating, as shown in Fig. 2. Moreover, the estimated thicknesses of all coatings were 260 ± 40 nm.

The microstructural images of the coating surface obtained using SEM are shown in Fig. 1. In the case of the ZnHAp1 coating, only polishing traces are visible in the image, whereas no features associated with the structure of the coating can be observed. This result indicates that the coating formed using chemical solution deposition with spin coating was homogeneous. However, the image of the ZnHAp2 surface reveals the formation of nanometer-sized granular precipitates (indicated by arrows), and the image of the ZnHAp3 coating shows a drastic increase in the amount of these precipitates, resulting in the surface being completely covered by the aggregation of these precipitates.

In order to analyze the chemical states of constituent elements in the coating surface, XPS spectra were measured in the Ca 2p, P

Please cite this article in press as: N. Ohtsu, et al., Antibacterial effect of zinc oxide/hydroxyapatite coatings prepared by chemical solution deposition, Appl. Surf. Sci. (2017), http://dx.doi.org/10.1016/j.apsusc.2017.09.101