Effect of annealing titanium on *in vitro* bioactivity

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In order to modify titanium surfaces for various biological applications, bioactive and pure titanium oxide thin films were coated on the titanium by thermal oxidation technique. The commercially pure titanium discs after polishing were heated at 500, 550, 600, 650 and 700 °C, respectively, for 10 min in air or in argon. To evaluate the ability of calcium phosphate formation, samples after annealing were soaked in the Eagle's minimum essential medium solution. Surface morphology and chemical composition of the samples before or after immersion were characterized by field emission – scanning electron microscopy and energy dispersive X-ray spectrometry.

1. Introduction

Bone-to-metallic implant fixation is often necessary in orthopedic, maxillofacial, and dental procedures. Achieving a stable bone–implant interface is an important factor in the long-term outcome of joint arthroplasty.

Titanium is one of the most important materials for implant because it has good biocompatibility compared to other metallic materials [1, 2]. Titanium is biologically inert and has a high corrosion resistance due to the spontaneous formation of titanium oxide (TiO_2) film on its surface in air and in electrolytes [3–7]. TiO_2 thin films naturally formed on titanium or its alloys can contribute to their good biocompatibility in human body [8]. However, it has been proven that such films were not bioactive enough to induce calcium phosphate (CaP) precipitation [9]. Thus, various surface modifications have been attempted to improve its bone conductivity. The most popular process is plasma spraying of ceramics on titanium [10–12]. However, the fracture has been reported on the interface of apatite and titanium, or inside of the apatite layer.

Many techniques, such as ion implantation, sand-blasting, micromachining, electropolishing and chemical treatment [13–17], have been applied to modify titanium surfaces to improve their biological applications.

Uchida *et al*. [18] have reported that *in vitro* apatite nucleation was effectively induced on anatase structures in Ti–OH groups. Furthermore, total surface area, pore size and pore volume were important parameters for determining the kinetics of apatite nucleation [19]. Hence, detailed knowledge about the oxide film formed on titanium surfaces and its evolution in biological environments is required to better understand the bioactivity of titanium.

To clearly investigate effect of the TiO_2 thin film on titanium by only the thermal oxidation on CaP forming ability, we selected mirror-polished titanium as a starting material to avoid surface-roughness effect. Effects of the TiO_2 thin films by different annealing temperatures on CaP forming ability were examined by *in vitro* test. During annealing, oxidation (in air) or reduction (in argon) atmosphere was adopted to investigate effect of the annealing condition on crystallinity of TiO_2 thin films.

2. Experimental procedure

Commercially pure titanium (c.p.Ti, NKK Co., Japan) discs with 14 mm in diameter and 1.5 mm in thickness sliced by diamond saw (Buehler Ltd., U.S.A.) were used. C.p.Ti discs were previously polished by SiC papers from No. 400 to 2000. Final polishing was done by graded alumina powders of 1, 0.3 and 0.05 μm. All the samples were ultrasonically cleaned in distilled water and acetone, respectively, for 10 min. Final rinsing was done by distilled water, and followed by drying in oven at 110 °C for 12 h in air.

In order to prepare TiO_2 films on titanium, the cleaned c.p.Ti discs were annealed at 500, 550, 600, 650 and

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700 °C, respectively, for 10 min in air or in argon (flow rate: 200–300 ml/min) in a preheated tube type-furnace (heating rate: ∼ 500 °C/min). Thickness of the TiO_2 film on titanium was estimated to be about 0.2–0.3 μm, which was confirmed by F20 (Filmetric, Inc., San Diego, CA, U.S.A.) using reflection spectrum. Spectral analysis of reflections from the top and bottom of the thin film provides thickness and optical constants (thickness accuracy: ± 1 nm at 500 nm thickness). *In vitro* bioactivity of the samples after annealing (five samples/each annealing temperature) was tested in an Eagle's minimum essential medium (MEM, Gibco BRL, Life Technologies, U.S.A.) solution. Each sample was placed in a sealed polystyrene vial and immersed in 15 ml MEM solution. The experiment was performed in a constant temperature-circulating bath (Model 90, Poly Science Co., U.S.A.) at a temperature of 36.5 °C. The MEM solution was refreshed everyday. The longest immersion time was 15 days. After immersion, the samples were thoroughly rinsed with distilled water and dried in an oven at 50 °C.

Before soaking, X-ray diffraction (XRD, D-Max-1200, Rigaku Co., Japan) analysis was used to determine the crystal structure of TiO_2 films on titanium. At the initial stage and at the end of the immersion test, surface morphology and chemical composition of the TiO_2 films before or after immersion were examined by field emission-scanning electron microscopy (FE-SEM, S-4700, Hitachi Co., Tokyo, Japan) and energy dispersive X-ray spectrometer (EDX, S-4700, Hitachi Co., Tokyo, Japan) equipped with a Robinson type-back-scattered electron detector.

3. Results and discussion

Figs. 1 and 2 show the XRD patterns for the TiO_2 films on titanium annealed at 500 °C and above in air or in argon. The rutile structure started to become visible with peak corresponding to the (1 1 0) spacing, for the sample heat treated at 700 °C in air, while relatively low annealing temperature was needed to prepare rutile crystalline for the sample annealed in argon. However, it is generally difficult to detect amorphous TiO_2 structure by using a conventional XRD. Furthermore, since an amorphous TiO_2 on titanium could be formed easily at low temperature, even in the room-temperature storage, it could be considered that samples used in our work were wholly covered with amorphous TiO_2 layer after annealing, although the samples annealed at low temperature exhibited no observable TiO_2 peaks. TiO_2 thin films naturally formed on titanium or its alloys can contribute to their good biocompatibility in human body. However, it has been proven that such films are not bioactive enough to induce CaP precipitation [9].

For the annealed samples, in order to confirm biomimetic CaP formation on TiO_2/Ti structure after soaking in MEM solution for 15 days, XRD analysis was done. However, in present work, it is difficult to obtain observable peaks according to CaP formation.

In Fig. 3 is shown FE-SEM photographs of as-polished titanium (a) and titanium surface after being soaked in MEM solution for 15 days at 36.5 °C (b). There was no Ca-P formation as well as other ions in MEM solution, such as Mg and Na, on the sample without annealing, as shown in Fig. 3(c). However, formation of the unknown coating, which was probably due to organics from the MEM solution, was clearly visible on the surface of the sample (see Fig. 3(b)), although EDX could not identify its composition.

Fig. 4 shows FE-SEM photographs of the TiO_2 films on titanium as a function of annealing temperatures from 500 to 700 °C in air for 10 min. Annealing of the titanium at 700 °C for 10 min produces a compact rutile-type titanium oxide layer, as previously shown in the XRD pattern of Fig. 1 and as shown in Fig. 4(e). While the TiO_2 film annealed at 650 °C and below exhibited a heterogeneous amorphous structure.

Fig. 5 shows FE-SEM photographs of the TiO_2 films

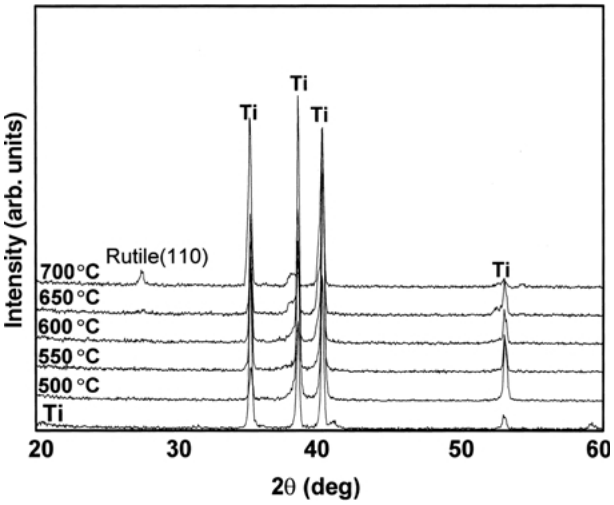

Figure 1 XRD patterns of TiO_2 layers on titanium as a function of annealing temperatures in air.

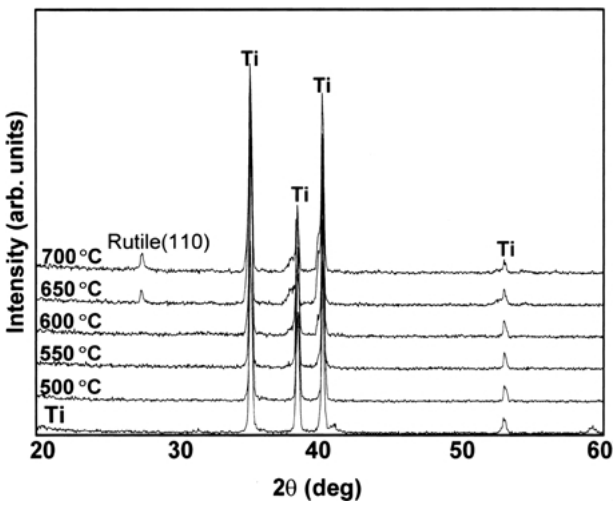

Figure 2 XRD patterns of TiO_2 layers on titanium as a function of annealing temperatures in argon.

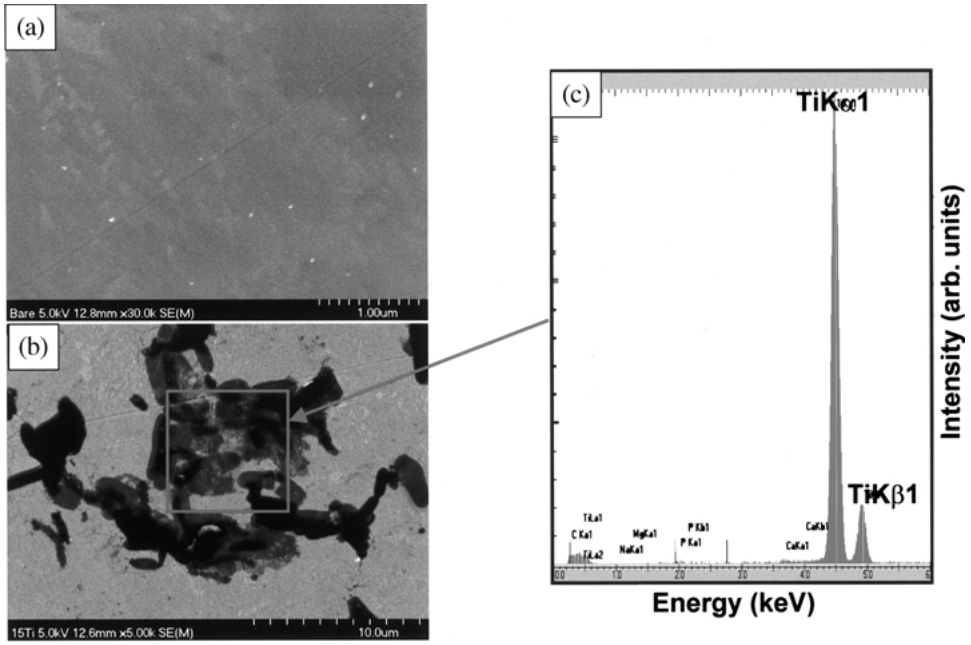

Figure 3 FE-SEM photographs of as-polished titanium (a), and morphology (b) and chemical composition (c) of titanium surface after immersion in MEM.

on titanium as a function of annealing temperatures in argon. TiO_2 layers annealed at 650 and 700 °C exhibited a well-grained crystalline structure in nature. On the other hand, for the TiO_2 films heat treated at 600 °C and below, no distinct crystal and grain boundary were identified. From the XRD and FE-SEM results, we confirmed that compact rutile structure could be formed by annealing at 700 °C in air for 10 min or at 650 and 700 °C in argon for 10 min.

The calcium and phosphate required for hydroxyapatite generation on the TiO_2 were extracted from MEM solution. This was indicated by increase of the concentration of the phosphate and calcium on TiO_2. Effects of the annealing temperatures or conditions on CaP forming ability were shown in Figs. 6–9 by FE-SEM and EDX for the samples after immersion for 15 days in MEM solution at 36.5 °C. As shown in Figs. 6 and 7, heterogeneous amorphous TiO_2 layer annealed at 650 °C and below in air showed the higher CaP forming ability, while TiO_2 layer composed by compact rutile crystals exhibited no CaP formation. Similarly, there was no CaP formation on the rutile surface annealed at 650 and 700 °C in argon, whereas CaP layer was identified at low-temperature annealing, 600 °C and below, as shown in Figs. 8 and 9.

The first step in the nucleation of hydroxyapatite in the presence of a bioactive oxide is thought to be the electrostatically driven adsorption of Ca^{2+} to ionized surface hydroxyl groups onto which phosphate is subsequently adsorbed [20, 21]. Moreover, amorphous TiO_2 has been shown to hydrolyze readily in simulated body fluid (SBF) [20]. In this work, since amorphous and heterogeneous TiO_2 layer easily induced CaP, changing the annealing temperature affected CaP forming ability the most.

As increase of the annealing temperatures at 700 °C in air or at 650 and 700 °C in argon, well-grained and compact rutile structure, which probably accompanying with the changes in porosity and surface area, was visible. Compared to a dense oxide film, a porous surface film is more open in the solution for ions to incorporate through the pores into the oxides. Moreover, from an electrostatic interaction point of view, surface hydroxyl groups present on or inside the oxide film tend to attract calcium ions from the solution [23]. As has been suggested, the amorphous structure, the porous Ti–O network, and the negative surface charge density of the TiO_2 layer may be responsible for the CaP formation. In our work, a newly formed amorphous TiO_2 layer with a heterogeneous structure corresponding to porosity and surface area was most probably responsible for the bioactivity of the annealed titanium.

Since the samples after annealing can induce the precipitation of a uniform CaP layer from MEM solution, our work provides a simple alternative for CaP formation on titanium.

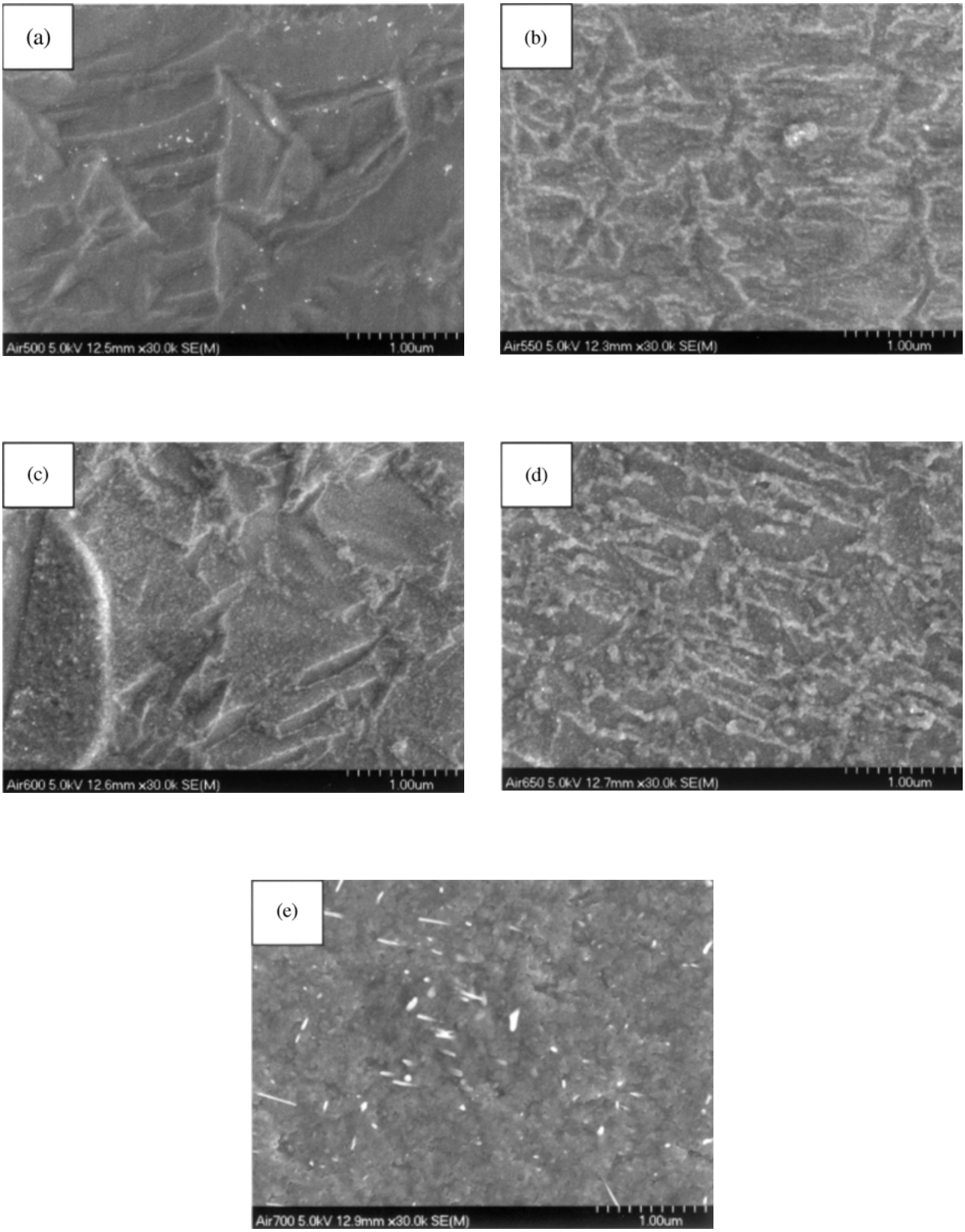

Figure 4 FE-SEM photographs of TiO_2 layers annealed in air for 10 min at 500 °C (a), 550 °C (b), 600 °C (c), 650 °C (d) and 700 °C (e).

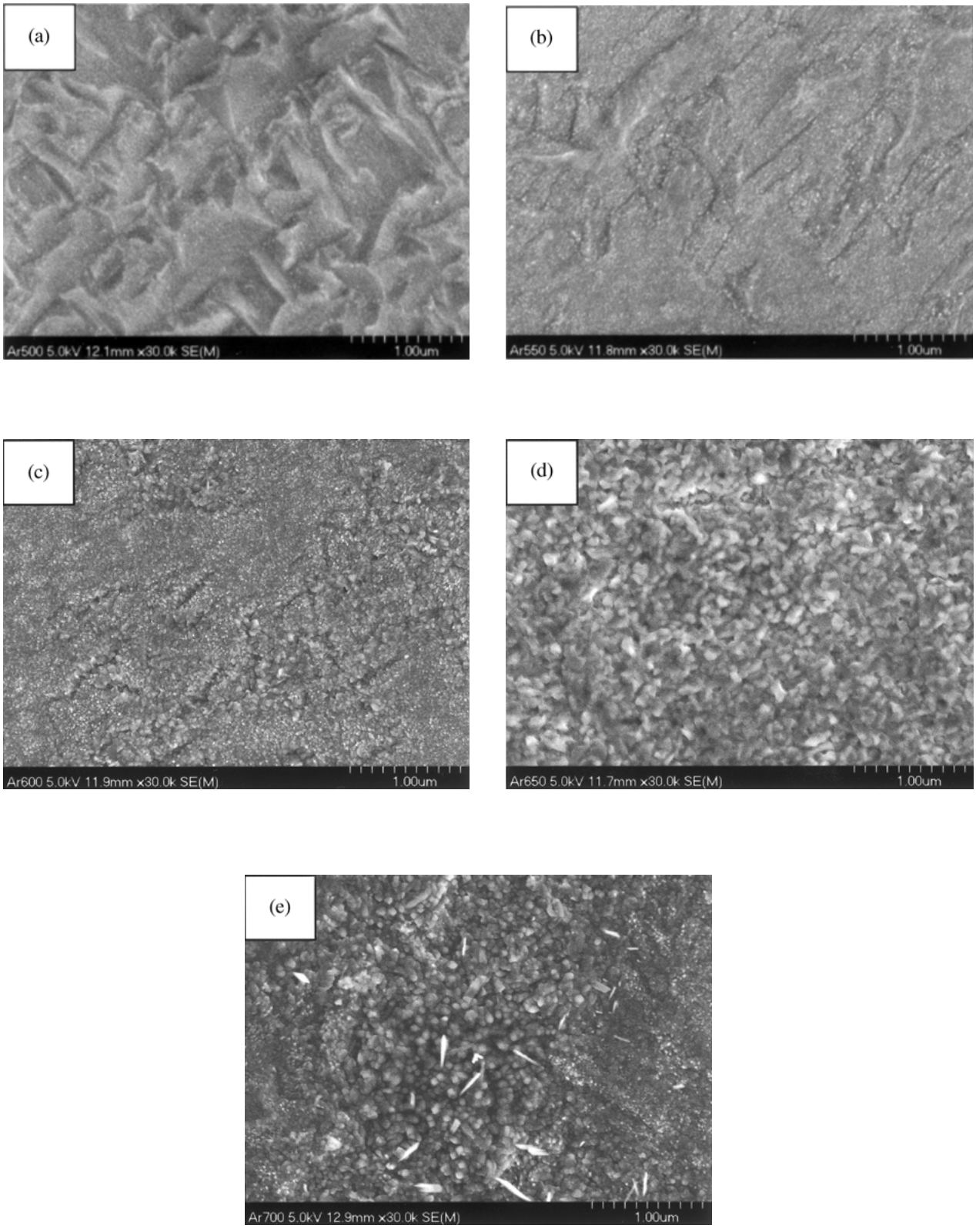

Figure 5 FE-SEM photographs of TiO_2 layers annealed in argon for 10 min at 500 °C (a), 550 °C (b), 600 °C (c), 650 °C (d) and 700 °C (e), respectively.

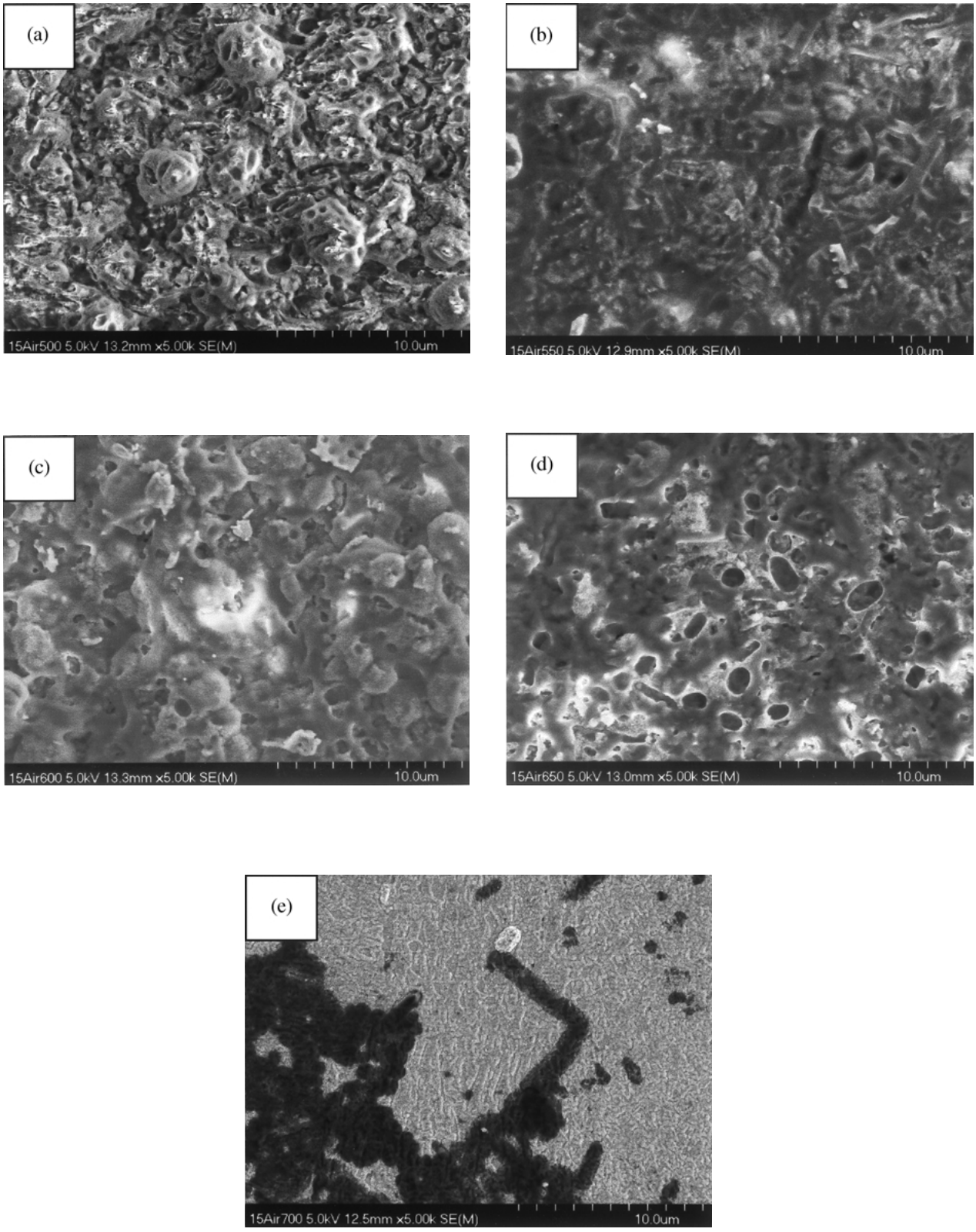

Figure 6 FE-SEM photographs of TiO_2 layers annealed in air for 10 min at 500 °C (a), 550 °C (b), 600 °C (c), 650 °C (d) and 700 °C (e), respectively, after soaking in MEM.

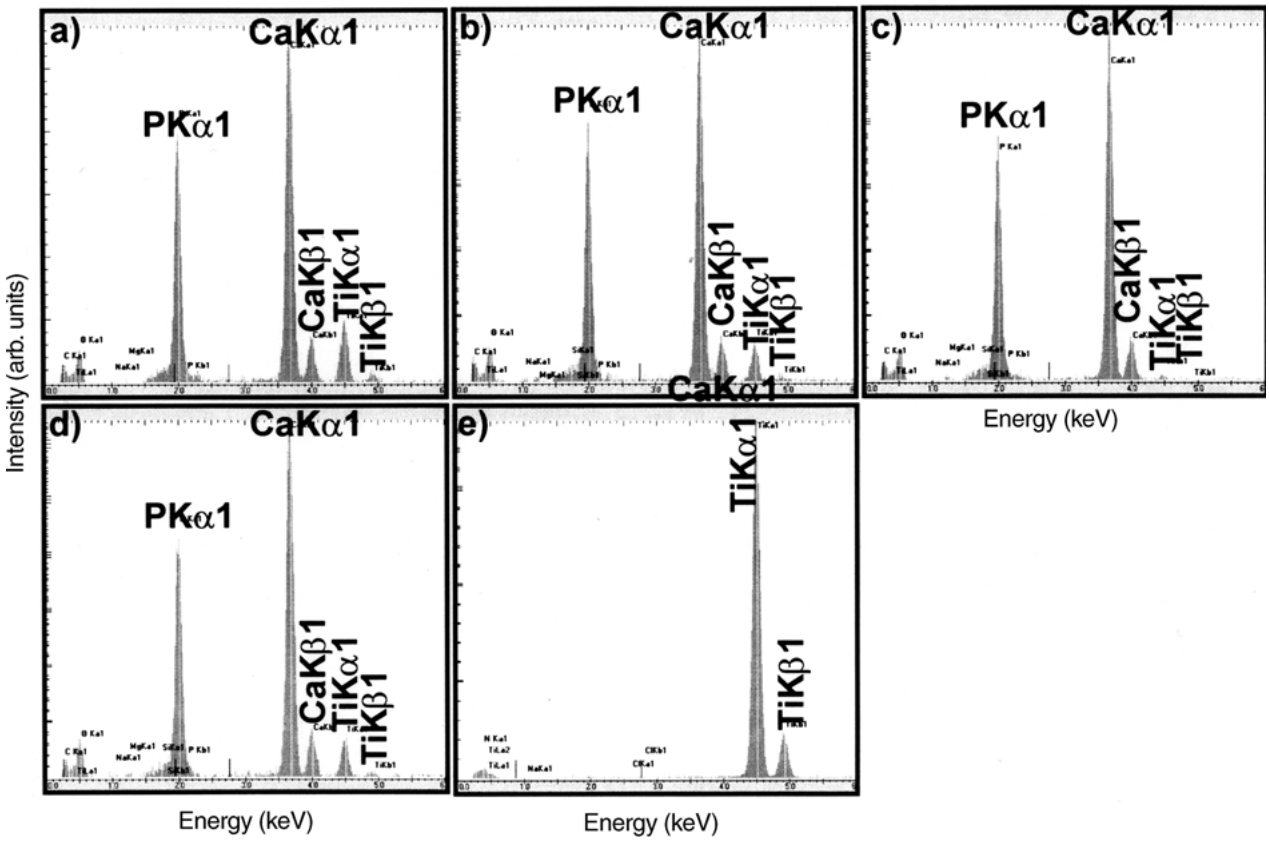

Figure 7 Chemical composition of TiO_2 layers annealed in air for 10 min at 500 °C (a), 550 °C (b), 600 °C (c), 650 °C (d) and 700 °C (e), respectively, after soaking in MEM.

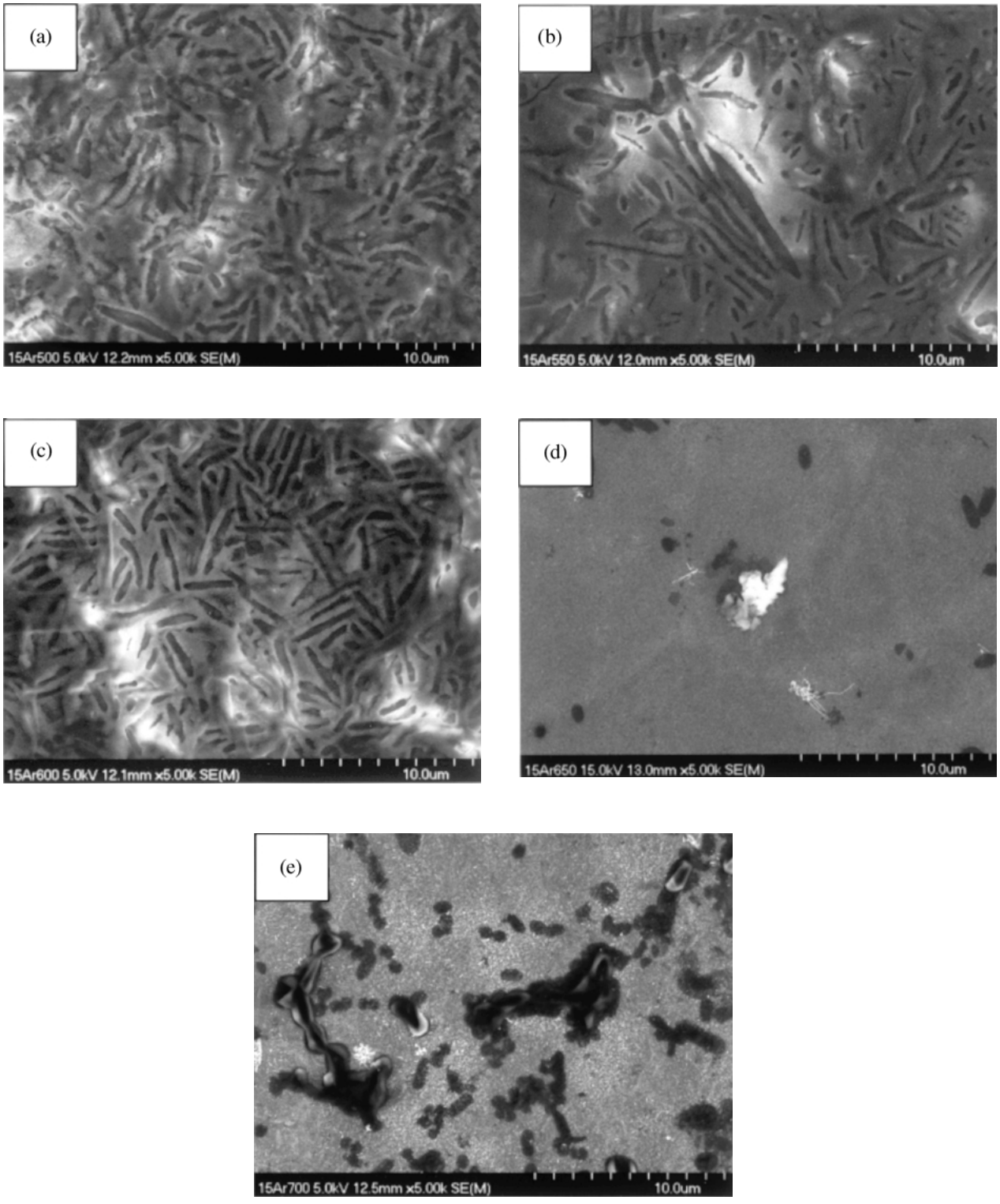

Figure 8 FE-SEM photographs of TiO_2 layers annealed in argon for 10 min at 500 °C (a), 550 °C (b), 600 °C (c), 650 °C (d) and 700 °C (e), respectively, after soaking in MEM.

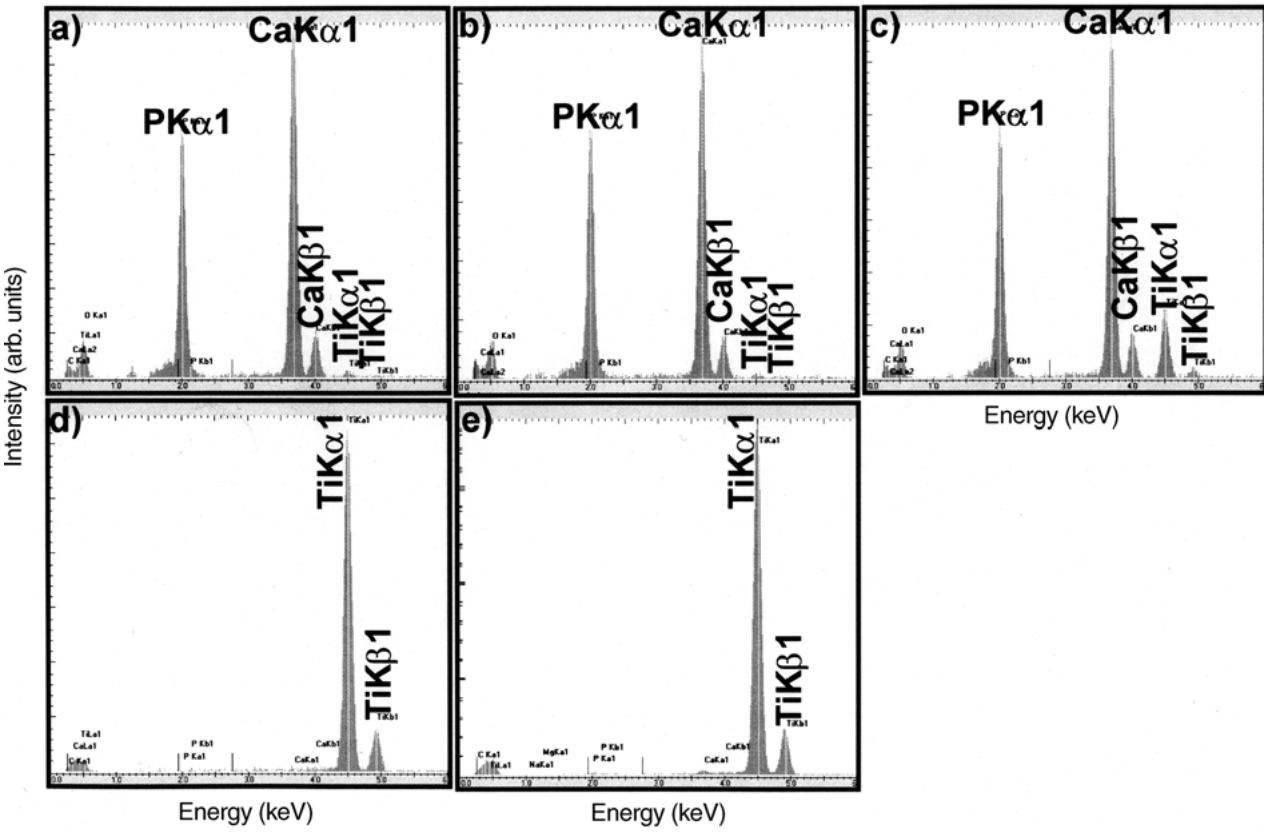

Figure 9 Chemical composition of TiO_2 layers annealed in air for 10 min at 500 °C (a), 550 °C (b), 600 °C (c), 650 °C (d) and 700 °C (e), respectively, after soaking in MEM.

4. Conclusion

In order to investigate influence of the crystallinity and surface morphology of the TiO_2 films on titanium, c.p.Ti discs were annealed at various temperatures in air or in argon. Well-grained and compact rutile structure could be formed by annealing at 700 °C in air or at 650 and 700 °C in argon. Amorphous TiO_2 layer with a heterogeneous structure annealed at 650 °C and below in air, and at 600 °C and below in argon showed the higher CaP forming ability. While TiO_2 layers composed by rutile crystals exhibited no CaP formation.

References

1. T. ALBREKTSSON, P. I. BRANEMARK, H. A. HANSSON, B. KASEMO, K. LARSSON, I. LUNDSTROM, D. H. MCQUEEN and R. SKALAK, *Ann. Biomed. Eng.* **11** (1983) 1.
2. D. F. WILLIAMS, in ''Biocompatibility of Clinic Implant Materials'', edited by D. F. Williams (CRC Press, Boca Raton, Florida, USA, 1981) pp. 9–44.
3. R. Z. LEGEROS and R. G. CRAIG, *J. Bone Miner. Res.* **8** (suppl. 2) (1993) S 583.
4. S. G. STEINEMANN, *Injury* **27** (suppl. 3) (1996) SC 16.
5. K. SUZUKI, K. AOKI and K. OHYA, *Bone* **21** (1997) 507.
6. J. J. JACOBS, J. L. GILBERT and R. M. URBAN, *J. Bone Joint Surg. Am.* **80** (1998) 268.
7. T. SAWASE, K. HAI, K. YOSHIDA, K. BABA, R. HATADA and M. ATSUTA, *J. Dent.* **26** (1998) 119.
8. B. KASEMO, *J. Prosthetic Dent.* **49** (1983) 832.
9. P. DUCHEYNE, P. BIANCO, S. RADIN and E. SCHEPERS, in ''Bone-bonding Biomaterials'', edited by P. Ducheyne, T. Kokubo and C. A. van Blitterswijk (Leiderdorp, Reed Healthcare Communications, The Netherlands, 1992) p. 1.
10. S. A. MCNALLY, J. A. SHEPPERD, C. V. MANN and J. P. WALCZAK, *J. Bone Joint Surg. Br.* **82** (2000) 378.
11. R. G. GEESINK and N. H. HOEFNAGELS, *J. Bone Joint Surg. Br.* **77** (1995) 534.
12. A. MORONI, S. TOKSVIG-LARSEN, M. C. MALTARELLO, L. ORIENTI, S. STEA and S. GIANNINI, *J. Bone Joint Surg. Am.* **80** (1998) 547.
13. A. J. PERRY, *Surf. Eng.* **3** (1987) 154.
14. A. PIATTELLI, A. SCARANO, M. PIATTELLI and L. CALABRESE, *Biomaterials* **17** (1996) 1015.
15. D. M. BRUNETTE, G. S. KENNER and T. R. L. GOULD, *J. Dent. Res.* **62** (1983) 1045.
16. J. LAUSMMA, B. KASEMO, H. MATTSSON and H. ODELIUS, *Appl. Surf. Sci.* **45** (1990) 189.
17. H. B. WEN, J. R. DE WIJN, F. Z. CUI and K. DE GROOT, *Biomaterials* **19** (1998) 215.
18. M. UCHIDA, H. M. KIM, T. KOKUBO, T. NAKAMURA, *Sixth World Biomater.* (2000) 1308.
19. T. PELTOLA, M. PÄTSI, H. RAHIALA, I. KANGASNIEMI and A. YLI-URPO, *J. Biomed. Mater. Res.* **41** (1998) 504.
20. S. HAYAKAWA, K. TSURU, C. OHTSUKI and A. OSAKA, *J. Amer. Ceram. Soc.* **8** (1999) 2155.
21. B. C. YANG, J. WENG, X. D. LI and X. D. ZHANG, *J. Biomed. Mater. Res.* **47** (1999) 213.
22. P. LI and K. DE GROOT, *J. Biomed. Mater. Res.* **27** (1993) 1495.
23. J. PAN, H. LIAO, C. LEYGRAF, D. THIERRY and J. LI, *J. Biomed. Mater. Res.* **40** (1998) 244.

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