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Preparation, cell response and apatite-forming ability of microarc oxidized coatings containing Si, Ca and Na on titanium

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Abstract

The formation, cell response and apatite-forming ability of novel TiO_2-based coatings containing Si, Ca and Na (SCN) on titanium formed by microarc oxidation (MAO) were investigated. The oxidation time has obvious effect on the surface morphology and SCN elements concentrations of the MAO coatings. The current results indicated that the MAO coatings containing SCN possess good apatite-forming ability, which is highly dependent on the applied voltage. And high applied voltage could facilitate for improving the apatite-forming ability of the MAO coatings due to the introduction of a large quantity of SCN elements. It was firstly observed that the induced biomimetic apatite could grow into the pores of MAO coatings, which could facilitate for the fixation of apatite layer. The A-type slightly substituted carbonated-HA and B-type slightly substituted carbonated-HA with HPO_4^{2-} group were obtained after immersion of the MAO coatings in a simulated body fluid. As expected, the MAO coatings show good biocompatibility according to the cells proliferation.

Keywords: D. Apatite; Microarc oxidation; Titanium; Cell response

1. Introduction

Microarc oxidation (MAO) is a relatively convenient and effective technique to deposit ceramic coatings on the surfaces of Ti, Al, Mg and their alloys [1]. This technique can introduce various desired elements into MAO coatings and produce various functional coatings with a porous structure [1]. Using MAO technique to deposit bioactive ceramic coatings on titanium and its alloys has received much attention in recent years [2–13]. In most studies, Ca and P elements were introduced into MAO coatings for preparing the bioactive MAO coatings on Ti and its alloys.

To form bioactive MAO coatings on titanium, the exploration for introducing various elements into the TiO_2-based MAO coatings has been continually noticed. In this work, novel MAO coatings containing Si, Ca and Na (SCN) elements were prepared on titanium. As well known, silicon plays an important role in bone mineralization and formation and is therefore used in a wide variety of medical implants and bone grafts [14]. And most of the MAO coatings containing Si are used for engineering applications [1]. In this work, SCN elements were doped into the MAO coatings to form novel TiO_2-based coatings. And the cell response and apatite-forming ability of the MAO coatings containing SCN were investigated.

2. Experimental procedure

2.1. Sample preparation

In the MAO experiment, Ti plates (10 mm × 10 mm × 1 mm) were used as anodes and stainless steel plates were used as cathodes in an electrolytic bath. The plates were ground with 400, 800 and 1000# abrasive papers, washed with acetone and distilled water, and dried at 40 °C. An electrolyte was prepared by the dissolution of reagent-grade chemicals of $Ca(CH_3COO)_2{\cdot}H_2O$ (8.8 g/l), Na_2SiO_3 (14.2 g/l), EDTA-2Na (15 g/l) and NaOH (20 g/l) into deionized water. The temperature of the electrolyte was kept at 40 °C by a cooling system. Firstly, to investigate the

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Table 1
Ion concentrations of the SBF and human blood plasma.

Ion	Concentrations (mmol/l)	
	SBF	Blood plasma
Na^+	142.0	142.0
K^+	5.0	5.0
Mg^{2+}	1.5	1.5
Ca^{2+}	2.5	2.5
Cl^-	147.8	103.8
HCO_3^{2-}	4.2	27
HPO_4^{2-}	1.0	1.0
SO_4^{2-}	0.5	0.5

formation of the MAO coatings containing SCN, the Ti plates were treated by MAO for 1, 3, 5, 10 and 30 min under an applied voltage, frequency and duty cycle of 400 V, 600 Hz and 8.0%. Then, to further investigate the effect of applied voltages on the cell and apatite-forming ability of the MAO coatings, different applied voltages of 200, 250, 300, 350, 400 and 450 V were used in MAO treatment for 5 min with the frequency and duty cycle of 600 Hz and 8.0%.

2.2. Immersion of the samples in a simulated body fluid

The MAO coatings were soaked in 15 mL simulated body fluid (SBF) [15] (Table 1) immersing for 14 and 28 days and the SBF was refreshed every other day. The SBF was prepared by dissolving reagent-grade chemicals of NaCl, $NaHCO_3$, KCl, $K_2HPO_4{\cdot}3H_2O$, $MgCl_2{\cdot}6H_2O$, $CaCl_2$, and Na_2SO_4 into deionized water and buffering at pH 7.40 with tris-hydroxymethylaminomethane ($(CH_2OH)_3CNH_2$) and 1.0 mol/L HCl at 37 °C.

2.3. Structure characterization

2.3.1. X-ray diffraction (XRD)

The phase composition of MAO samples after SBF immersion were analyzed by X-ray diffraction (XRD, D/max-γB, Japan) using a Cu Kα radiation with a continuous scanning mode at a rate of 4°/min, under an accelerating voltage of 40 kV and current of 50 mA.

2.3.2. Scanning electron microscopy (SEM) and energy dispersive X-ray spectrometer (EDS)

A scanning electron microscopy (SEM, Quanta 200, FEI Co., American) was used to observe the surface morphologies of the MAO coatings before and after SBF immersion. The surface was coated a very thin gold film to improve its electrical conductivity and secondary electron images of the surface were obtained under a voltage of 30 kV and chamber pressure of 5×10^{-3} Pa. In addition, the elemental compositions of the sample surfaces were detected by an energy dispersive X-ray spectrometer (EDS, EDAX, American) equipped on the SEM system.

2.3.3. Fourier transform infrared spectroscopy

Fourier transform infrared spectroscopy (FT-IR, Magna-IR 560 E.S.P., American) was used to analyze the apatite structure. In the FT-IR experiment, the scanning range and resolution were 4000–400 and 4 cm^{-1}, respectively. Approximately 1 mg of apatite layer on the samples mixed with about 500 mg of dry KBr powder was ground using an agate mortar and pestle. The mixed powder was pressed into transparent disks with a diameter of 13 mm for the FT-IR work.

2.3.4. Raman spectroscopy

The laser Raman spectroscopy measurements were performed on a HR800 spectrometer (Jobin Yvon, France) with argon ion laser under an output power of 20 mw and wave of 458 nm to detect the surface composition of the MAO coatings.

2.4. Cell proliferation

The biocompatibility of the MAO samples was preliminarily evaluated by cell tests. In the cell tests, the MG63 cells were used to characterize the cells proliferation behavior. The pre-incubated cell lines were plated onto samples with a cell density of 2×10^4 mL^{-1}, and then cultured in a humidified incubator with 5% CO_2 at 37 °C. Dulbecco's modified Eagle's medium (DMEM) with 10% fetal bovine serum (FBS) was used as the culturing medium. The proliferation behavior was determined by counting the number of cells after culturing them for 4 and 7 days. The cells were detached from the samples with 0.05% trypsin-EDTA and counted using a hemocytometer.

3. Results and discussion

3.1. The formation of the MAO coatings containing SCN

Fig. 1 shows the SEM morphology and EDS results of the titanium after MAO treatment for different times. It was observed that the change in the surface morphology is slightly, when the oxidized time increased from 1 to 3 min and 5 to 30 min. However, when the oxidized time increased from 3 to 5 min, the pores sizes of the MAO coatings increased evidently. And the cracks were observed on the surface of the MAO coating when oxidizing for 30 min.

The EDS results indicated that the SCN elements were introduced into the MAO coatings. And the atomic concentrations of SCN increased evidently when the oxidized time increased from 1 to 5 min, and that of Ti decreased significantly as shown Fig. 2. However, the change in the atomic concentrations is not obviously after oxidation more than 5 min, where the atomic concentrations of the Si, Ca and Na showed about 12, 5 and 4 at.%. In addition, the O atomic concentration did not change evidently during the whole oxidation process. Based on surface morphology and elemental concentrations of the MAO coatings, oxidized time of 5 min was recommended.

Fig. 3 shows the Raman spectra of the MAO coatings formed by oxidizing the titanium for 5 min at different applied voltages. The peaks were detected at 400, 518 and 641 cm^{-1},

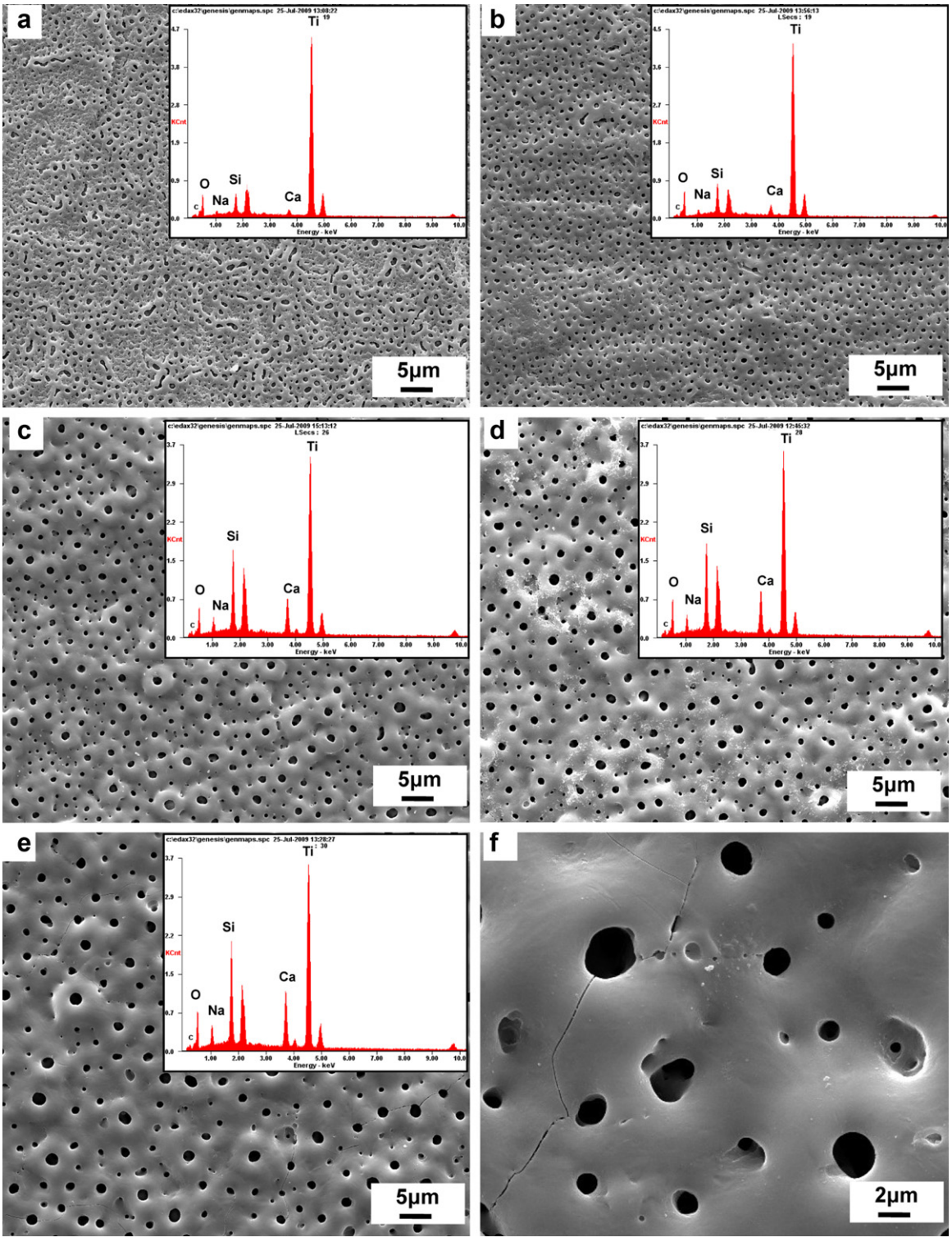

Fig. 1. SEM morphology and EDS results of titanium after MAO treatment for different times: (a) 1, (b) 3, (c) 5, (d) 10, (e) 30 min and (f) high magnification of (e).

corresponding to the anatase phase. In addition, previous investigated that the surface morphology of the MAO coating formed at different applied voltages is highly dependent on the applied voltage, and the pores size and surface roughness increased with increasing the applied voltage.

3.2. Apatite-forming ability of the MAO coatings

3.2.1. Phase composition

Fig. 4 shows the XRD patterns of the MAO coatings after SBF immersion for 14 days. All the surfaces of the MAO

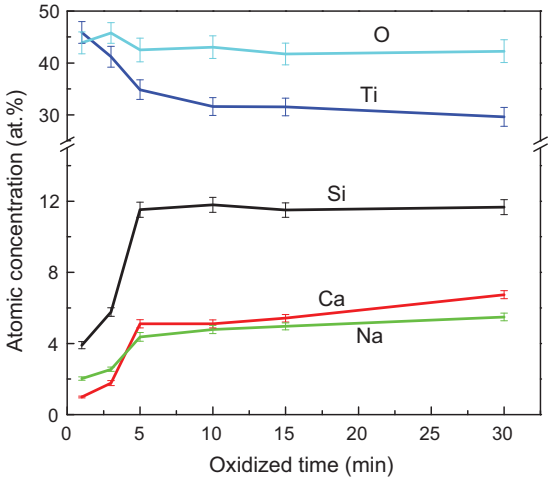

Fig. 2. The change in the atomic concentrations detected by EDS of the MAO coatings with increasing the oxidized time.

coatings show the apatite peaks, presenting apatite-forming ability. However, it was also observed that the apatite peaks became more intensive with increasing the applied voltages, suggesting that the apatite-forming ability of the MAO coatings containing SCN was improved by increasing the applied voltages.

3.2.2. Surface morphology and elemental composition

The surface morphologies of the MAO coating after soaking in the SBF for 14 and 28 days are shown in Figs. 5 and 6. In Fig. 5, local modification was observed on the surfaces of the MAO coatings formed at 200 and 250 V, while the entry surface of the MAO coatings formed at 300–450 V was modified by the apatite layer. The SEM results indicate that the ability to induce apatite formation is improved by increasing the applied voltage. At the higher magnification, lots of nano-rod like apatite crystals was found as shown in Fig. 5e. After immersion for 28 days, the entry surfaces of the MAO coatings formed at different applied voltages were covered by the apatite layers as shown in Fig. 6.

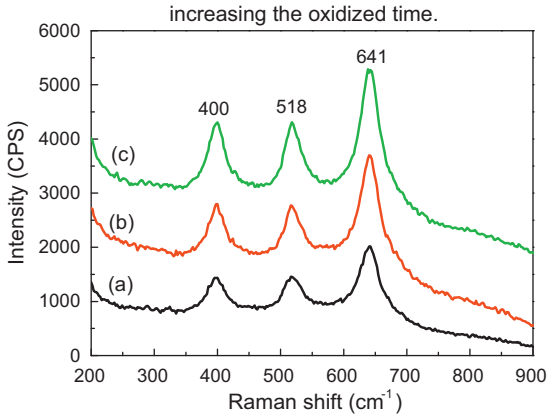

Fig. 3. Raman spectra of the MAO coatings prepared at various applied voltages: (a) 200, (b) 300 and (c) 400 V.

Fig. 7 shows the effect of applied voltage on the Ca/P ratio of induced apatite at different periods. Increasing the immersion time and applied voltage could improve the Ca/P ratio. After immersion for 2 and 3 weeks, the effect of applied voltage on the Ca/P ratio is obvious, compared to that after immersion for 4 weeks. At 4 weeks, the Ca/P ratios are at the level of 1.55–1.65. Totally, the Ca/P ratios of apatite are lower than that of HA. The apatite structure is very hospitable in allowing the substitutions of many other ions. The biomimetic apatite formed in SBF is similar to synthetic HA, but they differ from HA in composition, stoichiometry, and physical and mechanical properties. In fact, most investigations indicated that biomimetic apatites possess relative low Ca/P ratios due to the lack of Ca^{2+} ions in the apatite crystal, since the Ca^{2+} ions could be substituted by K^+, Na^+ and Mg^{2+} ions, and the PO_4^{3-} and OH^- groups could be substituted by HPO_4^{2-}, CO_3^{2-}, Cl^- and F^- ions. According to the EDS results, a small quantity of Na and Mg was detected in the apatite layers (not shown here).

Fig. 8 shows the elemental distributions on the surfaces of the MAO coating formed at 250 V after SBF immersion for 14 days after removing the apatite layer. It was interesting that apatite grew into the pores of the MAO coating according to the SEM morphology and elemental mapping results. As shown in Fig. 8b and c, the Ca and P concentrations in the pores were obviously higher compared to other places, indicating the deposition of apatite in the pores, which could facilitate the fixation of apatite on the surface. According to the current results, it was expected that new bone can grow into the pores of the MAO coatings after implanting, thus promoting the interface bonding between implants and bone.

3.2.3. FT-IR spectra

The FT-IR spectra of the MAO coatings after SBF incubation for 28 days are shown in Fig. 9. A broad absorption band at 3441 cm^{-1} and a bending mode at 1651 cm^{-1} indicate

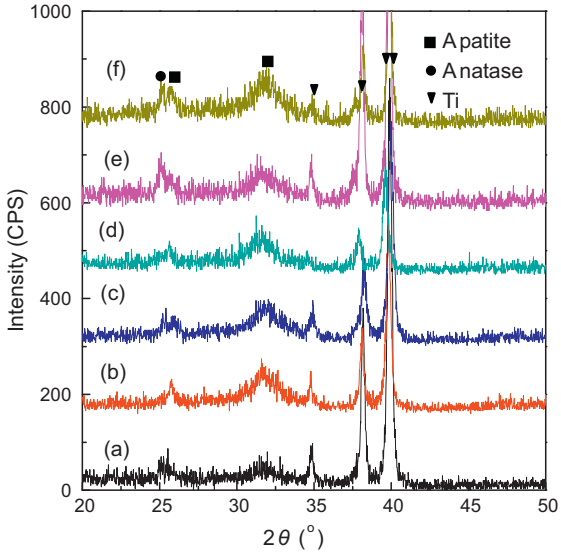

Fig. 4. XRD patterns of the MAO coatings prepared at various applied voltages after SBF immersion for 14 days: (a) 200, (b) 250, (c) 300, (d) 350, (e) 400 and (d) 450 V.

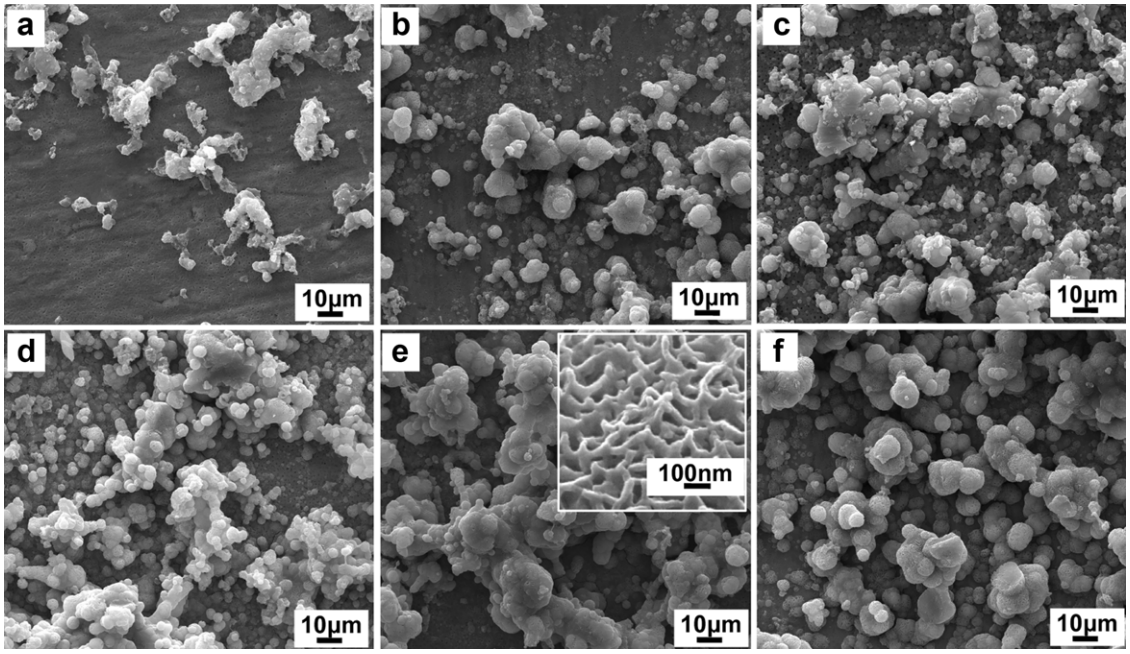

Fig. 5. SEM morphology of the MAO coatings prepared at various applied voltages after SBF immersion for 14 days: (a) 200, (b) 250, (c) 300, (d) 350, (e) 400 and (f) 450 V.

the presence of bonded water in the SBF incubated MAO coatings [16]. Absorption peaks of PO_4 bands were observed including the triply degenerated asymmetric stretching mode of v_3PO_4 band at 1033 cm^{-1}, triply degenerated bending mode of v_4PO_4 band at 602 and 566 cm^{-1} and double degenerated bending mode of v_2PO_4 band at 471 cm^{-1} [16]. In addition, the FT-IR spectrum shows the CO_3^{2-} absorption bands of the characteristic stretching mode of $v_1CO_3^{2-}$ group in A-type slightly substituted carbonated-HA at 1462 cm^{-1}, stretching mode of $v_1CO_3^{2-}$ group in B-type slightly substituted carbonated-HA at 1421 cm^{-1} and bending mode of (v_3 or v_4) CO_3^{2-} group in carbonated HA at 872 cm^{-1} [16]. Also, the HPO_4^{2-} groups were detected at characteristic peaks of 1099, 956 and 874 cm^{-1} [16]. The FT-IR result confirms that the apatite formed on the MAO coating has a carbonated structure.

3.2.4. Formation of apatite

The current results reveal that the MAO coatings containing SCN elements have good induction capability for the heterogeneous nucleation and growth of apatite in the

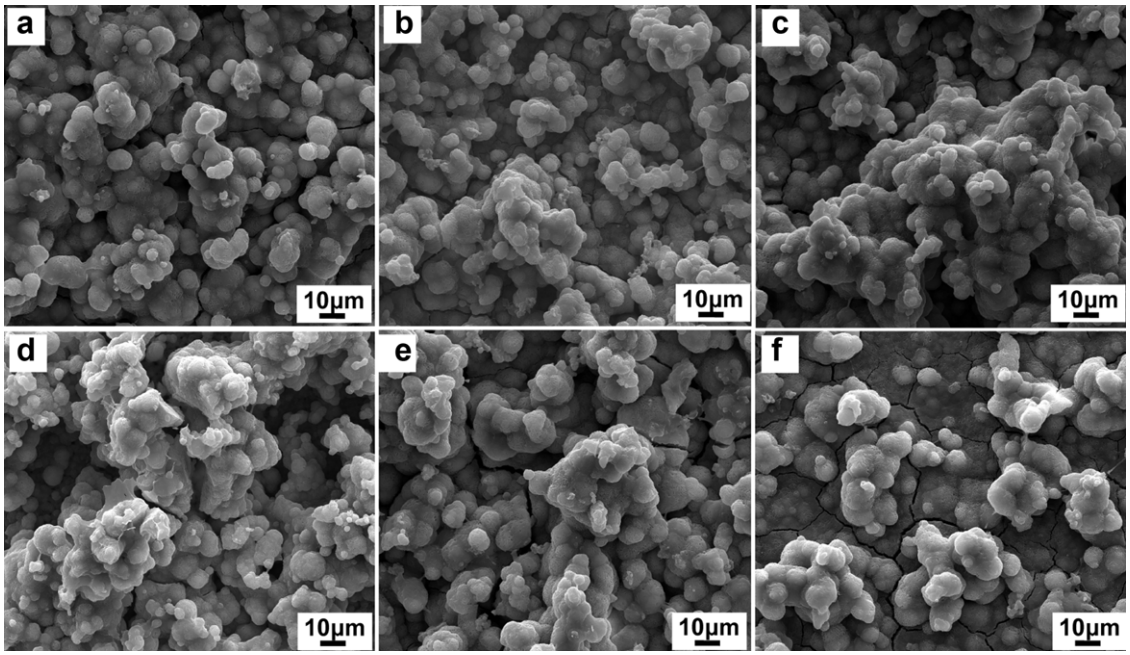

Fig. 6. SEM morphology of the MAO coatings prepared at various applied voltages after SBF immersion for 28 days: (a) 200, (b) 250, (c) 300, (d) 350, (e) 400 and (f) 450 V.

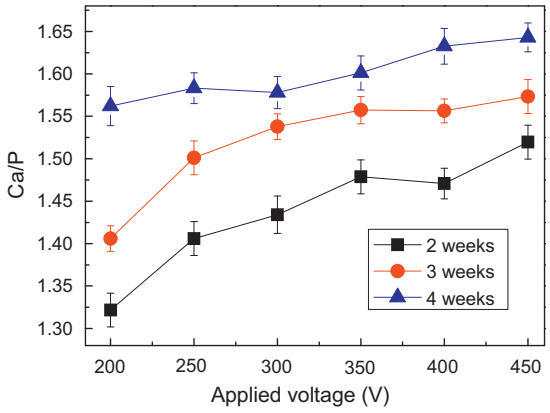

Fig. 7. Effect of the applied voltage on the Ca/P ratio of induced apatite at different periods.

SBF. The contribution of the SCN elements for the bioactivity is significant in various bioactive inorganic compounds such as bioactive glasses–ceramics, which can bond to bone tissues well because it can induce a layer of biologically active hydroxycarbonate apatite spontaneously under a physiological environment due to the presence of Si, Ca and Na, etc. Generally, bonelike apatite formation on biomaterial surface in SBF requires a chemical stimulus. The apatite-bonding structure is highly dependent on the chemical reactivity of the materials surface in fluids. In this work, the formation of apatite could be explained as following. Firstly, the deposition of apatite could be related with the formation of hydrated silica gel layer on the surfaces based on the previous researches [17], which could provide the chemical stimulus for the apatite deposition. The formation of hydrated silica gel is via dissolution of Na^+ ion from the surface based on the previous researches [17]:

$$Si(OSi)_3ONa + H_2O \rightarrow Si(OSi)_3OH + Na^+ + OH^- \quad (1)$$

$$Si(OSi)_3OH \rightarrow Si(OSi)_3O^- + H^+ \quad (2)$$

Moreover, the dissolution of Ca^{2+} ion into the SBF could also act as similar effect on the formation of OH group compared to Na^+ ion. At the same time, the dissolution of Ca^{2+} ion could further increase the local supersaturation near the surface of MAO coatings, promoting the deposition of calcium phosphates as shown in the following equation [17]:

$$Si(OSi)_3O^- + 2Ca^{2+} + P(V) \rightarrow Si(OSi)_3O^{d-}(Ca - O - PO_3 - Ca)^{d+} \quad (3)$$

It was observed that the increase of applied voltage could improve the apatite-forming ability of the MAO coatings. The reason for this is that increasing the applied voltage further augmented the concentrations of SCN in the MAO coatings, therefore improving the concentration of OH groups form by the dissolution of Na^+ and Ca^{2+} ions. Evidently, the formation of large quantity of OH groups facilitated for inducing the apatite deposition according to the above equation.

3.3. Cell proliferation

Fig. 10 shows the density of MG63 cells after proliferation for 4 and 7 days on the surfaces of titanium and MAO coatings. After cell proliferation for 7 days, the cell density increased obviously compared to that after 4 days. As expected, the MAO coating shows good biocompatibility according to the cells proliferation.

The previous research indicated the MAO coatings contain amorphous phase, which could be mainly composed of SCN

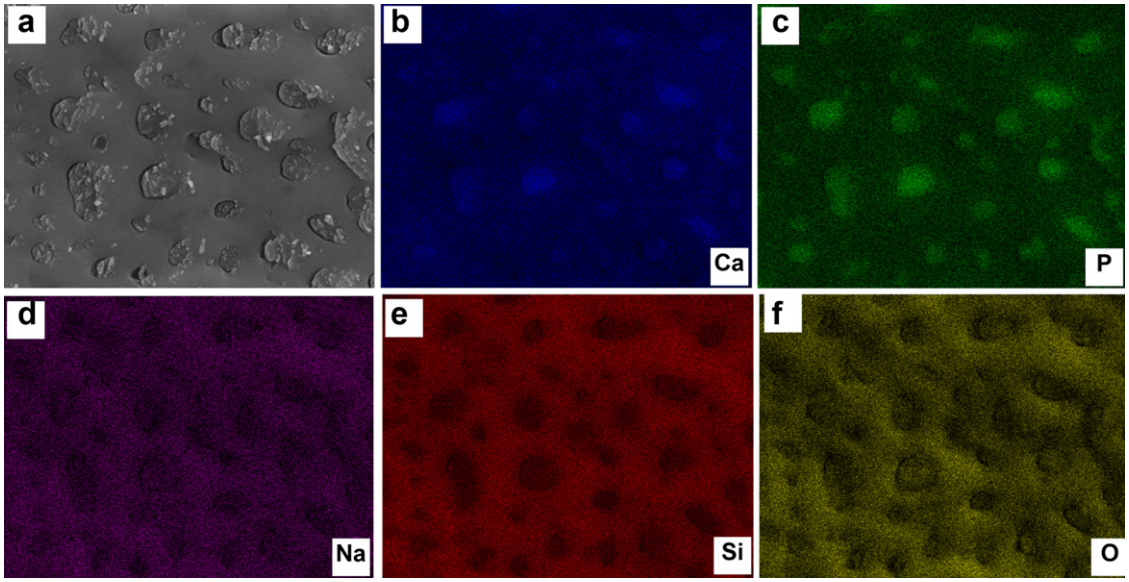

Fig. 8. Surface morphology and elemental distributions on the surfaces of the MAO coating formed at 250 V after SBF immersion for 14 days: (a) morphology, (b) Ca, (c) P, (d) Na, (e) Si and (f) O.

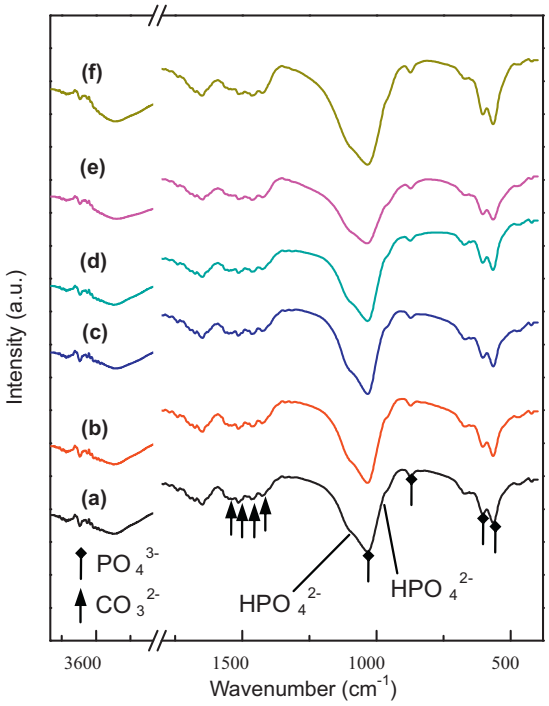

Fig. 9. FT-IR spectra of the MAO coatings formed at various voltages after SBF immersion for 28 days: (a) 200, (b) 250, (c) 300, (d) 350, (e) 400 and (f) 450 V.

and O elements. Recently, we found the MAO TiO_2-based coatings containing amorphous calcium phosphate has good cell proliferation and apatite-forming ability, while the cell proliferation and apatite-forming ability decreased after heat treatment due to the crystallization of amorphous phase in the MAO coatings [18]. Additionally, Nakamura et al. [19] also have believed that amorphous calcium phosphate phase facilitate the initial fixation of porous materials due to their excellent osteoconductive property. Based on these, we believe that the MAO coatings containing amorphous phase with composition of SCN elements is positive for the bioactivity and biocompatibility such as cell proliferation, apatite-forming ability, bone growth and bonding etc., which need more a thorough investigation in the future.

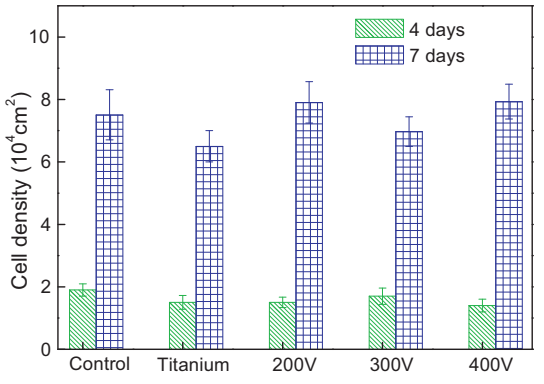

Fig. 10. Density of MG63 cells after proliferation for 4 and 7 days on the surfaces of titanium and MAO coatings formed at different applied voltages.

4. Conclusion

The formation, cell response and apatite-forming ability of MAO TiO_2-based coatings containing SCN on titanium were investigated. The oxidation time has obvious effect on the surface morphology and SCN elements concentrations of the MAO coatings. The current results indicated that the MAO coatings containing SCN possess good apatite-forming ability, which is highly dependent on the applied voltage. It was firstly observed that the induced biomimetic apatite could grow into the pores of MAO coatings. The A-type slightly substituted carbonated-HA and B-type slightly substituted carbonated-HA with HPO_4^{2-} group were obtained after immersion of the MAO coatings in simulated body fluid. As expected, the MAO coating containing SCN shows good biocompatibility according to the cells proliferation.

Acknowledgements

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References

[1] A.L. Yerokhin, X. Nie, A. Leyland, A. Matthews, S.J. Dowey, Plasma electrolysis for surface engineering, Surf. Coat. Technol. 122 (1999) 73–93.
[2] D.Q. Wei, Y. Zhou, D.C. Jia, Y.M. Wang, Characteristic and in vitro bioactivity of microarc oxidized TiO_2-based coating after chemical treatment, Acta Biomater. 3 (2007) 817–827.
[3] D.Q. Wei, Y. Zhou, D.C. Jia, Y.M. Wang, Effect of heat treatment on the structure and in vitro bioactivity of microarc-oxidized (MAO) titania coatings containing Ca and P ions, Surf. Coat. Technol. 201 (2007) 8723–8729.
[4] D.Q. Wei, Y. Zhou, D.C. Jia, Y.M. Wang, Effect of applied voltage on the structure of microarc oxidized TiO2-based bioceramic film, Mater. Chem. Phys. 104 (2007) 177–182.
[5] D.Q. Wei, Y. Zhou, Y.M. Wang, D.C. Jia, Characteristic of microarc oxidized coatings on titanium alloy formed in electrolytes containing chelate complex and nano-HA, Appl. Surf. Sci. 253 (2007) 5045–5050.
[6] D.Q. Wei, Y. Zhou, D.C. Jia, Y.M. Wang, Biomimetic apatite deposited on microarc oxidized anatase-based ceramic coating, Ceram. Int. 34 (2008) 1139–1144.
[7] M. Fini, A. Cigada, G. Rondelli, In vitro and in vivo behaviour of Ca- and P-enriched anodized titanium, Biomaterials 20 (1999) 1587–1594.
[8] X.L. Zhu, K.H. Kim, Y.S. Jeong, Anodic oxide films containing Ca and P of titanium biomaterial, Biomaterials 22 (2001) 2199–2206.
[9] X.L. Zhu, J.L. Ong, S.Y. Kim, K.H. Kim, Surface characteristics and structure of anodic oxide films containing Ca and P on a titanium implant material, J. Biomed. Mater. Res. 60 (2002) 333–338.
[10] Y. Han, S.H. Hong, K.W. Xu, Structure and in vitro bioactivity of titania-based films by micro-arc oxidation, Surf. Coat. Technol. 168 (2003) 249–258.
[11] V.M. Frauchiger, F. Schlottig, B. Gasser, M. Textor, Anodic plasma-chemical treatment of CP titanium surfaces for biomedical applications, Biomaterials 25 (2004) 593–606.
[12] L.H. Li, Y.M. Kong, H.W. Kim, Improved biological performance of Ti implants due to surface modification by micro-arc oxidation, Biomaterials 25 (2004) 2867–2875.

[13] W.H. Song, Y.K. Jun, Y. Han, S.H. Hong, Biomimetic apatite coatings on micro-arc oxidized titania, Biomaterials 25 (2004) 3341–3349.
[14] J.C. Heughebaert, G.H. Nancollas, Mineralization kinetics the role of octacalcium phosphate in the precipitation of calcium phosphates, Colloid Surf. 9 (1984) 89–93.
[15] A. Oyane, H.M. Kim, T. Furuya, T. Kokubo, T. Miyazaki, T. Nakamura, Biomimetic apatite coatings on micro-arc oxidized titania, J. Biomed. Mater. Res. 65A (2003) 188–195.
[16] L. Müller, F.A. Müller, Preparation of SBF with different HCO_3^- content and its influence on the composition of biomimetic apatites, Acta Biomater. 2 (2006) 181–189.
[17] S. Hayakawa, K. Tsuru, C. Ohtsuki, A. Osaka, Mechanism of apatite formation on a sodium silicate class in a simulated body fluid, J. Am. Ceram. Soc. 82 (1999) 2155–2160.
[18] D.Q. Wei, Y. Zhou, Characteristic and biocompatibility of the TiO_2-based coatings containing amorphous calcium phosphate before and after heat treatment, Appl. Surf. Sci. 255 (2009) 6232–6239.
[19] M. Nagano, T. Nakamura, T. Kokubo, M. Tanahashi, M. Ogawa, Differences of bone bonding ability and degradation behaviour in vivo between amorphous calcium phosphate and highly crystalline hydroxyapatite coating, Biomaterials 17 (1996) 1771–1777.