

Bioactive Co-Cr alloy for biomedical applications prepared by surface modification using self-assembled monolayers and poly- γ -glutamic acid

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Cobalt-chromium (Co-Cr) alloys are used in clinical practice for the hard tissue reconstruction because of their favorable biocompatibility and mechanical properties. However, their applications have been limited because of their poor bioactivity, making them poor at bone-bonding. In this study, the bioactivity of a Co-Cr alloy was evaluated following the immobilization of cross-linked poly- γ -glutamic acid (γ -PGA) onto its surface *via* the formation of 11-aminoundecylphosphonic acid self-assembled monolayers (SAMs). Results of X-ray photoelectron spectroscopy revealed the presence of a new P2p peak, which confirms SAMs formation. Furthermore, the surface became highly hydrophobic following the immobilization with γ -PGA. Subsequent treatment with CaCl₂ at 0.5 M or more and soaking in a simulated body fluid led to the formation of a low crystalline apatite. The present results show that chemical modification can be used to induce the formation of an apatite layer on the surface of a Co-Cr alloy in simulated body fluid.

Keywords: Co-Cr alloy, Poly- γ -glutamic acid, Self-assembled monolayers (SAMs), Simulated body fluid (SBF), Apatite

INTRODUCTION

Co-Cr alloys have been used in a wide variety of biomedical applications, including dental, cardiovascular and orthopedic devices^{1,2}. Alloys of this type generally demonstrate high levels of biocompatibility and also possess excellent mechanical properties, including resistance to pitting, wear, high abrasion and crevice corrosion, as well as showing high fatigue strength, malleability and ductility^{3,4}. Co-Cr alloys are frequently used in implant materials during joint replacement procedures involving hard tissues, such as tooth and bone, where they come into direct contact with blood, soft tissue and bone^{5,6}. Despite their many advantages and widespread application in biomedical devices, several issues have been reported with regard to the use of Co-Cr alloys in clinical studies, most notably a lack of bioactivity, leading to poor bone bonding.

An important requirement for a material to demonstrate bioactivity is the ability to form biologically active bone-like apatite on its surface⁷. The formation of apatite can even occur in simulated body fluid (SBF), which is a solution containing inorganic ion concentrations almost equal to those of human extracellular fluid^{8,9}. In a physiological environment, chemical surface modification processes such as alkali treatment and anodic oxidation can be used to convert Ti and its alloys¹⁰⁻¹⁴ and Ta¹⁵ into bioactive materials. However, the use of these modifications has been shown to be ineffective for Co-Cr alloys¹⁰. Although the application of a bioactive glass coating to the surface of Co-Cr alloys has been evaluated in detail¹⁶, very few studies have been reported pertaining to the precipitation

of apatite onto Co-Cr alloys in SBF.

It was recently reported that self-assembled monolayers (SAMs) can be successfully constructed on the surfaces of a variety of different metals, including Co, Co-Cr alloys, stainless steel, gold and Ti-45Nb alloy¹⁷⁻²¹. In addition, our group recently demonstrated that cross-linked poly- γ -glutamic acid (γ -PGA) can be used to induce bone-like apatite layers in SBF²²⁻²⁴. Taken together, these findings suggest that the immobilization of γ -PGA onto a Co-Cr alloy constructed from SAMs could be used to increase the bioactivity of the alloy.

In this study, we have prepared a series of SAMs based on 11-aminoundecylphosphonic acid (11-AUPA):NH₂(CH₂)₁₁PO₃H₂·HBr and subsequently immobilized γ -PGA. The apatite-forming properties of these layers were subsequently assessed in SBF. Ca²⁺ was also incorporated into the specimens to enhance their apatite-forming ability.

MATERIALS AND METHODS

The Co-Cr alloy used in the current study was purchased from Nihon Shika Kinzoku (Osaka, Japan), and its elemental composition is listed in Table 1. 11-AUPA was purchased from Dojindo Laboratories (Kumamoto, Japan). γ -PGA with a molecular weight in the range of 800 to 1,200 kDa was purchased from Meiji Seika (Tokyo, Japan). *N*-Hydroxysuccinimide (HOSu) was purchased from Wako Pure Chemical Industries (Osaka, Japan) and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC·HCl) was purchased from Tokyo Chemical Industry (Tokyo, Japan). All of the other reagents used in the current study were purchased from Nacalai Tesque (Kyoto, Japan). Ultrapure water with a resistivity of 18 M Ω ·cm was

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Table 1 Chemical composition of Co-Cr alloy

Elements	Co	Cr	Mo	Others
Content (Mass%)	58	30	6	6

Table 2 Reagents composition of PGA solution

Reagent	PGA	EDC·HCl	HOSu
Content (Mass%)	2.0	3.1	1.8

prepared using a water purification system (Direct-Q, Millipore, Billerica, MA, USA).

The Co-Cr alloy was cut into thick disks (15 mm diameter×1.0 mm), which were polished with #500, 1000 and 1500 SiC waterproof abrasive papers. The polished disks were then subjected to ultrasonic irradiation in a 0.5% sodium dodecyl sulfate aqueous solution, followed by ethanol and then acetone for 20 min each. The chemical surface modification was performed by soaking the disks in 5 mL of a 1 mM 11-AUPA solution in ethanol at 36.5°C for 2 days, followed by soaking in an aqueous solution of γ -PGA with stirring for 1 day at room temperature. The reagent composition of the γ -PGA solution is listed in Table 2. The untreated and treated specimens were subsequently immersed in CaCl₂ solutions of various concentrations at 36.5°C for 1 day. Finally, all the specimens were soaked in 30 mL of SBF (Na⁺:142.0, K⁺:5.0, Mg²⁺:2.5, Cl⁻:147.8, HCO₃⁻:4.2, HPO₄²⁻:1.0, and SO₄²⁻:0.5 mM) at 36.5°C for 7 days⁹. After soaking, the disks were removed from the SBF and immersed in ultrapure water for 1 day to remove any excess residual water-soluble salts before being dried at room temperature.

Changes in the chemical compositions and surface structures of the samples were characterized by X-ray photoelectron spectroscopy (XPS, KRATOS AXIS-His, Shimadzu, Kyoto, Japan), contact angle analysis (DME-200, Kyowa Interface Science, Saitama, Japan), thin-film X-ray diffraction (TF-XRD, MXP3V, Mac Science, Yokohama, Japan) and scanning electron microscopy (SEM, S-3500N, Hitachi, Tokyo, Japan). The binding energies were measured by XPS and corrected based on the binding energy of the C1s of the methylene groups of the hydrocarbon (284.6 eV) adsorbed onto the surface of the substrate.

RESULTS AND DISCUSSION

SEM images of the Co-Cr alloys before and after their sequential treatment with 11-AUPA and γ -PGA are shown in Fig. 1. Significant differences were not observed even after the treatments. The XPS spectra of the O1s, C1s, N1s and P2p regions of the Co-Cr alloys both before and after the treatment of the alloys with 11-AUPA are shown in Fig. 2. Notably, there was a significant increase in the intensity of the peak around

285 eV in the C1s spectrum following the treatment of the Co-Cr alloy with 11-AUPA. The O1s spectrum of the untreated alloy contained peaks at 530 and 532 eV, which were attributed to the metal oxide and hydroxide moieties, respectively²⁵. After being soaked in a solution of 11-AUPA, the XPS spectrum of the Co-Cr alloy contained a large peak at 531 eV, which was attributed to the P-OH or P=O bonds²⁶. The spectrum of the 11-AUPA-soaked material also contained two small peaks around 414.8 and 133 eV in the N1s and P2p regions, respectively. These results therefore suggested that 11-AUPA had been successfully immobilized onto the surface of the Co-Cr alloy. Contact angle images were recorded for the Co-Cr alloy with ultrapure water both before and after it had been soaked in a solution of 11-AUPA (Fig. 3). Contact angle images were also recorded in the same way following the soaking of the 11-AUPA-treated material in a solution of γ -PGA (Fig. 3). The results of these experiments demonstrated that the contact angle decreased from 73.6° to 54° and then to 24.4° after the 11-AUPA and γ -PGA treatments, respectively, which indicated that the surface of the alloy was becoming increasingly hydrophilic with each treatment.

The TF-XRD patterns of the Co-Cr alloys were measured before and after their sequential treatment with 11-AUPA, γ -PGA and a 1 M solution of CaCl₂ in SBF for 3 or 7 days, and the results are shown in Fig. 4. The TF-XRD pattern of the untreated Co-Cr alloy in the 2 θ mode contained peaks at 43° and 47°, which were assigned to Co-Cr and α -Co, respectively. No changes were observed in the positions of these peaks after the different soaking treatments. However, two new broad peaks appeared at 26° and 32° in the 2 θ mode for the Co-Cr alloys treated with 11-AUPA and γ -PGA after they had been soaked in SBF for 3 days. SEM images were collected for the Co-Cr alloys before and after their sequential treatment with 11-AUPA, γ -PGA and a 1 M solution of CaCl₂ in SBF for 3 or 7 days, and the results are shown in Fig. 5. The results revealed that a layer of fine particles formed in the alloys treated with CaCl₂ in SBF that almost covered their entire surface. This effect was not observed in the untreated samples.

The effect of the CaCl₂ concentration on the formation of apatite was also examined. The TF-XRD patterns of the Co-Cr alloy were recorded following

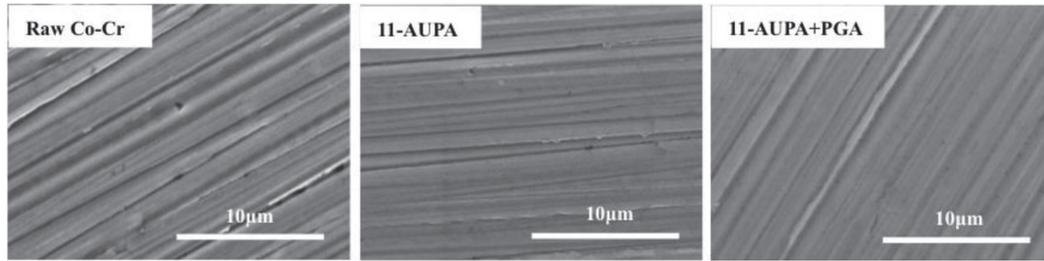


Fig. 1 SEM images of the surfaces of Co-Cr alloy with and without 11-AUPA and subsequent γ -PGA treatments.

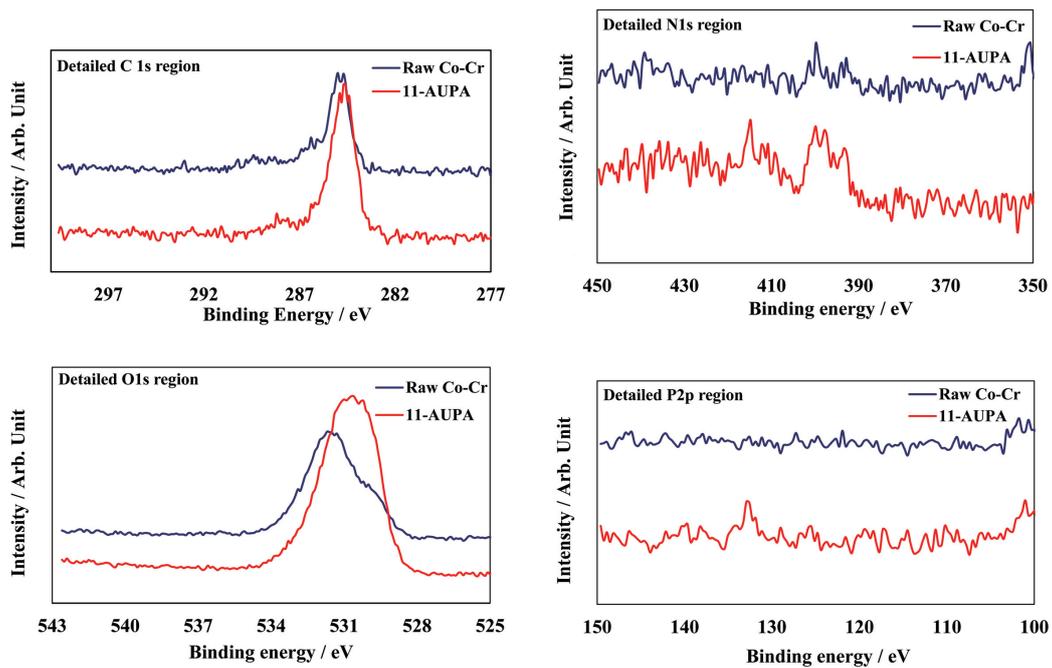


Fig. 2 XPS spectra of the O1s, C1s, N1s and P2p regions on Co-Cr alloy before and after treatment with the 11-AUPA solution.

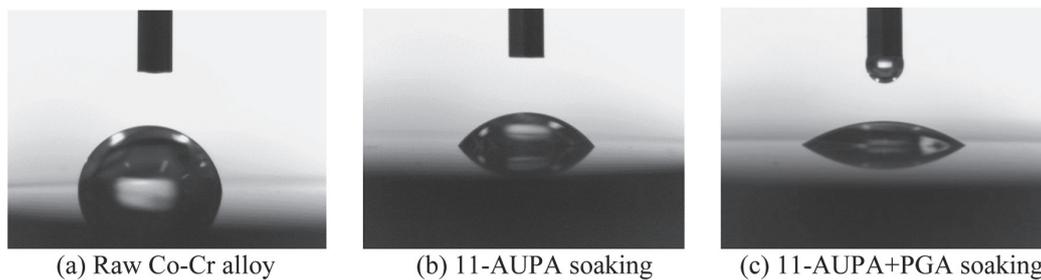


Fig. 3 Images of the contact angles of ultrapure water with the Co-Cr alloy before and after chemical surface modification.

their sequential treatment with 11-AUPA, γ -PGA and different concentrations of CaCl_2 in SBF for 7 days, and the results are shown in Fig. 6. Broad peaks corresponding to low-crystalline apatite were observed

in the samples treated with 0.5 and 1 M solutions of CaCl_2 , which indicated that that incorporation of Ca^{2+} ions into the alloy with a ≥ 0.5 M solution of CaCl_2 was necessary to allow for the formation of apatite on the

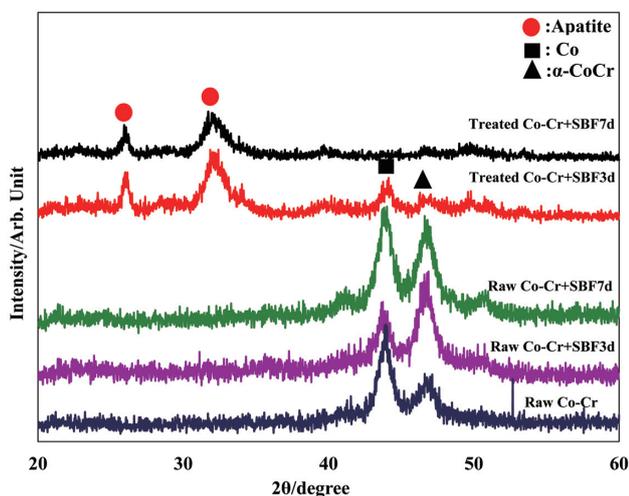


Fig. 4 TF-XRD patterns of the surfaces of the Co-Cr alloys with and without 11-AUPA, γ -PGA and 1 M CaCl_2 treatments and then soaking in SBF for 3 or 7 days.

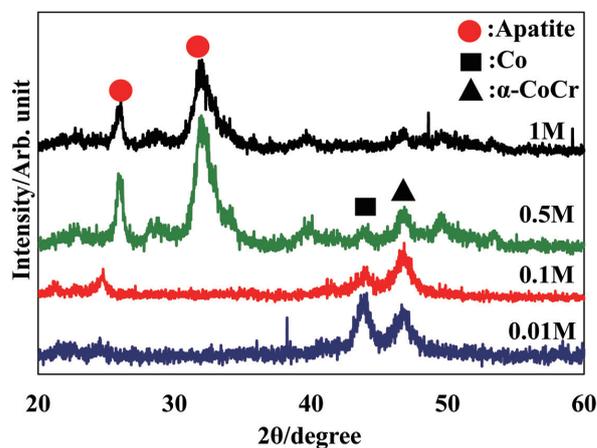


Fig. 6 TF-XRD patterns of the surfaces of the Co-Cr alloy treated with 11-AUPA, γ -PGA and various concentrations of CaCl_2 and then soaking in SBF for 7 days.

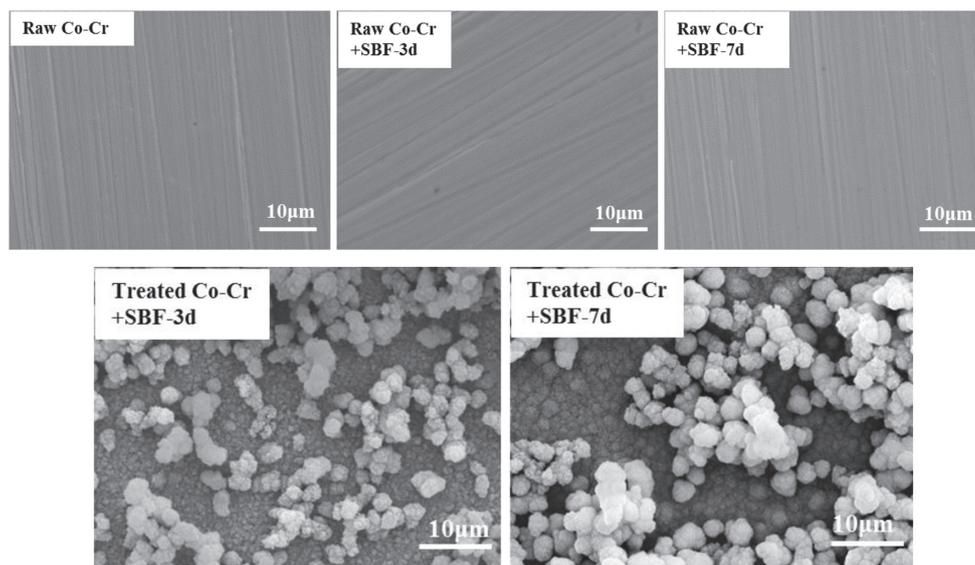


Fig. 5 SEM images of the surfaces of the Co-Cr alloys with and without 11-AUPA, γ -PGA and 1 M CaCl_2 treatments and then soaking in SBF for 3 or 7 days.

alloy.

These results indicate that γ -PGA had been successfully immobilized on the Co-Cr alloy following the sequential soaking of the alloy with a solutions of 11-AUPA and γ -PGA solution containing EDC·HCl and HOSu. Alginate can be immobilized on stainless steel surfaces that have been modified with 3-aminopropyltriethoxysilane *via* the formation of amide bonds²⁷. With this in mind, it was assumed that the γ -PGA used in the current study was also being immobilized on the surface of the Co-Cr alloy,

albeit through the formation of amino bonds, as shown schematically in Fig. 7. Namely, the 11-AUPA reacted with the Co-Cr alloy *via* a dehydration/condensation reaction between the P-OH groups of the 11-AUPA and the OH groups on the surface of the alloy. This result was confirmed by XPS analysis, which revealed the presence of N and P atoms derived from 11-AUPA after the treatment process (Fig. 2). When the 11-AUPA-treated alloy was subsequently immersed in a solution of γ -PGA containing HOSu and EDC·HCl, the amino groups of the 11-AUPA reacted with the carboxyl acid

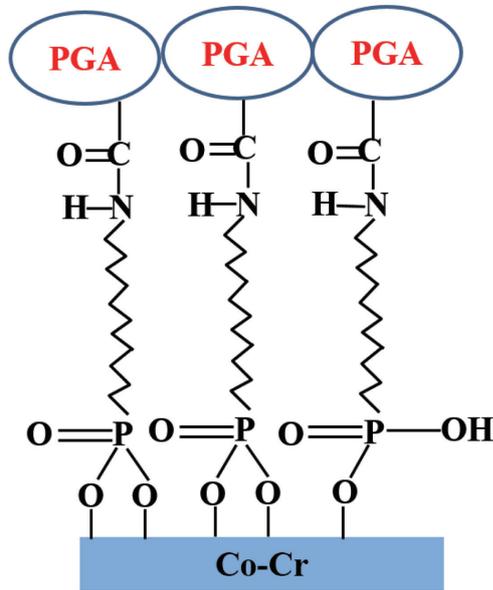


Fig. 7 Schematic illustration of γ -PGA-immobilization on Co-Cr alloy.

groups in γ -PGA to form the corresponding amide bond. The occurrence of this reaction was supported by the observed change in the contact angle of the alloy (Fig. 3). The formation of the SAMs was based on the naturally occurring OH groups on the surface of the alloy. If the density of the OH groups on the surface of the alloy could be increased with a pre-treatment process, then we would expect that the amount of immobilized 11-AUPA and γ -PGA on the surface of the alloy would also increase, which would consequently lead to an increase in the formation of apatite. The effect of the pre-treatment process on the reactivity of the alloy will be examined in greater detail as part of our future work.

The modification process described here allowed for the formation of apatite on the surface of a modified Co-Cr alloy in a simulated body environment. The mechanism of apatite formation on the Co-Cr alloy involved the formation of chemical bonds on the surface of the alloy between 11-AUPA and γ -PGA. The carboxyl groups on the γ -PGA units would interact with the Ca^{2+} , HPO_4^{2-} and PO_4^{3-} ions to allow for the heterogeneous nucleation of the apatite in SBF. Furthermore, sufficient Ca^{2+} ions would be released from the surface of the specimen into the SBF, leading to an increase in the degree of supersaturation with respect to the apatite when the sample was treated with an aqueous solution of CaCl_2 at concentrations in the range of 0.5 to 1.0 M.

CONCLUSION

SAMs functionalized with 11-AUPA were formed on the surfaces of Co-Cr alloys in aqueous solution. γ -PGA was then immobilized on the SAMs *via* a covalent cross-linking reaction. Apatite layers subsequently formed

on the surfaces of the modified alloys when they were submerged in SBF containing a sufficient amount of Ca^{2+} . The results of this study therefore represent a novel surface modification method that can be used to increase the bioactivity of bioinert materials.

REFERENCES

- 1) Carr BC, Goswami T. Knee implants-Review of models and biomechanics. *Mater Design* 2009; 30: 398-413.
- 2) Hsu RWW, Yang CC, Huang CA, Chen YS. Electrochemical corrosion studies on Co-Cr-Mo implant alloy in biological solutions. *Mater Chem Phys* 2005; 93: 531-538.
- 3) Sánchez-De Jesús F, Bolarín-Miró AM, Torres-Villaseñor CA, Cortés-Escobedo CA, Betancourt-Cantera JA. Mechanical alloying of biocompatible Co-28Cr-6Mo alloy. *J Mater Sci Mater Med* 2010; 21: 2021-2026.
- 4) Grgazka-Dahlke M, Dąbrowski JR, Dąbrowski B. Modification of mechanical properties of sintered implant materials on the base of Co-Cr-Mo alloy. *J Mater Process Tech* 2008; 204: 199-205.
- 5) Barnes CL, Deboer D, Corpe RS, Nambu S, Carroll M, Timmerman I. Wear performance of large-diameter differential-hardness hip bearings. *J Arthroplasty* 2008; 23: 56-60.
- 6) Katti KS. Biomaterials in total joint replacement. *Colloids Surf. B Biointerfaces* 2004; 39: 133-142.
- 7) Kokubo T, Kim HM, Kawashita M. Novel bioactive materials with different mechanical properties. *Biomaterials* 2003; 24: 2161-2175.
- 8) Cho SB, Nakanishi K, Kokubo T, Soga N, Ohtsuki C, Nakamura T, Kitsugi T, Yamamuro T. Dependence of apatite formation on silica gel on its structure: effect of heat treatment. *J Am Ceram Soc* 1995; 78: 1769-1774.
- 9) Kokubo T, Takadama H. How useful is SBF in predicting in vivo bone bioactivity? *Biomaterials* 2006; 27: 2907-2915.
- 10) Kim HM, Miyaji F, Kokubo T, Nakamura T. Preparation of bioactive Ti and its alloys via simple chemical surface treatment. *J Biomed Mater Res* 1996; 32: 409-417.
- 11) Yang B, Uchida M, Kim HM, Zhang X, Kokubo T. Preparation of bioactive titanium metal via anodic oxidation treatment. *Biomaterials* 2004; 25: 1003-1010.
- 12) Akahori T, Niinomi M, Nakai M, Fukuda H, Fukui H, Ogawa M. Bioactive ceramic surface modification of β -Type Ti-Nb-Ta-Zr system alloy by alkali solution treatment. *Mater Trans* 2007; 48: 293-300.
- 13) Kokubo T, Matsushita T, Takadama H, Kizuki T. Development of bioactive materials based on surface chemistry. *J Eur Ceram Soc* 2009; 29: 1267-1274.
- 14) Feng B, Chu X, Chen J, Wang J, Lu X, Weng J. Hydroxyapatite coating on titanium surface with titania nanotube layer and its bond strength to substrate. *J Porous Mater* 2010; 17: 453-458.
- 15) Miyazaki T, Kim HM, Miyaji F, Kokubo T, Nakamura T. Bioactive tantalum metal prepared by NaOH treatment. *J Biomed Mater Res* 2000; 50: 35-42.
- 16) Fujino S, Tokunaga H, Hata S, Saiz E, Tomsia AP. Graded glass coatings for Co-Cr implant alloys. *J Mater Sci* 2005; 40: 2499-2503.
- 17) Tanahashi M, Matsuda T. Surface functional group dependence on apatite formation on self-assembled monolayers in a simulated body fluid. *J Biomed Mater Res* 1997; 34: 305-315.
- 18) Zorn G, Gotman I, Gutmanas EY, Adadi R, Salitra G, Sukenik CN. Surface modification of Ti45Nb alloy with an alkylphosphonic acid self-assembled monolayer. *Chem Mater* 2005; 17: 4218-4226.
- 19) Raman A, Dubey M, Gouzman I, Gawalt ES. Formation of

- self-assembled monolayers of alkylphosphonic acid on the native oxide surface of SS316L. *Langmuir* 2006; 22: 6469-6472.
- 20) Devillers S, Hennart A, Delhalle J, Mekhalif Z. 1-Dodecanethiol self-assembled monolayers on cobalt. *Langmuir* 2011; 27: 14849-14860.
- 21) Bhure R, Mahapatro A, Bonner C, Abdel-Fattah TM. In vitro stability study of organophosphonic self assembled monolayers (SAMs) on cobalt chromium (Co-Cr) alloy. *Mater Sci Eng C* 2013; 33: 2050-2058.
- 22) Sugino A, Miyazaki T, Ohtsuki C. Apatite-forming ability of polyglutamic acid hydrogels in a body-simulating environment. *J Mater Sci Mater Med* 2008; 19: 2269-2274.
- 23) Miyazaki T, Kuramoto A, Hirakawa A, Shirosaki Y, Ohtsuki C. Biom mineralization on chemically synthesized collagen containing immobilized poly- γ -glutamic acid. *Dent Mater J* 2013; 32: 544-549.
- 24) Miyazaki T, Mukai J, Ishida E, Ohtsuki C. Apatite mineralization behavior on polyglutamic acid hydrogels in aqueous condition : effects of molecular weight. *Bio Med Mater Eng* 2013; 23: 339-347.
- 25) Tanaka Y, Saito H, Tsutsumi Y, Doi H, Imai H, Hanawa T. Active hydroxyl groups on surface oxide film of titanium, 316L stainless steel, and cobalt-chromium-molybdenum alloy and its effect on the immobilization of poly(Ethylene Glycol). *Mater Trans* 2008; 49: 805-811.
- 26) Bhure R, Abdel-Fattah TM, Bonner C, Hall F, Mahapatro A. Stability of phosphonic self assembled monolayers (SAMs) on cobalt chromium (Co-Cr) alloy under oxidative conditions. *Appl Surf Sci* 2011; 257: 5605-5612.
- 27) Yoshioka T, Tsuru K, Hayakawa S, Osaka A. Preparation of alginate acid layers on stainless-steel substrates for biomedical applications. *Biomaterials* 2003; 24: 2889-2994.