Technical Report

Bioactivity Treatment for Co-Cr-Mo Alloy by Precipitation of Low Crystalline Calcium Phosphate Using Simulated Body Fluid with Alkalinized Condition

Takeshi YABUTSUKA^{1*}, Hiroto MIZUTANI¹, Shigeomi TAKAI¹ and Takeshi YAO²

¹Dept. Fundamental Energy Science, Graduate School of Energy Science, Kyoto University, Yoshida-honmachi, Sakyo-ku, Kyoto 606-8501, Japan.

²National Institute of Technology, Kagawa College, Chokushi-cho, Takamatsu 761-8058, Japan.

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ABSTRACT

Roughened surface was formed on the surface of cobalt-chromium-molybdenum (Co-Cr-Mo) alloy substrate by sandblasting method using silicon carbide grinding particles with 3 μm in average diameter. In order to impart apatite formation ability to the Co-Cr-Mo alloy, the substrate was immersed in simulated body fluid (SBF) adjusted at higher pH in comparison with that of conventional SBF and subsequently the SBF was heated. When thus-treated substrate was immersed in physiological SBF, apatite formation was induced by the calcium phosphate film and the apatite covered the whole surface of the substrate within 1 day. The formed apatite layer adhered to the Co-Cr-Mo alloy substrate by mechanical anchoring effect.

KEY WORDS

cobalt-chromium-molybdenum alloy, calcium phosphate, simulated body fluid, apatite formation ability, mechanical anchoring effect

1 Introduction

Cobalt-chromium-molybdenum (Co-Cr-Mo) alloy is one of the most typical and attractive metallic biomaterials with excellent mechanical toughness and corrosion resistance. From these properties, Co-Cr-Mo alloy has been already used as biomaterials such as knee joint and dental devices. However, Co-Cr-Mo alloy is a bioinert material. Hence, this alloy cannot bond to living bone spontaneously. If Co-Cr-Mo alloy acquire spontaneous bone-bonding ability such as apatite formation ability, range of its clinical application is largely extended because of a possibility of cement-less treatment.

In recent years, there has been increasing interest in the preparation of calcium phosphate coatings on biomaterials by aqueous solution methods. From a viewpoint of hydroxyapatite coating on Co-Cr-Mo alloys, Wang et al. proposed preparation process of hydroxyapatite coating on Co-Cr-Mo implant alloys by employing an electrochemically-assisted deposition pretreatment followed by chemical immersion in a supersaturated calcification solution¹⁾.

When the pH and the temperature of a simulated body fluid (SBF) with inorganic ion concentrations nearly equal to those of human blood plasma^{2,3)} is raised, calcium phosphate (CaP) with low crystallinity is precipitated by increase of supersaturation respect to calcium phosphate. Recently, the authors found that the CaP behave as active precursors of apatite in SBF and apatite formation

was induced by the CaP in a short time^{4,5)}.

The CaP formation by utilizing the above process from a viewpoint of supersaturation of SBF respect to CaP is another efficient approach to forming bioactive calcium phosphate coatings on various kinds of bioinert materials such as metals, ceramics and polymers. In the previous study, we developed bioactive stainless steels (SUS316L) by applying the above-mentioned CaP formation process⁶⁾. Roughened surface was formed on SUS316L by sandblasting method using ceramics grinding particles with 3 μm for average particle size for an improvement of mechanical anchoring effect. Then the above-mentioned CaP were precipitated on the SUS316L and imparted high apatite formation ability to the SUS316L.

In the present study, applying the above-mentioned knowledge, we aimed to develop bioactive Co-Cr-Mo alloy. Roughened surface was formed on Co-Cr-Mo alloy substrate by the sandblasting method using ceramics grinding particles with 3 μm for average particle size for an improvement of mechanical anchoring effect. CaP was formed on the surface of Co-Cr-Mo alloy to imparted apatite formation ability. The apatite formation ability was tested by using SBF with physiological condition and the adhesive strength of the formed apatite layer was evaluated by a tensile test.

2 Materials and methods

2.1 Formation of roughened surface by sandblasting method

Co-Cr-Mo alloy disc (JIS T 6115, Dan Cobalt 400, Co: 61.9 wt%,

* Corresponding author, E-mail: yabutsuka@energy.kyoto-u.ac.jp

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Cr: 25.0 wt%, Mo: 7.3 wt%, W: 5.2 wt%, Others: 0.6 wt%, Nihon Shika Kinzoku Co., Ltd., Osaka, Japan) was used as a substrate. The surface of the substrate was treated by sandblasting method (PNEUMA-BLASTER[®] SFC-2, Fuji Manufacturing, Japan) at 0.85 MPa of discharge pressure using silicon carbide particles with $3.0 \pm 0.4 \mu\text{m}$ (JIS #4000) for average particle size. The substrates were washed with acetone, ethanol, then distilled water by ultrasonication. The surface of the substrate was analyzed by scanning electron microscopy (SEM; SU6600, Hitachi High-Technologies, Tokyo, Japan) and energy dispersive X-ray analysis (EDX; XFlash[®] 5010, Bruker, Germany).

2.2 Alkaline SBF treatment for impartation of apatite formation ability

SBF with ion composition shown in Table 1 was prepared by Kokubo's method²⁾. By dissolving tris(hydroxymethyl)aminomethane, the pH value of SBF was increased to 8.40 at 25.0°C. This solution is denoted as 'alkaline SBF' hereafter. The substrate was immersed in the alkaline SBF and pressed by cold isostatic press (CIP-SI, Kobe Steel, Ltd., Kobe, Japan) at 100 MPa for 60 min to penetrate the solution to the pores on the roughened surface. Then the alkaline SBF was held in an incubator held at 70.0°C for 24 hours while immersing the substrates. The substrate was washed with distilled water and air-dried. The surface of the substrate was analyzed by thin film X-ray diffraction (XRD; Rint 2500, Rigaku Corporation, Tokyo, Japan), SEM and EDX.

2.3 Evaluation of apatite formation ability

Apatite formation ability of thus-treated Co-Cr-Mo alloy was evaluated by immersing in SBF at pH 7.40, 36.5°C. The surface of the substrate was analyzed by XRD, SEM and EDX.

2.4 Evaluation of adhesive strength of apatite layer formed in physiological SBF

The adhesive strength between the Co-Cr-Mo alloy substrate and the apatite layer formed by immersion in SBF for 14 days by a modified ASTM C-633 method⁷⁾. Both surfaces of the substrate was fixed to stainless steel jigs by using Araldite[®] glue. Subsequently, a tensile load was applied at $1 \text{ mm} \cdot \text{min}^{-1}$ of cross-head speed until fracture occurred between the apatite layer and the substrate by using universal testing machine (AGS-H Autograph, Shimadzu Corporation, Kyoto, Japan).

3 Results and discussion

Fig. 1 shows the SEM photograph and the EDX spectrum of the surface of the untreated Co-Cr-Mo alloy substrate. Smooth surface was observed as shown in Fig. 1 (a). In the EDX analysis, peaks of cobalt, chromium, molybdenum and tungsten, constituents of the Co-Cr-Mo alloy, were observed as shown in Fig. 1 (b).

Fig. 2 shows the SEM photograph and the EDX spectrum of the surface of the Co-Cr-Mo alloy substrate after the sandblasting process. Roughened surface formed by the sandblasting was observed on the whole surface as shown in Fig. 2 (a). In the EDX

Table 1 Ion concentration of human blood plasma and simulated body fluid (SBF).

Ion	Concentration/mol·m ⁻³	
	Blood plasma	SBF
Na ⁺	142.0	142.0
K ⁺	5.0	5.0
Mg ²⁺	1.5	1.5
Ca ²⁺	2.5	2.5
Cl ⁻	103.0	147.8
HCO ₃ ⁻	27.0	4.2
HPO ₄ ²⁻	1.0	1.0
SO ₄ ²⁻	0.5	0.5

Fig. 1 (a) SEM photograph and (b) EDX spectrum of the surface of the untreated Co-Cr-Mo alloy substrate.

spectrum, a peak of silicon was observed as shown in Fig. 2 (b). This peak of silicon was attributed to grinding particles, consisted of silicon carbide, remained on the surface of the substrate even after the washing process using ultrasonication.

Fig. 3 shows the SEM photograph and the EDX spectrum of the surface of the Co-Cr-Mo alloy substrate after the alkaline SBF treatment. The surface morphology was changed and the shape of the surface became rounded off in comparison with Fig. 2 (a), after the sandblasting process, as shown in Fig. 3 (a). In the EDX spectrum, a peak of calcium and phosphorous was newly observed in comparison with Fig. 2 (b), after the sandblasting process, as shown in Fig. 3 (b). This result suggested that nucleation of calcium phosphate was progressed during the alkaline SBF treatment and the formed apatite nuclei induced formation of calcium phosphate. As a result, some kinds of CaP film was formed on the surface of the substrate.

Fig. 2 (a) SEM photograph and (b) EDX spectrum of the surface of the Co-Cr-Mo alloy substrate after the sandblasting process.

Fig. 3 (a) SEM photograph and (b) EDX spectrum of the surface of the Co-Cr-Mo alloy substrate treated with the sandblasting and the subsequent alkaline SBF.

These results indicate that raising pH of SBF promoted calcium phosphate formation. Ionic activity product (IP) of hydroxyapatite is given by the following formula where γ is the activity coefficient, and $[]$ is the concentration of each ion.

$$IP = (\gamma Ca^{2+})^{10} (\gamma PO_4^{3-})^6 (\gamma OH^-)^2 \times [Ca^{2+}]^{10} [PO_4^{3-}]^6 [OH^-]^2$$

In this study, the pH value of SBF is higher than that of conventional one. Hence, it is considered that IP was increased by raising $[OH^-]$

Fig. 4 XRD patterns of the surface of the Co-Cr-Mo alloy substrate treated with the sandblasting and the subsequent alkaline SBF after the immersion in SBF for 0 day, 1 day, 3 days and 7 days.

of SBF. In addition, it is considered that the formation reaction of calcium phosphate is accelerated by raising temperature because of an increase of collision frequencies of inorganic ions in SBF.

Fig. 4 shows the XRD patterns of the surface of thus-obtained calcium phosphate-coated substrate before and after the immersion in SBF for various periods. Before the immersion in SBF, diffraction peaks of hydroxyapatite was not observed. This result suggested that the CaP film formed by the alkaline SBF treatment possessed extremely low crystallinity. After the immersion in SBF for 1 day, diffraction peaks of hydroxyapatite were detected around $2\theta = 26^\circ$ and 31° . This result indicates that apatite formation was induced by the CaP film formed on the substrate in SBF. After the immersion in SBF for 3 days and 7 days, the intensity of diffraction peaks of hydroxyapatite increased in comparison with those of 1 day. This result indicates that apatite formation was proceeded in SBF and apatite grew on the surface of the substrate.

Fig. 5 shows the SEM photograph and the EDX spectrum of the surface of the CaP-formed Co-Cr-Mo alloy substrate after the immersion in SBF for 1 day. It was observed that the whole surface of the Co-Cr-Mo alloy substrate was covered with flake-like crystallites, which characterize apatite formed in SBF, as shown in Fig. 5 (a). In the EDX spectrum, intensities of peaks of calcium and phosphorous became larger in comparison with Fig. 3 (b), before the immersion in SBF, as shown in Fig. 5 (b). It is considered that apatite formation was induced by the CaP film and the formed apatite covered the whole surface of the Co-Cr-Mo alloy within 1 day in SBF. This result indicates that high apatite formation ability could be imparted to the surface of the Co-Cr-Mo alloy by conducting the alkaline SBF treatment as a surface modification. When the Co-Cr-Mo alloy substrate just treated with sandblasting was immersed in SBF

Fig. 5 (a) SEM photograph and (b) EDX spectrum of the surface of the Co-Cr-Mo alloy substrate treated with the sandblasting and the subsequent alkaline SBF after the immersion in SBF for 1 day.

for 14 days, on the other hand, no apatite formation was observed. This result means that the existence of the CaP film contributed to the apatite formation ability of the Co-Cr-Mo alloy.

The average adhesive strength of apatite layer formed by immersion in SBF for 14 days and standard deviation were 9.29 (3.94) MPa for 8 specimen. It is considered that the adhesion of apatite layer was obtained by mechanical anchoring effect between the apatite layer and the substrate with roughened surface.

The limitation of this study is about the crystallinity of the calcium phosphate film. Taking into consideration the result of SEM, EDX and XRD, it is speculated that the CaP obtained by raising pH of

SBF is amorphous calcium phosphate rather than crystalline calcium phosphate such as hydroxyapatite and octacalcium phosphate. In order to clarify this point, it is necessary to conduct another measurement such as transmission electron microscopy and electron diffraction. This point will be studied in a future study.

4 Summary

Roughened surface was formed on the surface of the Co-Cr-Mo alloy by the sandblasting process. Apatite formation ability was imparted to the Co-Cr-Mo alloy by the formation of the CaP film with low crystallinity by the alkaline SBF treatment. It was indicated that the CaP film induced apatite formation on the surface of the Co-Cr-Mo alloy within 1 day in SBF. The formed apatite layer adhered by mechanical anchoring respect to the Co-Cr-Mo alloy substrate with roughened surface. This method is effective to impart high apatite formation ability to the bioinert Co-Cr-Mo alloy, which has high corrosion resistance, high wear resistance and high mechanical toughness.

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