

Calcium Phosphate Formation on Ti-Ag Alloys in Simulated Body Fluid*

Masatoshi TAKAHASHI**, Masafumi KIKUCHI**, Kouki HATORI***, Yusuke ORII***, Keiichi SASAKI*** and Yukyo TAKADA**

**Division of Dental Biomaterials, Tohoku University Graduate School of Dentistry, 4-1 Seiryomachi, Aoba-ku, Sendai 980-8575, Japan

E-mail: m-tak@mail.tains.tohoku.ac.jp

***Division of Advanced Prosthetic Dentistry, Tohoku University Graduate School of Dentistry

Abstract

Ti-Ag alloys with 20 and 25 mass% Ag were prepared; their surfaces were polished using silicon carbide abrasive papers. The polished alloys were immersed in a simulated body fluid (SBF), and their surfaces were observed by scanning electron microscope (SEM) to investigate the spontaneous formation of calcium phosphates. The precipitates formed on the alloys were qualitatively and quantitatively analyzed using an electron probe microanalyzer (EPMA), with pure titanium and silver used as controls. Hardness and surface roughness, which was measured as the height parameter Ra , were examined, and their effect on calcium phosphate formation was elucidated. On immersion in SBF, calcium phosphates were formed on the Ti-Ag alloys and pure titanium but not on pure silver. The amount of the precipitate and the concentration ratios of calcium to phosphorus in the precipitates of the Ti-Ag alloys and pure titanium did not differ significantly. The Ti-Ag alloys showed significantly higher hardness and lower Ra values than pure titanium. The Ra values of the metals decreased as their hardness increased. The Ra values of the metals appeared to have very little effect on calcium phosphate formation. The results of this study have verified the biocompatibility of the Ti-Ag alloys, making them suitable for use as dental and orthopedic implants.

Key words: Biomaterial, Titanium Alloy, Calcium Phosphate, Simulated Body Fluid, Bioconductivity, Biocompatibility, Dental Implant

1. Introduction

Titanium is considered to be one of the most biocompatible metallic materials and has been widely used in the fabrication of dental and orthopedic implants. The high biocompatibility of pure titanium is generally attributed to the fact that titanium passivates in aqueous solutions and that the resultant passive film formed on its surface is stable even in a biological environment⁽¹⁾. This film consists of nonstoichiometric TiO_2 and is amorphous or exhibits low crystallinity^(2, 3). It has been demonstrated that the physical characteristics of the TiO_2 film (thickness, porosity, and crystallinity) strongly affect the in vivo performance of the titanium implants⁽²⁾. Some studies have revealed that the surface of titanium is covered by a TiO_2 film with a thickness of 2–5 nm before implantation⁽³⁾. Further, it has been found that in an electrolyte solution, calcium phosphates are easily deposited on the passive TiO_2 film^(3, 4). In a study conducted on pure titanium implants extracted from human bone, the presence of calcium and phosphorus and the growth of the TiO_2 film on the implants were confirmed using Auger electron spectroscopy (AES); this

result indicated that calcium and phosphorus get incorporated in the TiO₂ film during in vivo exposure⁽⁵⁾. The formation of calcium phosphate, whose structure is similar to the biologically produced apatite, favors the high biocompatibility of pure titanium. In addition, when titanium is inserted into human bone, the pure titanium gets assimilated into the bone by a process called osseointegration, observed in the case of dental and orthopedic implants^(1,6). Furthermore the biological apatite formed, which is nothing but the calcium phosphate precipitated on the surface of the implant in the presence of body fluids, serves as a matrix for osteogenic cell attachment and growth⁽⁶⁻⁸⁾. It is believed that the apatite activates signaling proteins that enable the cells to initiate the bone-formation process⁽⁷⁾. Therefore, the formation of calcium phosphate on the passive TiO₂ film enhances biocompatibility and facilitates osseointegration.

Pure titanium, however, has some disadvantages due to poor machinability (ability to undergo cutting or grinding) and insufficient strength, especially in the case of some dental applications that require relatively high strength. Most dental implants are developed using commercially pure titanium of grade 4, since it is stronger than titanium of other grades⁽⁹⁾. Titanium alloys, on the other hand, exhibit higher yield strength and better fatigue properties than pure titanium, and are mainly composed of Ti-6Al-4V alloy⁽⁹⁾. As a part of our efforts to develop a new dental titanium alloy with enhanced machinability and mechanical properties, which can be used as dental implants, we prepared experimental Ti-Ag alloys and investigated their properties⁽¹⁰⁻¹²⁾. The tensile and yield strengths of the Ti-Ag alloys with 5–20 mass% Ag and their Vickers hardness values were observed to increase with the concentration of Ag; moreover, the alloys exhibited sufficient elongation, making them suitable for dental applications⁽¹⁰⁾. The strength and hardness of the Ti-Ag alloy with 20 mass% Ag was 1.6 times and 1.8 times higher than the respective values of pure titanium⁽¹⁰⁾. The machinability of the Ti-Ag alloys with 20 and 30 mass% Ag was superior to that of pure titanium⁽¹¹⁾. The bond strengths between the Ti-Ag alloys with 10–20 mass% Ag and dental porcelain were found to be above the minimum value given by ISO 9693 specifications⁽¹²⁾. From these studies, the Ti-Ag alloys, especially those with Ag ≥ 20 mass%, were validated to be suitable for use in dental prostheses and implants.

Biocompatibility of a material is closely related to its corrosion resistance. By carrying out anode polarization test and evaluating the amount of ions released from the metals in 0.9% NaCl and 1% lactic acid solutions, we found that the corrosion resistance of the Ti-Ag alloys with up to 25% Ag was comparable to that of pure titanium^(13,14). Further, the rest potential measurements revealed that in electrolyte solutions, the Ti-Ag alloys became passive faster than that of pure titanium⁽¹⁴⁾. Therefore, we can conclude that the biocompatibility of Ti-Ag alloys may be comparable to that of pure titanium. However, another important factor that influences biocompatibility is the spontaneous formation of calcium phosphate on the Ti-Ag alloys in electrolyte solution, which has not been investigated thus far.

We used a simulated body fluid (SBF) to study the formation of calcium phosphate or its precipitation on the surfaces of biomaterials⁽¹⁵⁾. The in vivo behavior of the biomaterials can be predicted by performing in vitro tests on them by their immersion in the SBF⁽¹⁵⁾. Several studies have been conducted to investigate calcium phosphate formation on titanium alloys in SBF, in order to evaluate their bioconductivity^(16,17).

In the present study, we prepared experimental Ti-Ag alloys with 20 and 25 mass% Ag and investigated the spontaneous formation of calcium phosphate on the alloys by immersing them in SBF. Pure titanium and silver were used as control materials. We also evaluated the surface hardness and roughness of the alloys and elucidated the influence of the surface properties on calcium phosphate formation.

2. Materials and methods

2.1 Preparation of alloys

Two experimental alloys, Ti-20 mass% Ag alloy (20% Ag) and Ti-25 mass% Ag alloy (25% Ag), were prepared. The desired amounts of titanium sponge (purity > 99.8%, grade S-90, OSAKA Titanium technologies Co., Ltd., Amagasaki, Japan) and pure silver (purity > 99.99%, Ishifuku Metal Industry Co., Ltd., Tokyo, Japan) were melted in an argon-arc melting furnace (TAM-4S, Tachibana Riko, Sendai, Japan), and 15 g buttons of each alloy were prepared. Each button was melted six times and inverted between each melting (five times) to ensure homogeneity of the alloy. In addition, pure titanium (Ti) buttons were prepared from the titanium sponge by following the same procedure.

2.2 Preparation of specimens

Buttons of each of the Ti-Ag alloy and that of pure Ti were cast into plates (35 mm × 8.6 mm × 2.8 mm) using magnesia investment (Selevest CB, Selec Co., Osaka, Japan) in an argon-gas pressure dental casting machine (Castmatic-S, Iwatani, Osaka, Japan) at 473 K, the obtained cast plates were then bench cooled. The surfaces of all cast plates were abraded to a depth of 300 μm using silicon carbide abrasive paper to remove the hardened layer on the surface of the castings. These polished plates were then cut into square specimens (8.0 mm × 8.0 mm × 2.2 mm). The surfaces of each specimen were again polished using 180–1000 grit silicon carbide abrasive paper.

Pure silver (Ag) was melted on a gas burner to form 15 g buttons. The buttons were rolled into 2.2-mm-thick plates by a rolling mill. The plates were annealed at 873 K for 1.8 ks in air. These annealed plates were then cut into square specimens (8.0 mm × 8.0 mm × 2.2 mm). The surfaces of each specimen were polished using 180–1000 grit silicon carbide abrasive paper.

2.3 Bulk hardness and surface roughness tests

The bulk hardness of each specimens was determined by a Vickers microhardness tester (HM-102, Akashi, Yokohama, Japan) using a 1.961 N load and 30 s dwell time. The measurements were carried out on three randomly selected points on each specimen.

The polished specimens were ultrasonically washed in distilled water for 0.6 ks and dried in air. The surface roughness of the specimens was measured using a surface profilometer (Surfcom 480A, Tokyo Seimitsu, Tokyo, Japan); the evaluation length, stylus speed, and the cutoff value were set at 4.0 mm, 0.6 mm/s, and 0.8 mm, respectively. Surface roughness was measured in terms of the height parameter, R_a , which is the arithmetic mean of the absolute values of the deviations from the surface profiles, within the specified evaluation length. These measurements were carried out on three randomly selected areas on each specimen surface.

2.4 Tests on calcium phosphate formation

A protein-free SBF with ion concentrations almost equal to that of human blood plasma⁽¹⁵⁾ was used to carry out the tests on calcium phosphate formation. The concentrations of the different inorganic ions used in SBF are listed in Table 1. The polished specimens were ultrasonically cleaned in acetone for 0.6 ks and then in distilled water for 0.6 ks. The cleaned specimens were dried in air and immediately immersed in a polystyrene vial containing 20 ml of SBF with a pH 7.40 at 310 K for 1209.6 to 4838.4 ks (2 to 8 weeks). After the immersion of specified duration, the specimens were retrieved and immediately washed with distilled water and dried in air. The surfaces of the specimens before and after the immersion in the SBF were observed using a scanning electron microscope (SEM) (JSM-6060, JEOL, Tokyo, Japan). The precipitates formed on the surfaces were analyzed qualitatively and quantitatively using an electron probe microanalyzer (EPMA)

(JXA-8900R, JEOL, Tokyo, Japan). During the specified duration of immersion, the SBF used was the same.

Table 1 Ion concentrations (mM) of SBF and human blood plasma⁽¹⁵⁾.

Media	Na ⁺	K ⁺	Mg ²⁺	Ca ²⁺	Cl ⁻	HCO ³⁻	HPO ₄ ²⁻	SO ₄ ²⁻
SBF	142.0	5.0	1.5	2.5	148.8	4.2	1.0	0.5
Blood plasma	142.0	5.0	1.5	2.5	103.0	27.0	1.0	0.5

2.5 Statistical analysis

All the data collected were statistically analyzed by a one-way ANOVA and the Tukey HSD test at a significance level of $\alpha = 0.05$.

3. Results

3.1 Bulk hardness and surface roughness

Bulk hardness and *Ra* values of all the metal specimens are shown in Table 2. The measured hardness values of the Ti-Ag alloy specimens were significantly higher ($p < 0.05$) than that of pure Ti specimen. The mean hardness value of the Ti-Ag alloy with 25% Ag was higher than that of the Ti-Ag alloys with 20% Ag, although the difference between the two was not significant. The hardness values of pure Ag specimens were significantly lower ($p < 0.05$) than those of the other specimens.

The *Ra* values of the Ti-Ag alloy specimens were significantly lower ($p < 0.05$) than that of pure Ti specimen. The mean *Ra* value of the Ti-Ag alloy with 25% Ag was lower than that of the Ti-Ag alloy with 20% Ag, although the difference between the two values was not significant. The *Ra* value of pure Ag specimen was significantly higher ($p < 0.05$) than that of the other metal specimens. It was observed that the *Ra* values of all the metal specimens decreased as their hardness increased.

Table 2 Vickers hardness and surface roughness (*Ra*) values of specimens.

Metal	Pure Ti	20% Ag	25% Ag	Pure Ag
Hardness	156.5 (5.4) ^a	226.5 (13.8) ^b	236.7 (10.0) ^b	46.6 (3.6) ^c
<i>Ra</i>	0.182 (0.008) ^d	0.118 (0.008) ^e	0.116 (0.003) ^e	0.291 (0.024) ^f

Mean (SD); identical superscripts indicate no statistical differences ($p > 0.05$).

3.2 Calcium phosphate formation

On macroscopic examination of the SBF and the wall of the vials containing the specimens, it was found that precipitates such as AgCl were not deposited during the experiments. Images of the specimen surface before and after immersion are shown in Fig. 1; these images clearly demonstrate the changes in the surface due to immersion in the SBF. The surfaces of all the metals before immersion clearly show the presence of grinding marks. Moreover, the surfaces of the Ag specimens appear to be rougher than those of the other metals.

In the images taken after 2 weeks of immersion, the small number of precipitates was observed on the surfaces of the pure Ti and Ti-Ag alloy specimens, which was not observed in the images taken before immersion. The size of these precipitates was estimated to be less than 1 μm . The qualitative analysis of these specimens carried out by EPMA did not reveal the presence of any elements other than the matrix elements at the location where the precipitates were observed. After 4 weeks of immersion, a few precipitates, approximately 5 μm in size, were observed in the images of the surfaces of pure Ti and Ti-Ag alloy

specimens. The qualitative analysis of these specimens at this stage confirmed the presence of Ca or Ca and P, in addition to the matrix elements, at the positions where the precipitates were observed. However, the amount of precipitate formed was too small to be quantitatively analyzed using EPMA. After 8 weeks of immersion, many precipitates were observed on the pure Ti and Ti-Ag alloy surfaces; the size of these precipitates was found to be 5–10 μm . The qualitative analysis of these samples revealed that the precipitates consisted mainly of Ca and P. Other elements such as Na, K, Mg, Cl, C, and S, which were present in the SBF, were not detected in the precipitates. From the quantitative analysis of the precipitates, the ratios of the concentration of calcium to that of phosphorus (Ca/P molar ratios) in the precipitates were found to be approximately 1.5, and the values of the Ca/P molar ratios of the metals did not significantly differ. In fact, it was observed that the amount of precipitates formed on pure Ti, the alloy with 20% Ag, and the alloy with 25% Ag did not differ from each other at any immersion time. The surfaces of the pure Ag specimens were found to be corroded after immersion. The qualitative analysis of these surfaces revealed the presence of only Ag on the corroded surfaces.

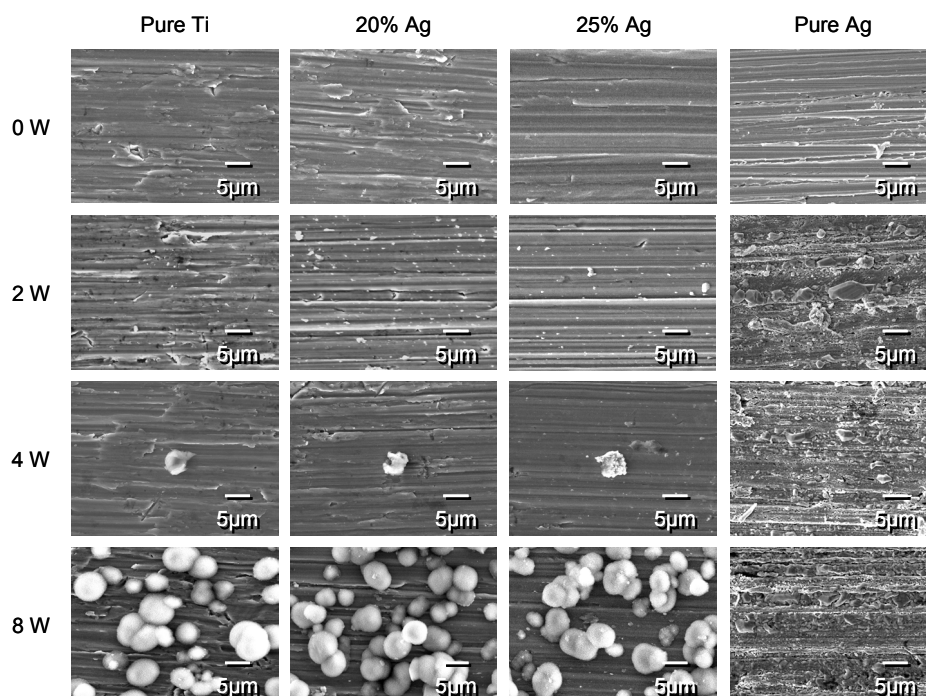


Fig. 1 Specimen surfaces after immersion in SBF for different durations of time.

4. Discussion

4.1 Surface properties

The hardness of the Ti-Ag alloys was found to be significantly higher than that of pure Ti. The increased hardness of the alloys was attributed to the occurrence of solid-solution hardening in the alpha phase of the alloys and the precipitation of Ti_2Ag in the alloys, as suggested in a previous study⁽¹⁰⁾. The mean hardness value of the alloy with 25% Ag was slightly higher than that of the alloy with 20% Ag. This is because in Ti-Ag alloys with Ag $\geq 20\%$, intermetallic compounds such as Ti_2Ag get precipitated, and thus, the amount of Ti_2Ag in the alloy with 25% Ag is more than that in the alloy with 20% Ag⁽¹³⁾. Although all the specimens were polished using silicon carbide abrasive papers with the same grit size, the surface roughness of the specimens of all metals differed significantly. This difference

in roughness is attributed to the different hardness of each metal. Since the depth of cut in a hard polished material is small, the surface roughness of the hard materials is also low. Therefore, the metals that exhibited higher hardness showed lower surface roughness. Since the hardness of the alloy with 20% Ag and 25% Ag did not differ significantly, the surface roughness of these alloys was also comparable. The surfaces of the Ag specimens appeared to be rougher than those of the other metal specimens in the SEM images taken before immersion. In this study, the *Ra* value was the highest in the case of Ag specimens. The *Ra* value was consistent with the results of the SEM observations.

4.2 Calcium phosphate formation

Hanawa et al. suggested the following mechanism of formation of calcium phosphate on pure titanium surfaces^(4, 18). The surface of the pure titanium film, which consists of titanium oxide and titanium oxyhydroxide, preferentially absorbs phosphate ions to form titanium phosphate. Then, calcium ions get adsorbed on the film, resulting in the formation of calcium phosphate or calcium titanium phosphate on the outermost surface of the film.

In the case of Ti-Ag alloys, an oxide layer is formed on their surfaces, making the alloys passive. The stability of the oxide layer on the surface of the Ti-Ag alloys would be comparable to that of the oxide layer formed on pure titanium because the passive current densities and the transpassive potentials of the alloys and pure titanium were found to be comparable⁽¹³⁾. Moreover, the Ti-Ag alloys became passive faster than pure titanium, since the time taken by the Ti-Ag alloys to attain the stable rest potential is less as compared to that taken by pure titanium⁽¹⁴⁾. Therefore, it is speculated that the mechanism for calcium phosphate formation in Ti-Ag alloys is similar to that in titanium. It should be noted that calcium phosphates are not spontaneously formed on all titanium alloys. Although the Ti-29Nb-13Ta-4.6Zr alloy, which was developed for use in biomedical applications, showed the formation of an oxide layer, the precipitation of calcium phosphate on their surface after immersion in SBF was detected only in the case of the samples that were subjected to surface treatments⁽¹⁶⁾. Moreover, the Ca/P ratio of the precipitate formed on pure titanium was closer to the stoichiometric Ca/P ratio of hydroxyapatite than the Ca/P molar ratios of the precipitates formed on other titanium alloys such as the Ti-6Al-4V alloy⁽³⁾. It is concluded that the precipitates observed on the surface of Ti-Ag alloys and pure Ti were calcium phosphates, because the qualitative analysis of these precipitates revealed the presence of Ca and P. Although quantitative analysis was not performed on all samples, the amount of the calcium phosphate precipitates formed on the Ti-Ag alloys and those on pure Ti appear to be similar. Further, the Ca/P molar ratios of the precipitates on the alloy specimens and those on pure Ti specimens were also found to be approximately equal. It is worth noting that although calcium phosphate was spontaneously formed on the Ti-Ag alloys as well as the pure Ti specimens, it was not formed on the pure Ag specimens. Therefore, it can be concluded that the addition of silver does not deteriorate the biocompatibility or bioconductivity of titanium. It is suggested that Ti-Ag alloys are also capable of undergoing osseointegration, as in the case of pure titanium; however, further detailed investigation is required to confirm this suggestion.

In this study, the precipitates formed after 8 weeks of immersion were confirmed to be calcium phosphates by EPMA. Although it was not confirmed, we believe that the precipitates formed after 2 weeks of immersion (1 μm in size) and the precipitates formed after 4 weeks of immersion (5 μm in size) were also calcium phosphates. In the study by Hanawa et al.^(3, 4), a minute amount of calcium phosphate was precipitated on pure titanium after immersion in Hanks' solution for 2592 ks (30 days); further, SEM observation of the surfaces of pure titanium showed no apparent change after immersion. Hanawa et al. claimed that the characterization of such a thin layer of precipitates should be carried out using surface analysis techniques such as X-ray photoelectron spectroscopy (XPS) with an

angle-dependent technique. By using XPS or AES, we could possibly confirm the presence of calcium phosphate in the precipitates formed during the immersion of less than 8 weeks. In order to increase the rate of formation of calcium phosphates and to increase the activity of the precipitated calcium phosphates, the use of various surface treatments for the materials, such as plasma-spraying, acid-etching, anodization, and ion implantation, were examined ⁽⁶⁾. Further, when the specimens were immersed in a fresh SBF every day, the Ca/P molar ratio of the precipitate was found to be close to the stoichiometric Ca/P ratio of hydroxyapatite ⁽¹⁹⁾. The main aim of this study was the investigation of the spontaneous formation of calcium phosphates on Ti-Ag alloys; therefore, research on surface treatment of the alloys for facilitating calcium phosphate formation will be conducted in the future.

In general, the surface roughness of pure titanium is known to affect calcium phosphate formation ⁽²⁰⁾. This is because pure titanium with high surface roughness has been reported to show increased calcium phosphate formation in the SBF as compared to that with a low surface roughness ⁽²⁰⁾. In our study, we expected that considerable calcium phosphate formation would be observed on the pure Ag specimens, since they exhibited the highest *Ra* value; however, calcium phosphates precipitate were not observed on the pure Ag specimens. Moreover, although the *Ra* values of the Ti-Ag alloys and pure Ti were significantly different, the amount of precipitates and the Ca/P molar ratios of precipitates formed on these two metals did not differ considerably. Therefore, in our study, the *Ra* values did not significantly influence calcium phosphate formation.

5. Conclusion

The spontaneous formation of calcium phosphates on experimental Ti-Ag alloys and on pure Ti on immersion in SBF has been confirmed. The amount and the Ca/P molar ratios of the precipitates formed on the different alloys and pure Ti did not differ significantly. Calcium phosphate was not formed on pure Ag. We have demonstrated that the bioconductivity and biocompatibility of Ti-Ag alloys make them suitable for use in the fabrication of dental and orthopedic implants.

Acknowledgments

The authors gratefully acknowledge Osaka Titanium technologies Co., Ltd. for supplying the high-purity titanium sponge.

References

- (1) Albrektsson, T., Brånemark, P. I., Hansson, H. A., Kasemo, B., Larsson, K., Lundström, I., MaQueen, D. H. and Skalak, R., The interface zone of inorganic implants in vivo: titanium implants in bone, *Annals of Biomedical Engineering*, Vol. 11 (1983), pp. 1-27.
- (2) Sul, Y. T., The significance of the surface properties of oxidized titanium to the bone response: special emphasis on potential biochemical bonding of oxidized titanium implant, *Biomaterials*, Vol. 24 (2003), pp. 3893-3907.
- (3) Hanawa, T. and Ota, M., Calcium phosphate naturally formed on titanium in electrolyte solution, *Biomaterials*, Vol. 12 (1991), pp. 767-774.
- (4) Hanawa, T. and Ota, M., Characterization of surface film formed on titanium in electrolyte using XPS, *Applied Surface Science*, Vol. 55 (1992), pp. 269-276.
- (5) Sundgren, J. E., Bodö, P. and Lundström, I., Auger electron spectroscopic studies of the interface between human tissue and implants of titanium and stainless steel, *Journal of Colloid and Interface Science*, Vol. 110 (1986), pp. 9-20.
- (6) Le Guéhennec, L. Soueidan, A., Layrolle, P. and Amourig, Y., Surface treatments of

- titanium dental implants for rapid osseointegration, *Dental Materials*, Vol. 23 (2007), pp. 844-854.
- (7) Kokubo, T., Kim, H. M., Kawashita, M. and Nakamura, T., What kinds of materials exhibit bone-bonding?, In Davies J. E. editor, *Bone Engineering*, Toronto, Canada, EM2, (2000), pp. 190-194.
 - (8) Davies, J. E., Understanding peri-implant endosseous healing, *Journal of Dental Education*, Vol. 67 (2003), pp. 932-949.
 - (9) Steinemann, S. G., Titanium - the material of choice?, *Periodontology 2000*, Vol. 17 (1998), pp. 7-21.
 - (10) Takahashi, M., Kikuchi, M., Takada, Y. and Okuno, O., Mechanical properties and microstructures of dental cast Ti-Ag and Ti-Cu alloys, *Dental Materials Journal*, Vol. 21 (2002), pp. 270-280.
 - (11) Kikuchi, M., Takahashi, M. and Okuno, O., Machinability of experimental Ti-Ag alloys, *Dental Materials Journal*, Vol. 27 (2008), pp. 216-220.
 - (12) Yoda, M., Konno, T., Takada, Y., Iijima, K., Griggs, J., Okuno, O., Kimura, K. and Okabe, T., Bond strength of binary titanium alloys to porcelain, *Biomaterials*, Vol. 22 (2001), pp. 1675-1681.
 - (13) Takahashi, M., Kikuchi, M., Takada, Y., Okabe, T. and Okuno, O., Electrochemical behavior of cast Ti-Ag alloys, *Dental Materials Journal*, Vol. 25 (2006), pp. 516-523.
 - (14) Takahashi, M., Takada, Y., Kikuchi, M. and Okuno, O., Released ions and microstructures of dental cast experimental Ti-Ag alloys, *Interface Oral Health Science 2007*, (2008), pp. 311-316.
 - (15) Kim, H. M., Miyaji, F., Kokubo, T. and Nakamura, T., Preparation of bioactive Ti and its alloys via simple chemical surface treatment, *Journal of Biomedical Materials Research*, Vol. 32 (1996), pp. 409-417.
 - (16) Li, S. J., Yang, R., Niinomi, M., Hao, Y. L. and Cui, Y. Y., Formation and growth of calcium phosphate on the surface of oxidized Ti-29Nb-13Ta-4.6Zr alloy, *Biomaterials*, Vol. 25 (2004), pp. 2525-2532.
 - (17) Xie, Y., Liu, X., Zheng, X. and Ding, C., Bioconductivity of plasma sprayed dicalcium silicate/titanium composite coatings on Ti-6Al-4V alloy, *Surface and Coatings Technology*, Vol. 199 (2005), pp. 105-111.
 - (18) Hanawa, T., Asami, K. and Asaoka, K., Repassivation of titanium and surface oxide film regenerated in simulated bioliquid, *Journal of Biomedical Materials Research Part A*, Vol. 40 (1998), pp. 530-538.
 - (19) Li, J., Liao, H. and Sjöström, M., Characterization of calcium phosphates precipitated from simulated body fluid of different buffering capacities, *Biomaterials*, Vol. 18 (1997), pp. 743-747.
 - (20) Vanzillotta, P. S., Sader, M. S., Bastos, I. N. and de Almeida Soares, G., Improvement of in vitro titanium bioactivity by three different surface treatments, *Dental Materials*, Vol. 22 (2006), pp. 275-282.