*Key Engineering Materials Vols. 284-286 (2005) pp. 473-476*
*online at http://www.scientific.net*


# Transformation of Electrodeposited Calcium Phosphate Coatings in Simulated Body Fluid and in Culture Medium

Ji-Ho Park[1,2,a], Yong-Keun Lee[1,2,b], Kwang-Mahn Kim[1,2,c], and Kyoung-Nam Kim[1,2,d,*]

[1] Department & Research Institute of Dental Biomaterials and Bioengineering, Yonsei University College of Dentistry, Shinchon-dong, Seodaemun-gu, Seoul 120-752, South Korea

[2] BK21 Project for Medical Science, Yonsei University, 134 Shinchon-dong, Seodaemun-gu, Seoul 120-752, South Korea

[a] parkjiho76@hotmail.com, [b] leeyk@yumc.yonsei.ac.kr, [c] kmkim@yumc.yonsei.ac.kr,

[d] kimkn@yumc.yonsei.ac.kr

* Corresponding author

**Keywords:** Bioactivity, Culture Medium, Electrodeposition, Hydroxyapatite (HA), MG63 cells, Simulated body fluid (SBF)

**Abstract.** The coatings formed on the $H_2O_2$-treated titanium substrate by electrodeposition were used in order to evaluate the difference of transformations in the simulated body fluid (SBF) and the culture medium with MG63 cells. A porous hydroxyapatite (HA) coating with relatively low crystallinity and large crystallites was formed on the $H_2O_2$-treated titanium substrate by electrodeposition. HA coating transformed for 5 days in the SBF consisted of densely-packed rod-shaped crystallites with various differentiated grains. Octacalcium phosphate (OCP) and HA coating transformed for 5 days in the culture medium consisted of both flake-shaped and rod-shaped crystallites with indistinct grains. MG-63 cells were well attached and proliferated during the transformation into this flaked-shaped OCP. This difference between transformations of the HA coatings in the acellular SBF and in the culture medium with MG63 cells is due to different ion composition in each solution and proteins in culture medium.

## Introduction

The bioactivity of a material is the ability to induce the direct, adherent, and strong bonding between the materials and the bone tissue [1]. Recently, formation of bonelike-apatite in simulated body fluid (SBF) and affinity to osteoblast-like cells *in vitro* have been measured in order to evaluate the bioactivity of materials including electrodeposited coating [2,3]. The apatite-forming ability and cell affinity of the coating have been known to be closely connected with transformation of the coatings in SBF and in culture medium, respectively. Especially, cell attachment on the coating was determined by surface roughness, surface composition, surface energy, and other surface properties [4].

The materials implanted in the human body come in contact with the fluid including protein and cell as well as various ions. These proteins and cells can affect the reactions at the biomaterials surface. However, there are few reports related to comparison between transformations of calcium phosphate coatings in the SBF and in the culture medium. We demonstrated that a $H_2O_2$ pretreatment was an effective surface pretreatment for producing more bioactive calcium phosphate coatings by electrodeposition [5]. Therefore, we used coatings electrodeposited on $H_2O_2$-pretreated titanium substrate for comparison between transformations of coatings in the SBF and in the culture medium. In this study, we investigated transformations of electrodeposited calcium phosphate coatings in the SBF and in the culture medium, and the MG63 cell attachment on the electrodeposited calcium phosphate coating in the culture medium.

## Materials and methods

Commercially pure titanium sheets (10 mm × 10 mm × 0.8 mm) were used as substrates for electrodeposition. Their surfaces were ground with #100 and #600 SiC paper. The edges of the titanium substrates were rounded to avoid an edge effect during electrodeposition. The $H_2O_2$-treated titanium substrate was prepared by immersing the titanium substrate in 10 ml of a 5*M* $H_2O_2$ solution at 60°C for 24 hours.

The electrodeposition of calcium phosphates was performed at 60°C for 1 hour in a conventional cell fitted with a saturated calomel electrode (SCE) maintaining a cathodic potential of –2 V (vs. SCE). A modified SBF was used as the electrolyte for electrodeposition. The modified SBF was prepared by dissolving reagent-grade NaCl, $NaHCO_3$, $K_2HPO_4{\cdot}3H_2O$, and $CaCl_2$ into double-distilled water, which was buffered at pH 7.4 at 60°C with tris-hydroxymethylaminomethane [$(CH_2OH)_3CNH_2$] and 1*M* hydrochloric acid (HCl). The $H_2O_2$-treated titanium substrate was used as cathodes for electrodeposition. A potentiostat/galvanostat (EG&G Instruments, princeton, NJ, USA) operating in potentiostatic mode was used to maintain the cathodic potential.

In order to evaluate the bioactivity, the coatings formed on the $H_2O_2$-treated titanium substrates by electrodeposition were immersed in 10 ml of acellular SBF and culture medium with MG63 cells, derived from a human osteosarcoma, under humidified air atmosphere of a 5% $CO_2$ at 36.5°C for 5 days, respectively. The acellular SBF with ion concentrations similar to that of human blood plasma was prepared by dissolving reagent grade NaCl, $NaHCO_3$, KCl, $K_2HPO_4{\cdot}3H_2O$, $MgCl_2{\bullet}6H_2O$, $CaCl_2$, and $Na_2SO_4$ in double-distilled water and buffering at pH 7.4 at 36.5°C with tris-hydroxymethylaminomethane [$(CH_2OH)_3CNH_2$] and 1M hydrochloric acid (HCl). The culture medium with MG63 cells was prepared by dissolving alpha-minimum essential medium (α-MEM; Gibco, Grand Island, NY, USA), 10% fetal bovine serum (FBS; Gibco, Grand Island, NY, USA), and 1% Penicillin-Streptomycin (PS; Gibco, Grand Island, NY, USA) in double-distilled water and adding MG-63 cells with a concentration of approximately $1{\times}10^5$ cells/ml. The SBF and the culture medium were renewed every day during the immersion test.

The crystallinity and structure of the coatings were examined by X-ray diffraction (XRD; Rigaku, Tokyo, Japan). The morphology of the coatings and the cell attachment after immersion in culture medium were observed using scanning electron microscopy (SEM; Hitachi, Tokyo, Japan). The chemical composition of the coatings was analyzed by energy dispersive spectroscopy (EDS; Kevex Instruments, Waltham, MA, USA).

## Results

Fig. 1 shows XRD patterns of the coating formed on the $H_2O_2$-treated titanium substrate after electrodeposition, the coating transformed on the $H_2O_2$-treated titanium substrate after electrodeposition and subsequent immersion in the SBF for 5 days, and the coating transformed on the H2O2-treated titanium substrate after electrodeposition and subsequent immersion in the culture medium for 5 days. The main structure of the coating formed on the $H_2O_2$-treated titanium substrate by electrodeposition was mainly HA with a low crystallinity and density. After immersion in the SBF for 5 days, the structure of the coating transformed poorly crystalline HA into highly crystalline HA. After immersion in the culture medium with MG63 cells for 5 days, the structure of the coating transformed poorly crystalline HA into highly crystalline octacalcium phosphate (OCP) and a little HA.

Fig. 2 shows SEM images showing the morphologies of the coating formed on the $H_2O_2$-treated titanium substrate after electrodeposition, the coating transformed on the $H_2O_2$-treated titanium substrate after electrodeposition and subsequent immersion in the SBF for 5 days, and the coating transformed on the $H_2O_2$-treated titanium substrate after electrodeposition and subsequent immersion in the culture medium for 5 days. The coating formed on the $H_2O_2$-treated titanium substrate after electrodeposition consisted of irregular crystallites with various pores. The coating transformed on

the $H_2O_2$-treated titanium substrate after electrodeposition and subsequent immersion in the SBF for 5 days was consisted of uniform and small rod-shaped crystallites with clearly distinguished grains. The coating transformed on the $H_2O_2$-treated titanium substrate after electrodeposition and subsequent immersion in the culture medium for 5 days consisted of both flake-shaped and rod-shaped crystallites with unclear grains.

The Ca/P atomic ratio of the coatings was measured to analyze the chemical composition of the coating transformed on the $H_2O_2$-treated titanium substrate after electrodeposition and subsequent immersion in the SBF for 5 days and the coating transformed on the $H_2O_2$-treated titanium substrate after electrodeposition and subsequent immersion in the culture medium for 5 days using EDS. The Ca/P atomic ratio (1.51) of HA coating transformed in the culture medium was lower than that (1.62) of HA coating transformed in the SBF.

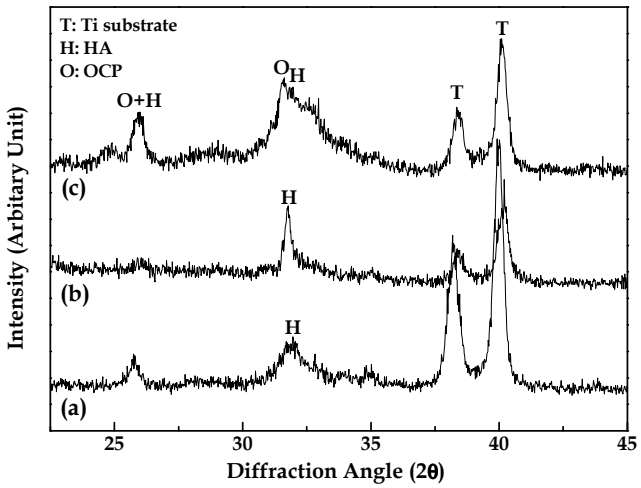


Fig. 1. XRD patterns of (a) the coating formed on the $H_2O_2$-treated titanium substrate after electrodeposition, (b) the coating transformed on the $H_2O_2$-treated titanium substrate after electrodeposition and subsequent immersion in the SBF for 5 days, and (c) the coating transformed on the $H_2O_2$-treated titanium substrate after electrodeposition and subsequent immersion in the culture medium for 5 days.

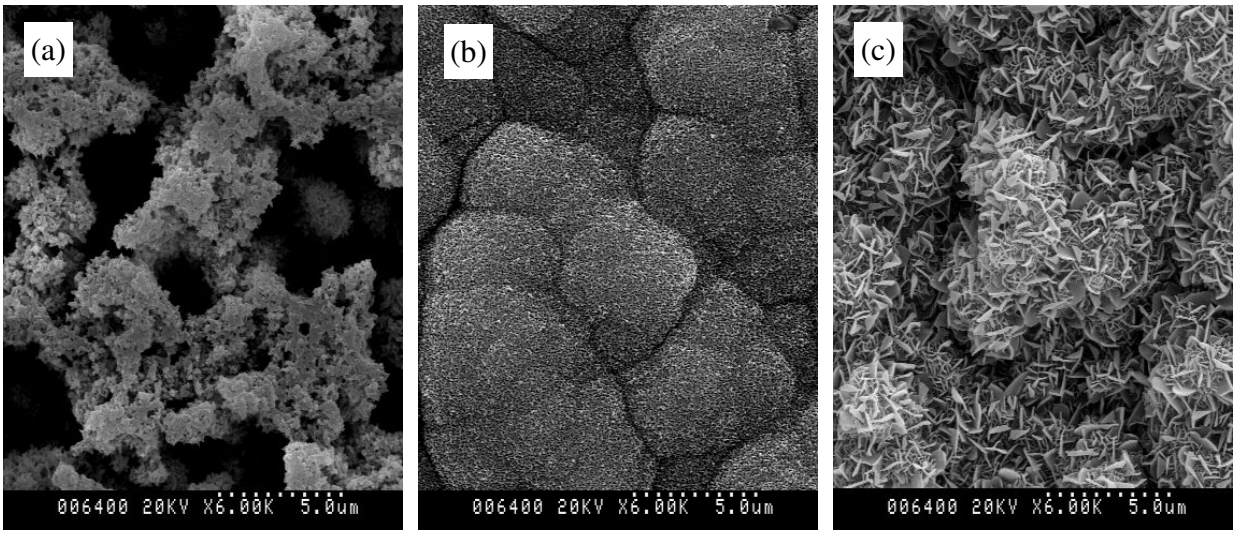


Fig. 2. SEM images showing the morphologies of (a) the coating formed on the $H_2O_2$-treated titanium substrate after electrodeposition, (b) the coating transformed on the $H_2O_2$-treated titanium substrate after electrodeposition and subsequent immersion in the SBF for 5 days, and (c) the coating transformed on the $H_2O_2$-treated titanium substrate after electrodeposition and subsequent immersion in the culture medium for 5 days.

Fig. 3 shows SEM morphologies of MG-63 cells after 1 day culturing and after 5 days culturing on the coating formed on the $H_2O_2$-treated titanium substrate. After 1 day culturing, the cells were well attached on the surface of the electrodeposited coating with extending cytoplasmic process. After 5 days culturing, the cells were spread more flatly with the increase of the number of the cells.

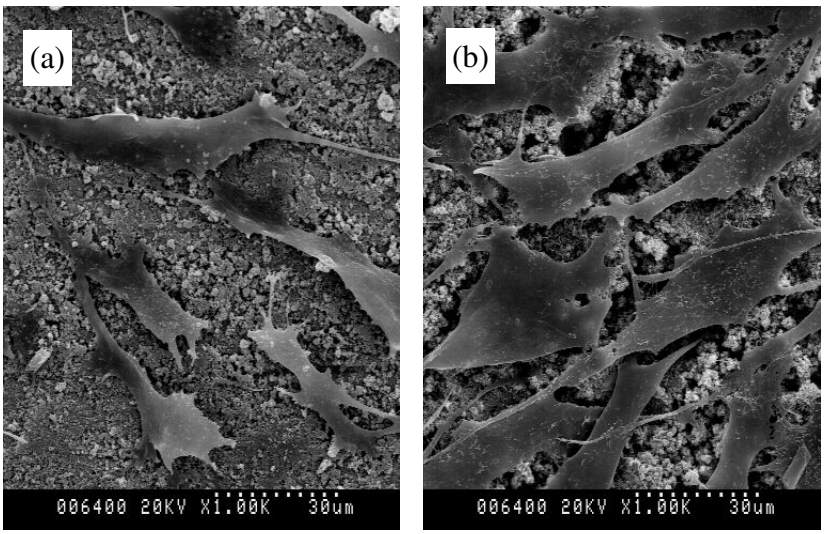


Fig. 3. SEM images showing the morphologies of MG-63 cells (a) after 1 day culturing and (b) after 5 days culturing on the coating formed on the $H_2O_2$-treated titanium substrate.

## Discussion and conclusion

The more crystalline OCP, prephase of HA, was formed on the electrodeposited coating 5 days after immersion in the culture medium. It indicates that the coating in the culture medium transformed into bonelike HA more slowly than that in the SBF preventing proteins in the culture medium from transforming poorly crystalline HA into bonelike HA. However, MG-63 cells were well attached and proliferated during the transformation into this flaked-shaped OCP. It also means that the OCP generated during the transformation into bonelike HA shows proper biocompatibility. The difference between transformations of the HA coatings in the acellular SBF and in the culture medium with MG63 cells is due to different ion composition in each solution and the presence of proteins in the culture medium. Furthermore, immersion test in culture medium with cells is more recommended in order to evaluate the bioactivity due to the presence of protein and cells similar to the fluid in the human body.

## Acknowledgements

This work was supported by grant No. R13-2003-13 from the Medical Science and Engineering Research Program of the Korea Science and Engineering Foundation.

## Literature References

[1] L.L. Hench: J. Am. Ceram. Soc. Vol 74 (1994), p. 1487.
[2] M. Kumar *et al.*: J. Biomed. Mater. Res. Vol 45 (1999) p. 302.
[3] J. Wang *et al.*: BiomaterialsVol 25 (2004), p. 583.
[4] B. Kasemo: J. Prosthet. Dent. Vol 49 (1983) p. 832.
[5] J.H. Park *et al.*: Surf. Coat. Technol. (submitted).