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Osteoconductivity of Anodized Titanium with Controlled Micron-Level Surface Roughness

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The aim of this study was to elucidate the relationship between the surface roughness and osteoconductivity of anodized titanium surfaces. Before anodizing, titanium substrates with different surface roughness were prepared by wet-polishing. These substrates were anodized at various voltages in H_3PO_4, H_2SO_4, and NaOH aqueous solutions, and their surface roughness was controlled simultaneously at the micron level. Surface roughness of these coatings was expressed with the arithmetical means (Ra). The osteoconductivity of anodized samples was evaluated by *in vivo* tests. In *in vivo* tests, samples were implanted in rats' tibia for 14 d. Anatase type TiO_2 films were formed on all of the anodized samples for *in vivo* tests. It was newly found that TiO_2 film with small Ra value exhibited high osteoconductivity than that with high Ra value, especially when Ra value was $<0.3\,\mu m$. In addition, the osteoconductivity of anodized samples with $Ra/\mu m > 0.3$ was not improved by anodizing, showing the same low osteoconductivity of as-polished samples. These tendencies were observed for all of the TiO_2 films regardless of the type of electrolytes. [doi:10.2320/matertrans.M2011049]

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1. Introduction

Titanium (Ti) is widely used in dental and orthopedic implants because of its good biocompatibility and high corrosion resistance. The long-term success rates of Ti implants have been well documented.[1,2] However, many failures occur when the bone is of poor quality.[3,4] Various methods to improve the clinical performance of Ti implants in poor-quality bone and to shorten the healing period have been assessed. For example, hydroxyapatite (HAp), which is the main inorganic component of natural bone, is usually used in the form of a coating on a metallic substrate to compensate for its poor intrinsic mechanical properties. In our previous studies, HAp with various crystal structures,[5–9] carbonate apatite (CO_3Ap), $CO_3Ap/CaCO_3$ composite films, HAp/collagen[10] and HAp/gelatin composite films,[11] have been fabricated on Ti substrates using the thermal substrate method and we have investigated their osteoconductivity in *in vivo* tests in rats' tibia. These studies have revealed that a specific crystal structure strongly accelerates calcification in the cancellous bone part.

Titanium dioxide (TiO_2) is in the spotlight as an osteoconductive substance, similar to HAp. TiO_2 has been shown to exhibit strong physicochemical bonding between an Ti implant and living bone because of its ability to induce bone-like apatite in a body environment.[12] It is thought that the surface properties of Ti implants influence the biological responses at the interface between the bone tissue and the implant.[13–18] Therefore, the optimization of the surface properties of TiO_2 coatings is key point to improve the osteoconductivity of implants. There are many types of TiO_2 coating methods for Ti substrates, such as thermal treatment,[19] chemical methods,[20–22] physical vapor deposition,[23,24] and anodizing.[25–28] Among these methods, we chose anodizing as a processing route involving hydroprocessing, as a hydrous environment is similar to the internal environment of the body. Anodizing can form uniform TiO_2 thin films on Ti substrates. Furthermore, thin adhesive film can even be formed on substrates with a complicated topography. The surface morphology and surface roughness of TiO_2 films can be controlled using the initial substrate roughness and anodizing conditions, such as the applied voltage and type of aqueous solution.

For Ti implants, it has been reported that a macro-rough surface enhances bone formation.[14,17,29] On the other hand, for TiO_2 coatings, it is not clear how the micron-level surface roughness influences osteoconductivity. In this study, TiO_2 coatings were fabricated, and their surface roughness was controlled using anodizing. These samples were implanted into rats' tibia for a period of 14 d, and the influence of the surface roughness on the osteoconductivity was investigated.

2. Materials and Methods

2.1 Preparation of Ti substrates

Cp-Ti plates (for evaluating the coatings, area $= 1.13\,cm^2$) and rods (for *in vivo* testing, dimensions $= \phi 2 \times 5\,mm$) were used as the substrates, and these were covered with epoxy resin, except for the face that would be in contact with the aqueous solution. Various degrees of surface roughness were obtained by polishing the samples in the same direction with emery paper (grid = #120, #220, and #400) followed by buffing using Al_2O_3 particles (particle size $= 0.05\,\mu m$). After polishing, the substrates were cleaned and then degreased with ethanol.

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2.2 Anodizing in aqueous solutions

Ti substrates were used as the working electrode, and a Pt coil was used as the counter electrode. The aqueous solution was stirred while anodizing and was kept at a constant temperature (298 K) in a water bath. Aqueous 0.1 M H_2SO_4, 0.1 M H_3PO_4, and 0.1 M NaOH solutions were used as electrolytic baths, because they are often used in *in vitro* studies of anodized titanium.[30,31] A rapid rate of increase in the voltage made it difficult to control the surface roughness of the substrates, so the anodizing potential was increased slowly (0.1 V s^{-1}) up to a potential of 200 V. All the samples were evaluated after sterilization, and sterilization did not influence on evaluated surface properties of samples.

2.3 Analysis of coatings

All the samples were sterilized using an autoclave unit at 394 K for a period of 20 min. before analysis. The surface morphology of the substrates was observed using a scanning electron microscope (SEM). The coated films were identified using a thin-film X-ray diffraction (XRD) and a X-ray photoelectron spectroscopy (XPS). The surface roughness measurements were conducted by means of contactless probing using a confocal laser scanning microscope with a measurement area of 150 μm × 112 μm. The arithmetical means of the surface roughness (Ra) was used, as this value was not distorted by any local scarring of the sample.

2.4 *In vivo* tests

Since the experimental procedure for our *in vivo* study was almost the same as described in previous reports,[9] it is described in brief here. Before surgery, all the implants were cleaned in distilled water and immersed in a chlorhexidine gluconate solution. Ten-week-old male Sprague Dawley rats (Charles River Japan, Inc., Japan) were used in our experimental procedures. The samples were implanted in the tibial metaphysis of rats to contact with both cortical bone part and cancellous bone part. A slightly oversized hole, which did not pass through to the rear side of the bone, was created using a low-speed rotary drill. Subsequently, the implants were inserted into these holes, and then the subcutaneous tissue and skin were closed and sterilized.

The rats were sacrificed after a period of 14 d, and the implants with their surrounding tissue were retrieved. The samples were fixed in a 10% neutral buffered formalin solution, dehydrated in a graded series of ethanol, and embedded in methylmethacrylate. Following polymerization, each implant block was sectioned longitudinally into 20 μm thick slices. These sections were then stained with toluidine blue.

Optical microscope was used for the observation of interface between bone and implant. The sum of the linear bone contact with the implant surface was measured and was expressed as a percentage over the entire implant length (the bone-implant contact ratio, $R_{B\text{-}I}$) in the cancellous bone and the cortical bone parts. Significant differences in the bone-implant contact ratio were analyzed statistically using the Tukey-Kramer method.[32] Differences were considered statistically significant at the $p < 0.05$ level (described as dotted lines and stars in Fig. 5). This animal study was conducted in the laboratory of AAALAC International (Association for Assessment and Accreditation of Laboratory Animal Care International).

3. Results and Discussion

3.1 Surface properties

Representative SEM images of Ti substrate surface before and after anodizing are shown in Fig. 1. No obvious scratches were observed on a buffed as-polished substrate (Fig. 1(a)) indicating that Ra/μm = 0.057, but scratches along the same direction were clearly observed for the #400 polished substrate (Fig. 1(b)), which showed that Ra had increased to 0.225 μm.

A TiO_2 film was formed on the Ti substrate after anodizing in a 0.1 M H_2SO_4 solution (Fig. 2(a)). When anodized under a potential of 100 V, an anatase peak was observed in the XRD data. The XPS data showed that the film contained SO_4^{3-}, which seemed to have derived from the aqueous solution (Fig. 3). This film showed a yellow interference color, indicating a film thickness of about 120 nm.[33] Regarding the buffed substrates, although small swellings were observed in spots on the film, other area were as flat as the as-polished ones. Because of these small swellings, the Ra value changed slightly from 0.057 μm to 0.084 μm. Regarding the #400 polished substrate after anodizing, scratch marks were clearly observed, and the value of Ra was almost equal to that of the as-polished sample, which implied that the initial surface morphology was maintained after anodizing at 100 V. When a potential of 200 V was applied to the substrates, the film became thicker and the surface morphology of both buffed and #400 polished substrates changed. Regarding the buffed substrate, the small swellings observed at a potential of 100 V increased, and some pores were observed in the film, which showed an increase in Ra to 0.110 μm. In the case of the #400 polished substrates, pores were observed across the entire film, and no scratches were observed, resulting in an increase in the value of Ra. These observations seemed to be the result of a breakdown of the dielectric. Figure 4 shows the relationship between the applied voltage and the current density. From Fig. 4, a fluctuation in the current density at relatively high current densities was observed at potentials above 170 V, which represented a local breakdown in the film. As the applied voltage increased, the anatase peak became more dominant and a weak rutile was also detected, as shown in Fig. 2(a). A similar oxide film was obtained by Cui *et al.*[34]

In the case of anodizing at 100 V and 80 V in 0.1 M H_3PO_4 and 0.1 M NaOH solutions, respectively, each of the films showed a yellow interference color, which was composed of an anatase-type TiO_2 (Fig. 2(b)), indicating a similar value of Ra to the initial Ra value (Fig. 1(a)(4) or (6), Fig. 1(b)(4) or (6)), shown by the film anodized in a 0.1 M H_2SO_4 solution. Both anions and cations were also contained in the films anodized in H_3PO_4 and NaOH (Fig. 3). When anodized at 200 V, the same behavior was observed as shown by the film anodized in a 0.1 M H_2SO_4 solution. A similar oxide film was previously obtained by Kuromoto *et al.*[35]

From the above results, it can be said that the surface roughness and morphology of the substrate could be controlled by changing the applied voltage. Application of

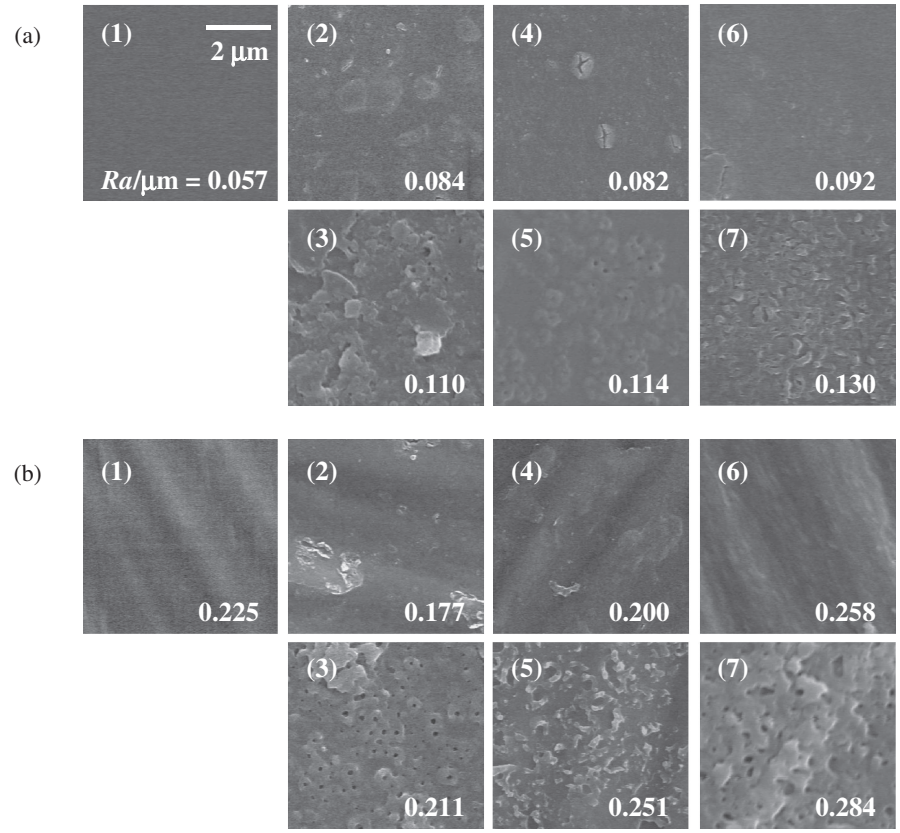

Fig. 1 SEM images of a substrate surface of an as-polished Ti sample, and after anodizing with surface roughness of Ra: (a) buffed sample, (b) polished with grid = #400, (1) as-polished, (2) anodized in H_2SO_4 at 100 V, (3) anodized in H_2SO_4 at 200 V, (4) anodized in H_3PO_4 at 100 V, (5) anodized in H_3PO_4 at 200 V, (6) anodized in NaOH at 80 V, and (7) anodized in NaOH at 200 V.

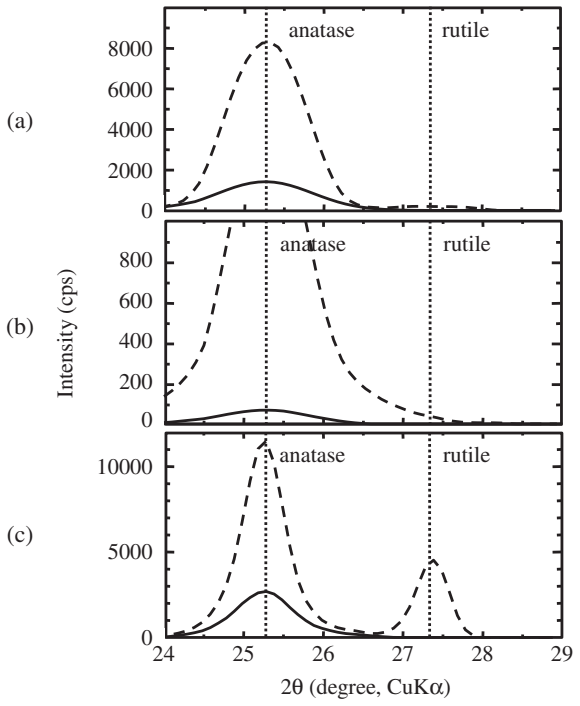

Fig. 2 XRD patterns of Ti substrates after anodizing at room temperature in (a) 0.1 M H_2SO_4, (b) 0.1 M H_3PO_4, and (c) 0.1 M NaOH aqueous solutions. —: 100 (80) V, – –: 200 V.

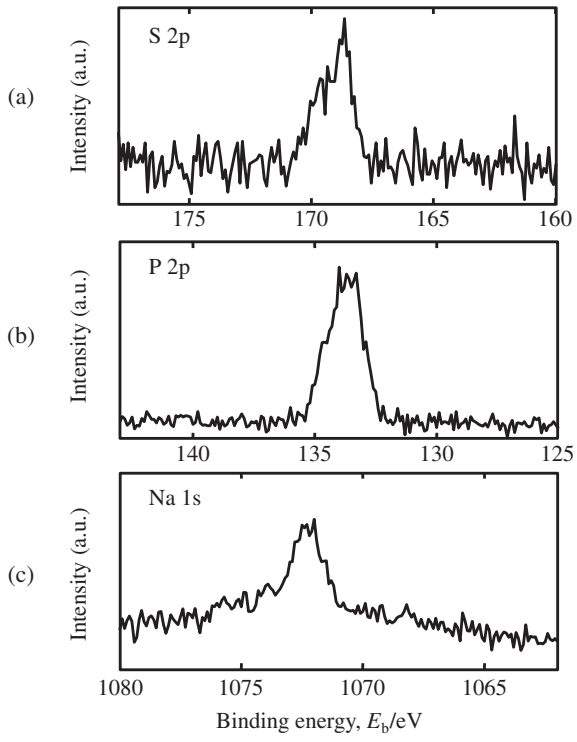

Fig. 3 High-resolution XPS spectra of the titanium substrate anodized at 100 V in: (a) 0.1 M H_2SO_4, (b) 0.1 M H_3PO_4, and (c) 0.1 M NaOH aqueous solutions.

a potential of 100 or 80 V did not increase the surface roughness, and the initial morphology was maintained after anodizing, regardless of the degree of the initial surface roughness. However, application of a potential of 200 V to the Ti substrate brought about a serious change in the surface morphology in all of the aqueous solutions, resulted in the increase of Ra value. Previous papers reported that crystal structure and film thickness could affect the apatite formation ability or osteoconductivity.[31,34,36] Furthermore, we have already found that the crystallinity of anatase film and anions/cations contained in TiO_2 film could influence on their osteoconductivity. These findings will be reported in next papers. Therefore, samples for *in vivo* test should be prepared so that crystal structure, crystallinity, film thickness, and anions/cations in the film do not influence on the osteoconductivity of samples in evaluating the influence of surface roughness. From above viewpoints, samples with various surface roughness prepared by anodizing in 0.1 M H_2SO_4, H_3PO_4, and NaOH aqueous solutions at 100 or 80 V were used for the following *in vivo* test and evaluated for every electrolytes.

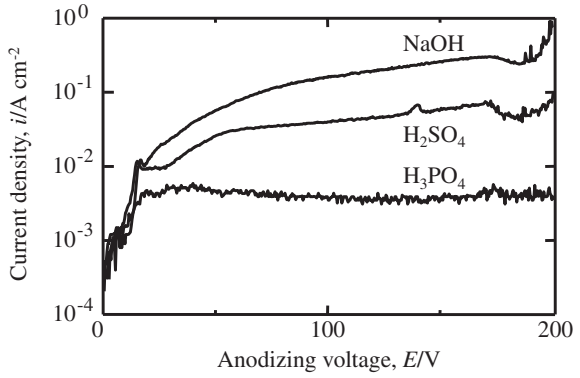

Fig. 4 Change in current density with applied voltage to Ti substrates in 0.1 M H_2SO_4, 0.1 M H_3PO_4, and 0.1 M NaOH at 298 K.

3.2 *In vivo* studies

Figure 5 shows the effect of the surface roughness on the osteoconductivity in different aqueous solutions. In the cortical bone parts, the as-polished Ti implant had a low $R_{B\text{-}I}$ value, <20%, irrespective of Ra value (Fig. 5(1)). The surface roughness of Ti has been reported to influence the osteoconductivity,[14,17,29] but this tendency was not seen in the region of Ra studied in this work, showing almost constant low $R_{B\text{-}I}$ value of 20%. Similar low $R_{B\text{-}I}$ values were also obtained for the samples with Ra/μm > 0.3 after anodizing in H_2SO_4 aqueous solution. However, the samples with Ra/μm < 0.3 had higher $R_{B\text{-}I}$ value of near 40% after anodizing in H_2SO_4 aqueous solution. This result was not unique in the case of H_2SO_4, but also shown in the case of H_3PO_4 and NaOH aqueous solutions. Furthermore, the same tendencies were also obtained in cancellous bone part. In our previous studies, Kuroda *et al.* precipitated HAp with a needle-like crystal structure on Ti substrates and implanted them in rats' tibia for 14 d.[9] The value of $R_{B\text{-}I}$ was 34% in the cortical bone parts, which was similar to the $R_{B\text{-}I}$ values of the anodized implants in this study. This demonstrates that TiO_2 films with Ra/μm < 0.3 formed in this study had high osteoconductivity equivalent to HAp. About 30% of hard tissue formation was observed by measuring the ratio of hard tissue formation inside of cancellous bone, which was considered as natural hard tissue ratio in cancellous bone. Since implants with Ra/μm < 0.3 anodized in H_2SO_4 and H_3PO_4 solution had higher $R_{B\text{-}I}$ value than 30%, enough amount of hard tissue was formed. However, the influence of surface roughness on the mechanism of bone formation was

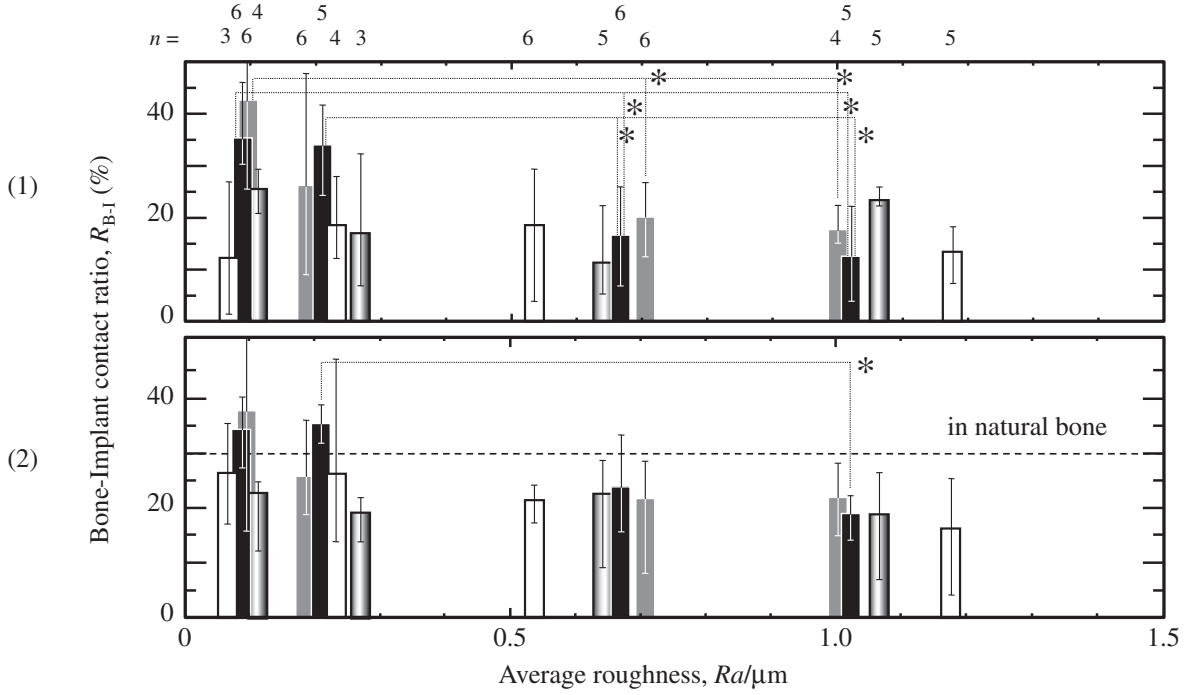

Fig. 5 Influence of the surface roughness, Ra, on the bone-implant contact ratio, $R_{B\text{-}I}$: (1) cortical bone part, and (2) cancellous bone part. Key: □ = as-polished, anodized in ■ = 0.1 M H_2SO_4, ■ = 0.1 M H_3PO_4, and ■ = 0.1 M NaOH, *: $p < 0.05$.

not clear yet. This was because enough data has not been obtained to discuss the mechanism since the results of *in vitro* evaluations[37–39] did not always agree with the results of *in vivo* evaluations.[40] Nevertheless, it was significant result that TiO_2 coatings with fine surface in micron-level tended to show high $R_{B\text{-}I}$ value.

4. Conclusions

In this research, the influence of micron-level controlled surface roughness on the osteoconductivity of anodized TiO_2 coatings was investigated, and following findings were obtained.

(1) Anatase-type TiO_2 films were obtained by anodizing cp-Ti in 0.1 M H_2SO_4 at 100 V, 0.1 M H_3PO_4 at 100 V, and 0.1 M NaOH at 80 V. Surface roughness and morphology of these films were almost the same as those of initial substrates.

(2) When Ra value was <0.3 μm, the anodized Ti implants induced a high level of hard tissue formation at the interface between the implant and the bone.

(3) The osteoconductivity of anodized samples with Ra/μm > 0.3 was not improved by anodizing, showing the same low osteoconductivity of as-polished samples.

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REFERENCES

1) R. Adell, B. Eriksson, U. Lekholm, P. I. Branemark and T. Jemt: Int. J. Oral Maxillofac Implants. **5** (1990) 347–359.
2) D. van Steenberghe, U. Lekholm, C. Bolender, T. Folmer, P. Henry, I. Herrmann, K. Higuchi, W. Laney, U. Linden and P. Astrand: Int. J. Oral Maxillofac Implants. **5** (1990) 272–281.
3) R. A. Jaffin and C. L. Berman: J. Periodontol. **62** (1991) 2–4.
4) W. Khang, S. Feldman, C. E. Hawley and J. Gunsolley: J. Periodontol. **72** (2001) 1384–1390.
5) K. Kuroda, R. Ichino, M. Okido and O. Takai: J. Biomed. Mater. Res. **59** (2002) 390–397.
6) K. Kuroda, R. Ichino, M. Okido and O. Takai: J. Biomed. Mater. Res. **61** (2002) 354–359.
7) K. Kuroda, Y. Miyashita, R. Ichino, M. Okido and O. Takai: Mater. Trans. **43** (2002) 3015–3019.
8) K. Kuroda, S. Nakamoto, R. Ichino, M. Okido and R. M. Pilliar: Mater. Trans. **46** (2005) 1633–1635.
9) K. Kuroda, S. Nakamoto, Y. Miyashita, R. Ichino and M. Okido: Mater. Trans. **47** (2006) 1391–1394.
10) K. Kuroda, M. Moriyama, R. Ichino, M. Okido and A. Seki: Mater. Trans. **49** (2008) 1434–1440.
11) K. Kuroda, M. Moriyama, R. Ichino, M. Okido and A. Seki: Mater. Trans. **50** (2009) 1190–1195.
12) R. Hazan, R. Brener and U. Oron: Biomater. **14** (1993) 570–574.
13) D. Buser, N. Broggini, M. Wieland, R. K. Schenk, A. J. Denzer, D. L. Cochran, B. Hoffmann, A. Lussi and S. G. Steinemann: J. Dent. Res. **83** (2004) 529–533.
14) D. L. Cochran, D. Buser, C. M. ten Bruggenkate, D. Weingart, T. M. Taylor, J. P. Bernald, F. Peters and J. P. Simpson: Clin Oral Implants Res. **13** (2002) 144–153.
15) C. Eriksson, H. Nygren and K. Ohlson: Biomater. **25** (2004) 4759–4766.
16) J.-W. Park, K.-B. Park and J.-Y. Suh: Biomater. **28** (2007) 3306–3313.
17) G. B. Schneider, R. Zaharias, D. Seabold, J. Keller and C. Stanford: J. Biomed. Mater. Res. **69** (2004) 462–468.
18) G. Zhao, Z. Schwartz, M. Wieland, F. Rupp, J. Geis-Gerstorfer, D. L. Cochran and B. D. Boyan: J. Biomed. Mater. Res. **74** (2005) 49–58.
19) S. Fujibayashi, M. Neo, H.-M. Kim, T. Kokubo and T. Nakamura: Biomater. **25** (2004) 443–450.
20) L. Jonasova, F. A. Muller, A. Helebrant, J. Strnad and P. Greil: Biomater. **25** (2004) 1187–1194.
21) F. Xiao, K. Tsuru, S. Hayakawa and A. Osaka: Thin Solid Films. **441** (2003) 271–276.
22) J.-M. Wu, S. Hayakawa, K. Tsuru and A. Osaka: Scr. Mater. **46** (2002) 101–106.
23) K.-R. Wu, C.-H. Ting, W.-C. Lie, C.-H. Lin and J.-K. Wu: Thin Solid Films **500** (2006) 110–116.
24) L. S. Hsu, R. Rujkorakarn, J. R. Sites and C. Y. She: J. Appl. Phys. **59** (1986) 3475–3480.
25) Y.-T. Sul, C. B. Johansson, S. Petronis, A. Krozer, Y. S. Jeong, A. Wennerberg and T. Albreksson: Biomater. **23** (2002) 491–501.
26) J. P. Schreckenbach, G. Marx, F. Schlotigg, M. Textor and N. D. Spencer: J. Mater. Sci. Mater. Med. **10** (1999) 453–457.
27) B. Yang, M. Uchida, H.-M. Kim, X. Zhang and T. Kokubo: Biomater. **25** (2004) 1003–1010.
28) L. A. de Sena, N. C. C. Rocha, M. C. Andrade and G. A. Soares: Surf. Coating Tech. **166** (2003) 254–258.
29) G.-L. Yang, F.-M. He, X.-F. Yang, X.-X. Wang and S.-F. Zhao: Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endod. **106** (2008) 516–524.
30) H.-J. Song, S.-H. Park, S.-H. Jeong and Y.-J. Park: J. Mater. Proc. Tech. **209** (2009) 864–870.
31) K. Das, S. Bose and A. Bandyopadhyay: Acta Biomater. **3** (2007) 573–585.
32) C. Y. Kramer: Biometrics **12** (1956) 307–310.
33) S. V. Gils, P. Mast, E. Stijns and H. Terryn: Surf. Coating Tech. **185** (2004) 303–310.
34) X. Cui, H.-M. Kim, M. Kawashita, L. Wang, T. Xiong, T. Kokubo and T. Nakamura: Dent. Mater. **25** (2009) 80–86.
35) N. K. Kuromoto, R. A. Simao and G. A. Soares: Mater. Charact. **58** (2007) 114–121.
36) C. Larsson, P. Thomsen, J. Lausmaa, M. Rodahl, B. Kasemo and L. E. Ericson: Biomater. **15** (1994) 1062–1074.
37) B. Feng, J. Weng, B. C. Yang, S. X. Qu and X. D. Zhang: Biomater. **24** (2003) 4663–4670.
38) D. de Santis, C. Guerriero, P. F. Nocini, A. Ungersbock, G. Richards, P. Gotte and U. Armato: J. Mater. Sci. Mater. Med. **7** (1996) 21–28.
39) J. Y. Martin, Z. Schwartz, T. W. Hummert, D. M. Schraub, J. Simpson, Jr. J. Lankford, D. D. Dean, D. L. Cochran and B. D. Boyan: J. Biomed. Mater. Res. **29** (1995) 389–401.
40) D. Buser, R. K. Schnek, N. Steineman, J. P. Fiorelini, C. H. Fox and H. Stich: J. Biomed. Mater. Res. **25** (1991) 889–902.