

Osteoinductivity of Titania/Hydroxyapatite Composite Films Formed Using Pulse Electrolysis

Kensuke Kuroda, Hironobu Shidu*, Ryoichi Ichino and Masazumi Okido

Department of Materials Science & Engineering, Graduate School of Engineering,
Nagoya University, Nagoya 464-8603, Japan

Titania (TiO₂)/hydroxyapatite (HAp) composite films were prepared on commercially pure titanium rods (2 mm in diameter, 5 mm in length) by anodic-cathodic pulse electrolysis in an aqueous solution consisting of 0.3 mM Ca(H₂PO₄)₂ and 0.7 mM CaCl₂ and pH = 5.5. The electrolysis was carried out in an autoclave at 120°C for 30 min. using +8.7 V vs. Ag/AgCl sat. KCl as the anodic potential and -9.3 V as the cathodic potential, and changing the electrolysis cycle (60 ~ 600 s) and duty ratio. We obtained TiO₂/HAp composite films in which fine HAp particles were uniformly dispersed on a thin TiO₂ layer. The coated rods were implanted in the tibiae of 10-week-old male rats. The constructs were retrieved 14 days postimplantation and examined for new bone formation and tissue response in the cancellous and cortical bone. They were compared with HAp-coated titanium rods, TiO₂-coated rods (anodizing in an aqueous solution), TiO₂/HAp-coated rods formed by the high temperature oxidization of specimens coated with HAp by cathodic electrolysis, and uncoated titanium rods. Fourteen days after implantation, new bone had formed on all the coated samples (HAp, TiO₂, and TiO₂/HAp) and noncoated titanium rods in the cancellous bone and cortical bone. In particular, TiO₂/HAp composite films prepared by pulse electrolysis had very high osteoinductivity, which resulted from a synergistic effect of HAp and TiO₂ on the bioactivity. [doi:10.2320/matertrans.48.328]

(Received August 28, 2006; Accepted October 27, 2006; Published February 25, 2007)

Keywords: hydroxyapatite, titania, composite coating, pulse electrolysis, anodizing, hydroprocess, *in vivo*

1. Introduction

It is well known that both hydroxyapatite (HAp) and TiO₂ ceramic have excellent biocompatibility, and many researchers have focused on their bioactivity.¹⁻⁴⁾ It is thought that the preparation of TiO₂/HAp composite coatings is a more promising approach than the individual coating of HAp or TiO₂, as they may have a synergistic effect in their bioactivity. In previous papers on TiO₂/HAp composite films, various preparation methods were reported, including HAp coating on the TiO₂ substrate,⁵⁾ composite HAp coating from a solution to which TiO₂ particles were added,⁶⁾ and sol-gel coating using media containing HAp and TiO₂ particles.⁷⁾ In general, these processes required two or more operation steps.

Some reports discussed the sandwich structure Ti-TiO₂-HAp, in which the intermediate TiO₂ layer, is effective in improving the adhesion of HAp with the Ti substrate.⁸⁾ Where the TiO₂ was completely covered with dense, thick HAp, however, the high osteoinductivity of TiO₂ was ineffective, because there was no TiO₂ on the outer face of the specimen to influence the bioactivity. We have proposed anodic-cathodic pulse electrolysis in aqueous solutions to form TiO₂/HAp composite films on a Ti substrate in a single-step process and, with this method, we were able to obtain TiO₂/HAp composite films with fine HAp particles dispersed uniformly on a thin TiO₂ layer.⁹⁾ In this paper, TiO₂/HAp composite coatings were prepared on Ti rods by anodic-cathodic pulse electrolysis and implanted in rat tibiae for 14 days to evaluate the extent of new bone formation.

2. Materials and Methods

2.1 TiO₂/HAp composite coating

As the experimental apparatus and procedure are almost

the same as those used in a previous report,⁹⁾ they are described only briefly here. The aqueous solution for the electrolysis consisted of 0.3 mM Ca(H₂PO₄)₂, and 0.7 mM CaCl₂. The pH of the solution was adjusted to 5.5 at room temperature by adding aqueous NaOH solution. Commercially pure titanium rods ($\phi 2 \times 5$ mm) were polished with #400 SiC emery paper. The working electrode (Ti rod), counter (Pt coil), quasi-reference (Pt), and thermocouple were immersed in 0.2 dm³ of aqueous solution. A magnetic stirrer agitated the electrolyte solution during the experiments. The electrolysis was conducted in an autoclave, and the temperature was maintained at 120°C by an external heater. The pulse electrolysis experiments were carried out for 1800 s using +8.7 V as the anodic potential and -9.3 V as the cathodic potential, and changing the electrolysis cycle and duty ratio to anodic 30 s and cathodic 30 s (A30-C30), anodic 30 s and cathodic 60 s (A30-C60), or anodic 60 s and cathodic 60 s (A60-C60). The electrode potential was calibrated and expressed as a reference of Ag/AgCl sat. KCl in this paper. The coatings were identified by using X-ray diffraction (XRD), and the surfaces and cross-sections of the samples were observed by scanning electron microscopy (SEM).

2.2 Other coatings for comparison with TiO₂/HAp composite

To allow comparison in *in vivo* evaluations, the following specimens were also prepared: (i) TiO₂ only coatings formed by anodizing in aqueous solution, (ii) TiO₂/HAp composite films formed by high temperature oxidization after coating with HAp by cathodic electrolysis (two-step operation), (iii) coating with HAp by the thermal substrate method in aqueous solution, and (iv) control Ti rods polished with #400 SiC emery paper.

2.2.1 TiO₂ coatings by anodizing in the aqueous solution

TiO₂ coatings were made by anodizing at +8.7 V for 900 s at 120°C in a solution consisting of 0.3 mM Ca(H₂PO₄)₂ and

*Graduate Student, Nagoya University

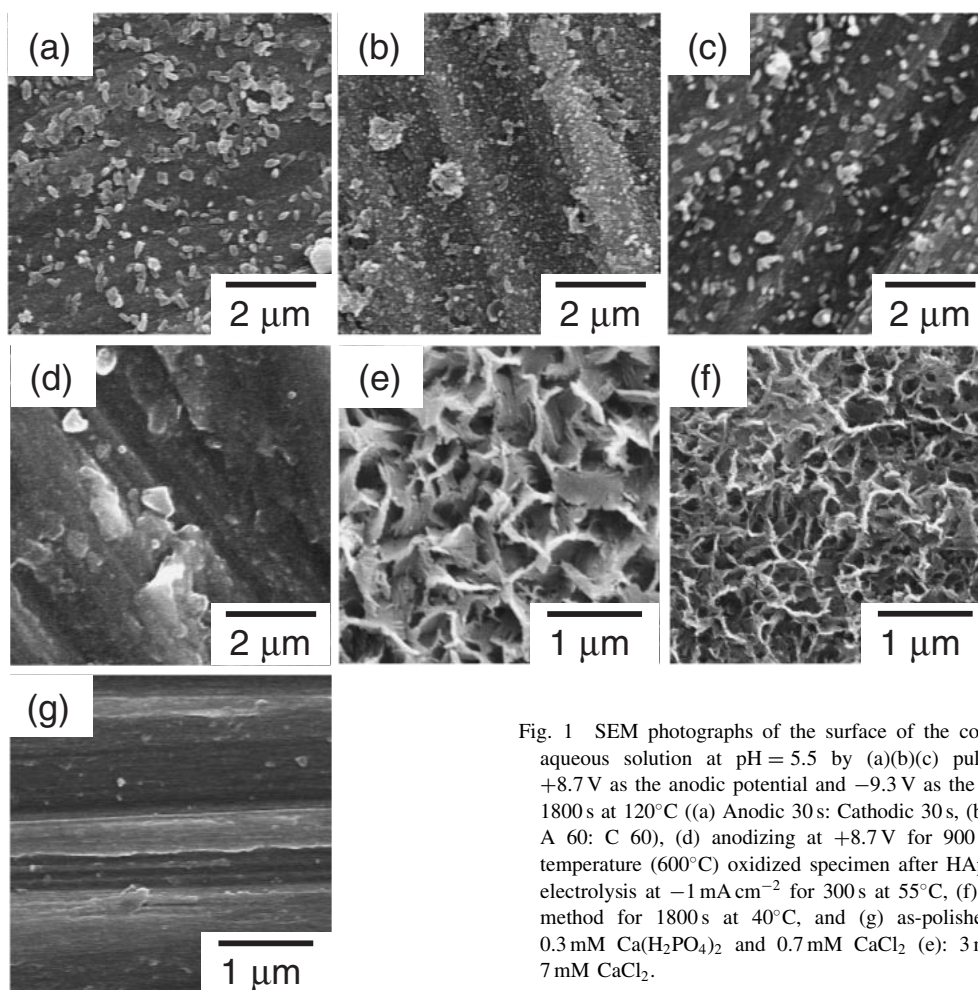


Fig. 1 SEM photographs of the surface of the coated specimens in an aqueous solution at pH = 5.5 by (a)(b)(c) pulse electrolysis using +8.7 V as the anodic potential and -9.3 V as the cathodic potential for 1800 s at 120°C ((a) Anodic 30 s: Cathodic 30 s, (b) A 30: C 60, and (c) A 60: C 60), (d) anodizing at +8.7 V for 900 s at 120°C, (e) high temperature (600°C) oxidized specimen after HAp coating by cathodic electrolysis at -1 mA cm^{-2} for 300 s at 55°C, (f) the thermal substrate method for 1800 s at 40°C, and (g) as-polished Ti. (a)(b)(c)(d)(f): 0.3 mM $\text{Ca}(\text{H}_2\text{PO}_4)_2$ and 0.7 mM CaCl_2 (e): 3 mM $\text{Ca}(\text{H}_2\text{PO}_4)_2$ and 7 mM CaCl_2 .

0.7 mM CaCl_2 , which was the solution used in the pulse electrolysis.

2.2.2 TiO_2/HAp composite films by high temperature oxidation after HAp coating by cathodic electrolysis

A cathodic current (-1 mA cm^{-2}) was applied to Ti substrates (1 cm^2) at 55°C for 300 s in an aqueous solution of 3 mM $\text{Ca}(\text{H}_2\text{PO}_4)_2$ and 7 mM CaCl_2 with pH = 5.5. The HAp coated specimens were then heated in an Ar-3% H_2 atmosphere at a heating rate of 5°C min^{-1} . On reaching 600°C, the furnace was turned off and the specimens were cooled to room temperature in the furnace in the same atmosphere. Titanium is strongly active with oxygen and is oxidized to TiO_2 at very low partial pressures of oxygen ($p_{\text{O}_2} = 10^{-47} \text{ atm}$ at 600°C¹⁰). Therefore, the slightly reductive atmosphere, Ar-3% H_2 , was sufficient at 600°C for the formation of a thin TiO_2 layer on the Ti substrate.

2.2.3 HAp coatings by the thermal substrate method in aqueous solution^{4,11)}

An alternating current was passed through Ti specimens in an aqueous solution consisting of 0.3 mM $\text{Ca}(\text{H}_2\text{PO}_4)_2$, 0.7 mM CaCl_2 , and 1 M ethanol and pH = 5.5. The temperature of the specimens was maintained at 40°C for 1800 s. An ultrasonic wave (100 kHz) was applied to the specimens during the coating to accelerate HAp nucleation and growth.¹²⁾ As the surface morphology of the HAp film

influenced the osteoinductivity,¹¹⁾ the above coating conditions in the thermal substrate method were selected to obtain an HAp coating with surface morphology as similar as possible to that obtained by cathodic electrolysis (ii).

2.3 *in vivo* evaluation

As the *in vivo* evaluation is also the same in a previous report,¹¹⁾ it is described only briefly here. Following the above treatments to produce the coatings, samples were implanted in the tibial metaphysis of rats, and the bioactivities of the coatings were evaluated from new bone formation and tissue response.

Before surgery, all implants were cleaned ultrasonically in distilled water and sterilized in a steam autoclave (121°C, 20 min.). For the experimental procedure, 340–400 g 10-week-old male Sprague Dawley rats (Charles River Japan, Inc.) were used. Prior to surgery, the rats were anesthetized and the incision sites (right and left) and surrounding area were shaved and cleaned. The implants were inserted in slightly oversized holes created with a low speed rotary drill. The rats were sacrificed after 14 days. This period was selected to evaluate osteoinductivity in the early implantation stage. After the sacrifice, the implants and their surrounding tissue were retrieved. The specimens were fixed in 10% neutral-buffered formalin solution, dehydrated in a graded series of ethanols, and embedded in methylmethacrylate.

Following polymerization, each implant block was sectioned to 20 μm thick slices. The final sections were stained with toluidine blue.

Bone-implant contact was determined by linear measurement of direct bone contact with the implant surface.¹¹ The sum of the length of the bone formation on the implant surface was measured and expressed as a percentage of the total implant length (bone-implant contact ratio, R_{B-I}) in the cancellous bone and the cortical bone. Differences in R_{B-I} between the HAp-, TiO_2 -, and TiO_2/HAp -coated implants and non-coated Ti were statistically analyzed using the Tukey-Kramer method. Differences were considered statistically significant at $p < 0.05$.

3. Results and Discussion

3.1 HAp-, TiO_2 -, and TiO_2/HAp -coating

Typical SEM photographs and XRD patterns for the surfaces of the specimens are shown in Figs. 1 and 2, respectively. The letters indicate the treatments as follows: pulse electrolyzed as described above for (a) A30-C30, (b) A60-C60, and (c) A30-C60; (d) specimen anodized according to procedure (i); (e) high temperature oxidized specimen after HAp coating by cathodic electrolysis according to procedure (ii); (f) HAp coating by the thermal substrate method according to procedure (iii), and (g) Ti polished with #400 SiC emery paper. Thus, TiO_2/HAp composite coatings were obtained using pulse electrolysis (a), (b), and (c) and by the two-step operation (high temperature oxidization after HAp coating) (e), a TiO_2 film was obtained by anodizing (d), and a HAp film was obtained by the thermal substrate method (f). The thicknesses of the anodized TiO_2 films ((a)-(d)) were estimated to be less than 50 nm,⁹ and the HAp coatings ((e) and (f)) were less than 3 μm according to the SEM-EDX analysis of the cross-section of the specimens. It was thought that the TiO_2/HAp composite film's structure of HAp deposited on the thin TiO_2 films occurred because anodized TiO_2 films formed more rapidly than HAp in the electrolysis, and the positive potential was applied initially for TiO_2 formation ahead of HAp precipitation. Pulse electrolysis ((a), (b), and (c)) gave finer HAp particles, uniformly dispersed on the TiO_2 film on the Ti substrates.⁹ On the other hand, in the TiO_2/HAp coating from the two-step operation (e), it was found that the entire surface of the specimen was covered with HAp, and similarly with the HAp coating by the thermal substrate method (f), even though XRD detected TiO_2 . Therefore, it was thought that a TiO_2 layer was formed only on the Ti substrate as an inner layer and that there was not TiO_2 near the surface of the composite coating, because the HAp formed by cathodic electrolysis was about 3 μm thick, and did not have a dense structure¹³ but had many pores and a net-like structure.

The difference in the crystal structure of the titanium oxide (TiO_2) between anatase and rutile was not considered in this paper, because its effect on osteoinductivity could not be found.¹⁴ It will be described in detail in our next report.

3.2 *in vivo* evaluation

During the sterilization in a steam autoclave (121°C, 20 min.), the surface morphology of the coated films did not

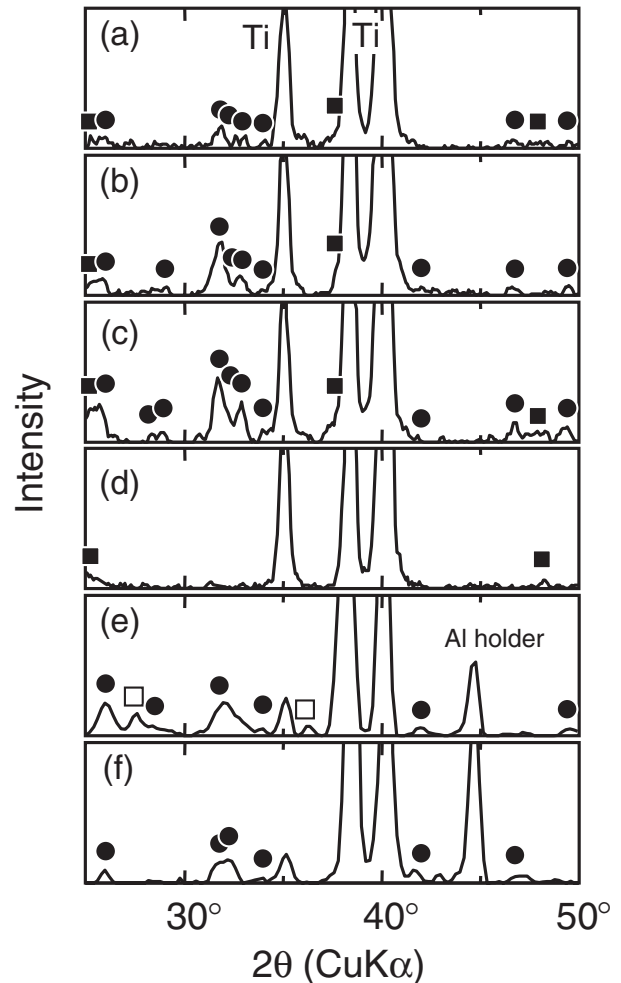


Fig. 2 XRD patterns of the coated specimens in an aqueous solution at $\text{pH} = 5.5$ by (a)(b)(c) pulse electrolysis using +8.7 V as the anodic potential and -9.3 V as the cathodic potential for 1800 s at 120°C ((a) Anodic 30 s: Cathodic 30 s, (b) A 30: C 60, and (c) A 60: C 60), (d) anodizing at +8.7 V for 900 s at 120°C, (e) high temperature (600°C) oxidized specimen after HAp coating by cathodic electrolysis at -1 mA cm^{-2} for 300 s at 55°C, (f) the thermal substrate method for 1800 s at 40°C, and (g) as-polished Ti. (a)(b)(c)(d)(f): 0.3 mM $\text{Ca}(\text{H}_2\text{PO}_4)_2$ and 0.7 mM CaCl_2 (e): 3 mM $\text{Ca}(\text{H}_2\text{PO}_4)_2$ and 7 mM CaCl_2 ●: HAp, ■: anatase, □: rutile.

change. The bone-implant contact ratio, R_{B-I} , of the implants with TiO_2/HAp composite coatings by pulse electrolysis are presented in Fig. 3, compared with the other coatings and control Ti.

The TiO_2/HAp composite coatings by pulse electrolysis ((a), (b), and (c)) had higher R_{B-I} values than did the individual coatings of TiO_2 (d) or HAp (f) in cancellous bone and cortical bone. The value of R_{B-I} for TiO_2/HAp in the cortical bone is the same or higher than the sum of the values for TiO_2 (d) and HAp (f), indicating that TiO_2 and HAp did not obstruct each other's osteoinductivity but that TiO_2 acted synergistically with HAp and the composite coating enhanced the ability.

On the other hand, R_{B-I} for TiO_2/HAp coatings by two-step operation (e) agreed with the individual coating of HAp (f), despite being composite. As the SEM photographs show, very much more HAp was created on the surface of the TiO_2/HAp coatings by the two-step operation. It is thought that the

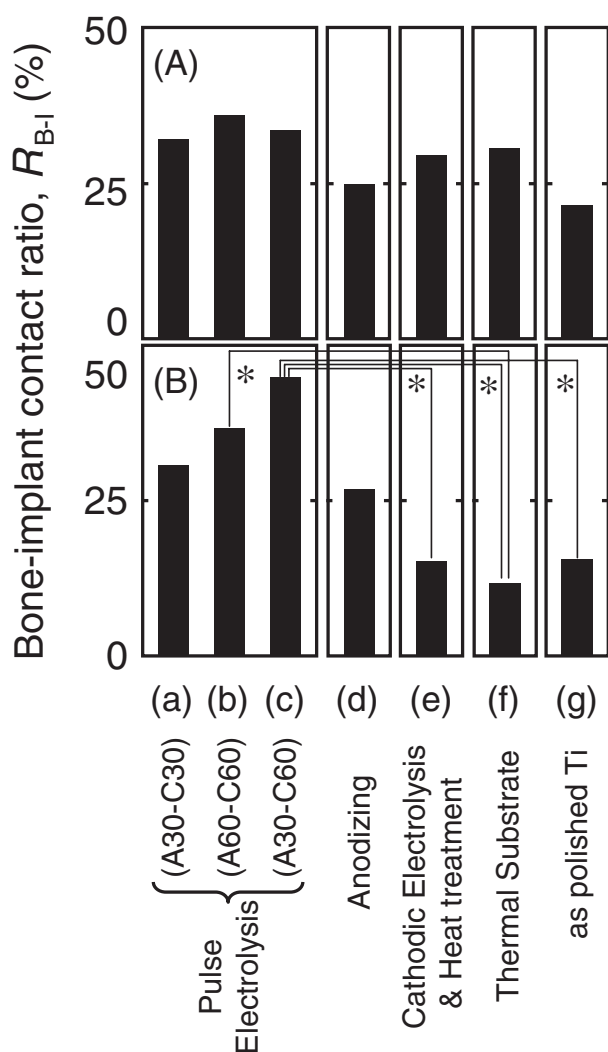


Fig. 3 Bone-implant contact ratio, R_{B-I} , for the coated specimens in the aqueous solution at pH = 5.5 by (a)(b)(c) pulse electrolysis using +8.7 V as the anodic potential and -9.3 V as the cathodic potential for 1800 s at 120°C ((a) Anodic 30 s: Cathodic 30 s, (b) A 30: C 60, and (c) A 60: C 60), (d) anodizing at +8.7 V for 900 s at 120°C, (e) high temperature (600°C) oxidized specimen after HAp coating by cathodic electrolysis at -1 mA cm⁻² for 300 s at 55°C, (f) the thermal substrate method for 1800 s at 40°C, and (g) as-polished Ti. (a)(b)(c)(d)(f): 0.3 mM Ca(H₂PO₄)₂ and 0.7 mM CaCl₂ (e): 3 mM Ca(H₂PO₄)₂ and 7 mM CaCl₂ (A) cancellous bone, (B) cortical bone. *: $p < 0.05$.

TiO₂/HAp composite coatings, in which TiO₂ existed only in the inner layer, could not benefit from the osteoinductivity of TiO₂, because the amount of HAp that overlay the TiO₂ reduced the influence of TiO₂ on the osteoinductivity. Therefore, the R_{B-I} of the TiO₂/HAp coating from the two-step operation was not as high as that for the coating from

pulse electrolysis. However, simple cathodic electrolysis could not produce the surface morphology with fine HAp particles dispersed on the Ti substrate that was obtained with pulse electrolysis. In this study, we could not determine the optimal value for osteoinductivity of the abundance ratio, TiO₂ : HAp, on the surface of the TiO₂/HAp coating by pulse electrolysis. Nonetheless, it bears mentioning that the TiO₂/HAp coating formed by pulse electrolysis, in which fine HAp particles were dispersed uniformly on a thin TiO₂ layer, had a higher osteoinductivity than did the other individual coatings in the early implantation stage.

4. Conclusions

- (1) TiO₂/HAp composite coatings by pulse electrolysis, in which fine HAp particles were dispersed uniformly on thin TiO₂, have higher R_{B-I} values compared with the other coatings studied (TiO₂ by anodizing and HAp by the thermal substrate method in cancellous bone and cortical bone, respectively). Therefore, the composite coating enhanced osteoinductive ability *in vivo*.
- (2) However, in the TiO₂/HAp composite with too much HAp on the surface, the TiO₂ layer existed as an inner layer, and thus these implants could not exploit the osteoinductivity of TiO₂.

REFERENCES

- 1) L. L. Hench and J. Wilson: *An Introduction to Bioceramics*, (World Scientific, Singapore, 1993).
- 2) R. Hazan, R. Brener and U. Oron: *Biomater.* **14** (1993) 570–574.
- 3) M. Keshmiri and T. Troczynski: *J. Non-cryst. Solids* **324** (2003) 289–294.
- 4) K. Kuroda, R. Ichino, M. Okido and O. Takai: *J. Biomed. Mater. Res.* **59** (2002) 390–397.
- 5) W. Song, Y. Jun, Y. Han and A. Hong: *Biomater.* **25** (2004) 3341–3349.
- 6) X. F. Xiao, R. F. Liu and Y. Z. Zheng: *Surf. Coat. Technol.* **200** (2006) 4406–4413.
- 7) P. A. Ramires, A. Wennerberg, C. B. Johansson, F. Cosentino, S. Tundo and E. Milella: *J. Mater. Sci. Mater. Med.* **14** (2003) 539–545.
- 8) W. Xu, W. Hu, M. Li and C. Wen: *Mater. Lett.* **60** (2006) 1575–1578.
- 9) K. Kuroda, H. Shidu, R. Ichino and M. Okido: *Mater. Trans.* **48** (2007) 322–327.
- 10) Nippon-kinzoku-gakkai, *Kinzoku-Seiren-Kougaku*, (Maruzen, 1999) p. 187.
- 11) K. Kuroda, S. Nakamoto, R. Ichino and M. Okido: *Mater. Trans.* **47** (2006) 1391–1394.
- 12) Y. Fang, D. K. Agarwal, D. M. Roy, R. Roy and P. W. Brown: *J. Mater. Res.* **7** (1992) 2294–2298.
- 13) M. Okido, K. Nishikawa, K. Kuroda, R. Ichino, Z. Zhao and O. Takai: *Mater. Trans.* **43** (2002) 3010–3014.
- 14) H. Shimizu, S. Nakamoto, K. Kuroda, R. Ichino and M. Okido: *Proc. of the spring meeting of Japan Institute of Metals*, (2005), p. 241.