

Hydroxyapatite/Titania Hybrid Coatings on Titanium by Sol-Gel Process

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(Received October 16, 2006/Accepted November 15, 2006)

Sol-gel thin films of hydroxyapatite and titania have received a great deal of attention in the area of bioactive surface modification of titanium implants. Sol-gel process offers lots of advantages over other coating techniques, e.g. increased homogeneity due to atomic level mixing; finer grain microstructure and lower temperature of the crystallization. In this study, we fabricated hydroxyapatite/titania hybrid coatings on titanium by sol-gel method to combine advantages of both materials: the adhesion strength of the titanium dioxide on the substrate and the bioactivity of the hydroxyapatite. Sol-gel coatings of pure hydroxyapatite and titania, and four hydroxyapatite composites with 10~70 mol% titania were developed on titanium substrates. The characteristics of coatings, such as crystallinity, roughness and composition of surface, were observed. The composite coatings showed the characteristic peaks of pure hydroxyapatite and titania with anatase and rutile structures. When the titania amounts adding into the hydroxyapatite sol increased, the surface of the composite coatings became slightly rougher. The critical load strength between coating and substrate slightly increased to 3.151, 4.168, and 5.389 N when the amount of titania added into hydroxyapatite sol increased to 30, 50, and 70 mol%, respectively. For bioactivity test, calcium phosphate deposits were observed on the film surfaces after the soaking in SBF for 1 week, except of titania coated-substrate. The in vitro cellular responses to the coatings were assessed in terms of cell attachment and proliferation. Hydroxyapatite composite coating with 70 mol% titania had the most excellent attachment of MG63 cells as cells tend to attach more readily to surfaces with a rougher microtopography. Statistically analysis revealed that there were no significant differences between the proliferation of osteoblastic cells on the various materials ($p > 0.05$).

Key words: Hydroxyapatite, Titania, Sol-gel method, Hybrid coating, Bonding strength, Bioactivity, Cellular response

INTRODUCTION

Titanium (Ti) and Ti alloys are proven to be potentially very suitable materials for load bearing in bioimplant applications due to their good and reliable mechanical properties. Unfortunately, like most metals, Ti exhibits poor bioactive properties. Therefore, the metallic implants coated with bioactive materials are of interest in the biomedical application. Among the bioactive coating materials, hydroxyapatite(HA) coatings on Ti have shown good fixation to the host bone.^{1,2)} The improved biocompatibility driven by the HA coatings was attributed to the chemical and biological similarity of HA to host tissues, as well as its osteoconductivity.³⁾ Various techniques, such as plasma spray, ion beam assisted deposition, radiofrequency magnetron sputtering, have been used to produce coatings on implants. Some drawbacks have been noticed regarding the long-term performance of the obtained coatings: coating resorption, poor mechanical properties, high thickness, non-homogeneity, lack of adherence.⁴⁾ Sol-gel processing represents an alternative

approach for the coating preparation with potential advantages, such as higher purity and homogeneity, lower processing temperatures, reduced thickness, simple and cheap method of preparation. Moreover, materials prepared by sol-gel process have shown to be more bioactive than those with the same composition but prepared with different methods. Recently, HA and titania (TiO_2) composite coatings have been studied to improve the low strength of pure HA coatings since TiO_2 has high chemical affinity between HA and Ti. The objective of this study is to fabricate HA/ TiO_2 hybrid coatings on Ti substrate by sol-gel method to combine advantages of both materials: the adhesion strength of the titanium dioxide on the substrate and the bioactivity of the HA. Various mol% amounts of TiO_2 sols were added to HA sols to make hybrid sols. Ti substrates were coated with the composite sols by spin-coater. The phase identification, morphology, roughness, and chemical composition of sol-gel coatings on Ti were determined, and the bonding strength between coatings and substrates were tested by scratch test. Biological performances such as cell attachment and proliferation were evaluated using MG63 osteoblast-like cell. Therefore, the purpose is to optimize the composition of coatings

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with not only excellent bonding strength but also excellent bioactive and biological properties.

MATERIALS AND METHODS

Preparation of HA, TiO₂ and HA/TiO₂ Sols

Calcium nitrate tetrahydrate [Ca(NO₃)₂·4H₂O] and triethyl phosphite [TEP; P(OC₂H₅)₃] as the Ca and P source were used to obtain a pure HA phase in an ethanol-water mixed solution. In order to make Ca solution, calcium nitrate tetrahydrate (Sigma-Aldrich, U.S.A.) of 2 M was dissolved in ethanol. A stoichiometric amount (Ca/P = 1.67) of triethyl phosphite (Sigma-Aldrich, USA) was hydrolyzed in ethanol-water mixed solution. After mixing the Ca and P solutions, 3 wt% NH₄OH was added to the mixed solutions, and the solutions were stirred for an additional 30 min. Finally, the HA sol was obtained by aging for 72 h. To produce a TiO₂ sol, titanium propoxide (Sigma-Aldrich, U.S.A.) was hydrolyzed within an ethanol-based solution, containing diethanolamine [(HOCH₂CH₂)₂NH, Aldrich, USA] and distilled water. The TiO₂ sol was obtained by additional aging for 72 h. The prepared TiO₂ sol was added to the prepared HA sol with various ratios. HT10, HT30, HT50, and HT70 were regarded as the TiO₂ amount of 10, 30, 50, and 70 mol% added to HA sol as shown in Table 1. For the purpose of comparison, pure HA (PH) and TiO₂ (PT) sols were prepared without mixing.

Sol-gel Coatings on Titanium Substrate

Commercially pure Ti (cp Ti, grade III) disc was purchased from Dynamet, USA. Pure Ti disks were cut into 10×10 mm size as coating substrates, and were prepared after polishing with silicon carbide paper (#1500 grit), and then cleaning in acetone and ethanol. The prepared composite sols were dropped onto the Ti substrate and then spin-coated at 2000 rpm for 10 s. After drying for 24 h, samples were heat treated at 500°C for 2 h in air at a heating and cooling rate of 1°C/min.

Characterization of Coatings on Ti

The phase of the HA, TiO₂ and composite layers on Ti

was investigated using X-ray diffractometer (XRD; X'pert PW1830, Philips, Japan) with Ni-filtered Cu- α ray. The crystalline structures were identified according to JCPDS software (PCPDFWIN1.30, JCPDS-ICDD). The cross section of coatings was observed using scanning electron microscopy (SEM; Hitachi, Japan). Roughness of the coating surface was measured at a speed of 0.1 mm/s using a surface profiler (Surfcorder SEF-30D, Kosaka Laboratory Ltd., Japan). Energy dispersive spectroscopy (EDS; Inka, Oxford, UK) was used to determine the Ca/P ratio of coating surface on Ti.

Scratch Test

The adhesion of the films obtained was assessed using a scratch tester (CSEM-REVETEST, Swiss) with a spherical Rockwell C diamond stylus of 200 μ m-radius. The scratches were generated on the coatings by constantly increasing the load at the rate of 50 N/min from initial load 2 N while the specimen was displaced at the constant speed of 5 mm/min. The point of adhesion failure of the coating from the substrate was detected by a burst increase in friction force from the sample. The load at which total peeling-off of the coating from the substrate occurs is referred to as the "critical load". The scratch track was observed using SEM with the EDS mapping of Ca.

Bioactivity Test

The bioactivity tests were performed using a simulated body fluid (SBF), which was buffered at physiologic pH 7.40 at 37°C with the same ionic concentration approximately as that of human blood plasma.⁵⁾ The ratio of the coating surface area (microscopic; SA) to SBF solution volume (V) was fixed to 0.1 cm⁻¹.⁶⁾ After immersion in SBF for 1 week at 37°C, the specimens were rinsed in distilled water, dried in a vacuum desiccator. SEM was used to observe the morphology of the precipitates after gold sputtering.

In Vitro Cell Test

All specimens for the cell tests were prepared after sterilization at 121°C for 20 min. For initial cell attachment tests, the

Table 1. Chemical composition, surface roughness and critical load of the coating layers on Ti.

Samples		Amount of TiO ₂ added to HA sol (mol %)	Roughness, Ra (μ m)	Critical Load (N)
Control Group	PH	Pure hydroxyapatite	0.831±0.014 ^a	< 2 ^A
	PT	Pure titania	0.923±0.043 ^e	9.636±2.347 ^D
Experimental Group	HT10	10	0.868±0.027 ^{ab}	< 2 ^A
	HT30	30	0.895±0.021 ^{bcd}	3.151±0.931 ^B
	HT50	50	0.931±0.032 ^{cde}	4.168±1.244 ^{BC}
	HT70	70	0.969±0.019 ^{de}	5.389±1.465 ^C

Ra: average height above center line.

Significant differences ($p < 0.05$) between a, b, c, d, and e, and between A, B, C and D.

cells were plated at a density of 5×10^4 cells/mL in 0.1 mL medium on all the specimens (10×10 mm) in individual wells of a 24-well plate and cultured for 6 h to allow the cells to attach. At the period, the cells were washed with PBS solution to eliminate the non-adherent cells.⁷⁾ MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] reagent was added onto the specimens. The mitochondria dehydrogenated from the living cells would reduce the MTT reagent into water-insoluble blue crystals.⁸⁾ After removal of the media, dimethylsulfoxide (DMSO) was added onto the specimens to dissolve the blue crystals. The optical density (OD) of the dissolved solute was then measured by an ELISA reader under a light source of 570 nm wavelength. For cell proliferation tests, the cells seeded at a density of 5×10^4 cells/mL were allowed to attach for 6 h, and then the samples were placed into new plates and cultured for up to 7 days in 1.5 mL medium in an incubator of 37°C. At each culture period (2, 4, and 7 days), MTT was added to each well and incubated at 37°C for 4 h. The blue formazan product was dissolved by DMSO, and the absorbance was measured at 570 nm using an ELISA reader. The relate value to initial density was calculated.

Statistical Analysis

The statistical significant difference of the results between the experimental and control groups were analyzed using one-way Anova and Tukey statistical test at a level of 0.05.

RESULTS

The XRD patterns (Figure 1(a), (f)) of the HA and TiO₂ coat-

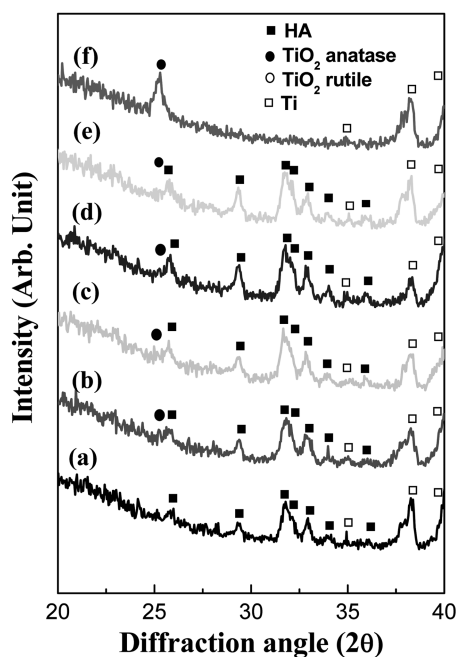


Figure 1. XRD patterns of the coatings; (a) PH, (b) HT10, (c) HT30, (d) HT50, (e) HT70, and (f) PT coatings.

ing layers had the characteristic peaks of the pure HA and TiO₂ phase with anatase, respectively. The characteristic HA and TiO₂ peaks were well developed after heat treatment. In Figure 1(b)-(e), the composite coatings had the representative peaks of the HA and TiO₂ anatase phase. As TiO₂ increased, TiO₂ anatase peak of composite coatings was increased, and there were no secondary phases detected. The roughness of coatings was shown in Table 1. The values of the coating roughness were about 0.9 μm . When the TiO₂ amounts adding into the HA sol increased, the morphology of the composite coatings became slightly rougher. Figure 2 shows the EDS spectra of the coating layer where HA sol was contained. In Figure 2, The Ca/P ratio of the PH, HT10, HT30, HT50, and HT70 coatings was 1.73, 1.67, 1.58, 1.54, and 1.58, respectively. In Figure 3, cross-section images of the coatings on Ti substrate showed homogeneous structures. The coating layer appeared to consist of very small grains throughout the coating layers. However, the HA and TiO₂ phases could not be discerned.

The diagram of friction versus load for the scratch test performed on the specimens is shown in Figure 4. In Figure 4(a) and (b), the diagram for PH and HT10 coatings represented higher slope and rougher striation than that of others from the initial scratch load of 2 N. Obviously, the HT10 coated film was scratched off the substrate and the wear debris were found on both sides of the scratch line at the initial stage in Figure 5(a). The EDS mappings of Ca also indicate that most of HT10 coating was removed from the specimen at the beginning of scratch, as shown in Figure 5(b). As can be seen from the diagram, TiO₂ coatings had the highest adhesion strength, and the adhesion strength between coating and sub-

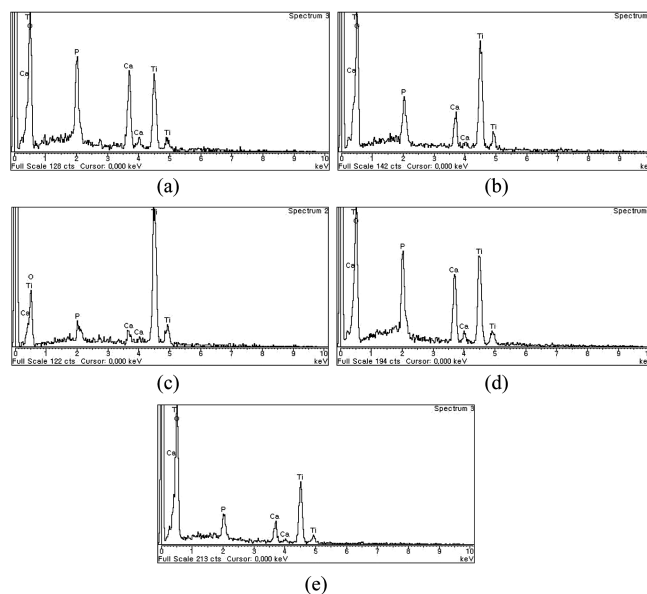


Figure 2. EDS spectra of the coating on Ti substrate; (a) PH, (b) HT10, (c) HT30, (d) HT50, and (e) HT70 coatings.

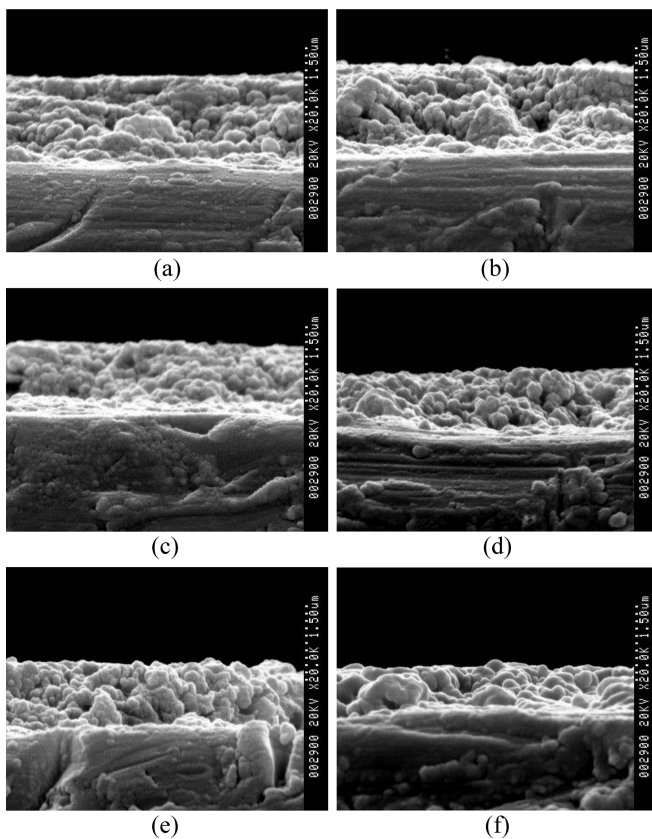


Figure 3. SEM morphologies of the cross section of the coating layers on Ti substrate; (a) PH, (b) HT10, (c) HT30, (d) HT50, (e) HT70, and (f) PT coating layers.

strate slightly increased to 3.151, 4.168, and 5.389 N when the amount of TiO_2 added into HA sol increased to 30, 50, and 70 mol%, respectively (Table 1). Consistently, SEM observations of the scratch line also exhibit two stages in the Figure 5(c). The first, HT70-coated film bore the wear stress and little wear debris was found (2-5 N). Secondly, HT70 coatings was crushed in advance and greatly worn away. The EDS mappings of Ca also indicate that little coating was worn away at the first stage, but much more at the second stage, as shown in Figure 5(d).

Figure 6 shows SEM images of the coating surfaces on the Ti substrate after immersion in the SBF for 7 days. Calcium phosphate deposits were observed on the film surfaces. The in vitro cellular responses to the sol-gel-derived coatings on Ti substrate were assessed in terms of cell attachment and proliferation. The initial cell attachment on the films is quantified in Figure 7 after culturing for 6 h. With respect to the cell attachment on the coatings for 6 h, cell numbers attached on all the composite coatings except HT70 have no statistical difference, compared to that on PH coatings ($p > 0.05$). The proliferation levels on the films with culturing for up to 7 days is shown in Figure 8, as assessed by an MTT method. The cells on all samples proliferated actively with culture period. Statistically anal-

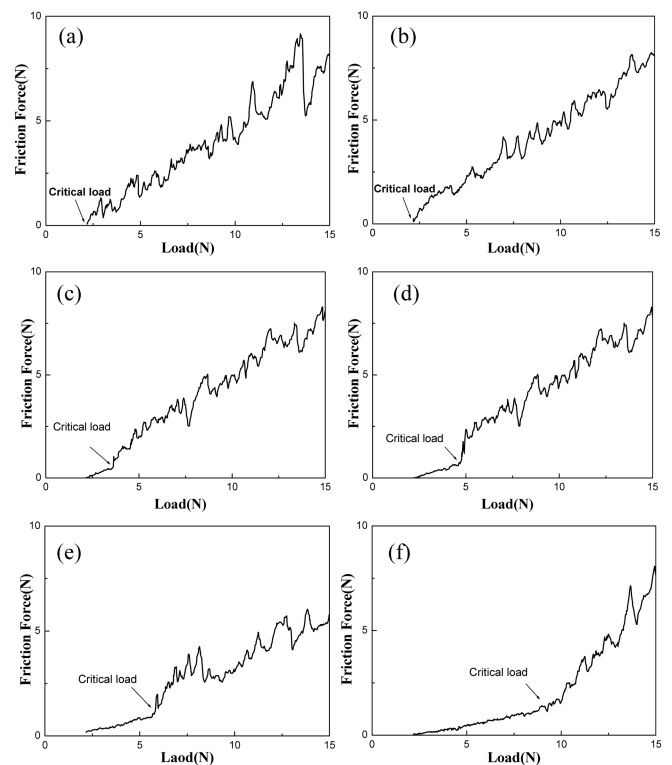


Figure 4. Diagrams of friction force versus load for the scratch tests on the coatings; (a) PH, (b) HT10, (c) HT30, (d) HT50, (e) HT70, and (f) PT coatings.

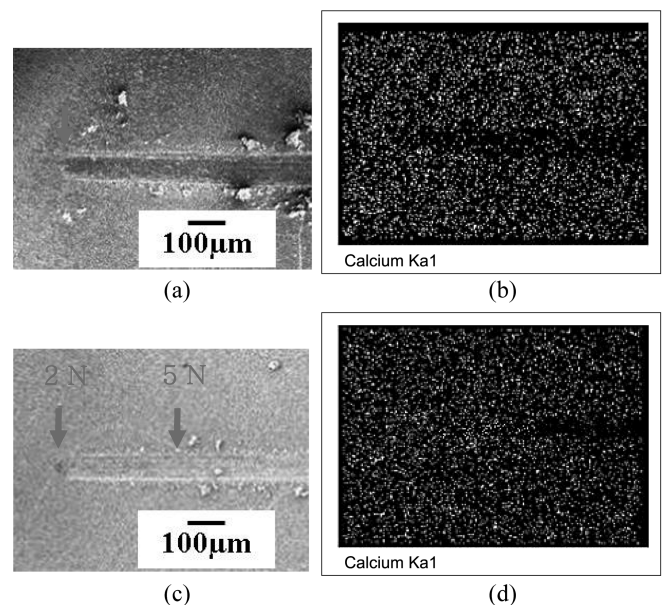


Figure 5. Scratch track of (a) HT10 and (c) HT70, and EDS Ca-mapping of HT10 and (d) HT70.

ysis revealed that there were no significant differences between the proliferation of osteoblast-like cells on the various materials ($p > 0.05$).

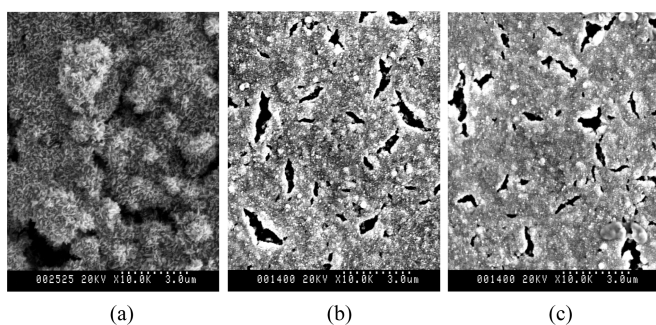


Figure 6. SEM morphologies of the coating surface after immersion of specimens in SBF for 7 days; (a) PH, (b) HT30, and (c) HT70 coatings.

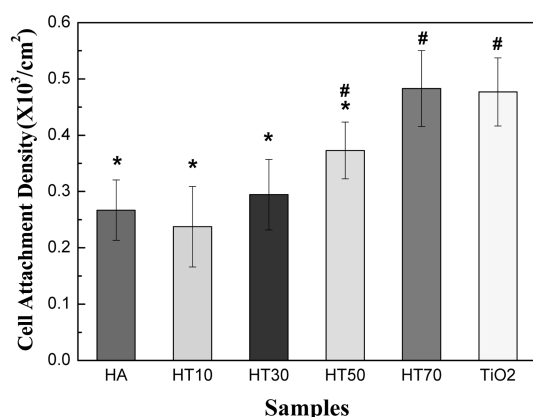


Figure 7. MG63 cell attachment density on the coatings on Ti with culturing for 6 h. Significant differences ($p < 0.05$) between * and #.

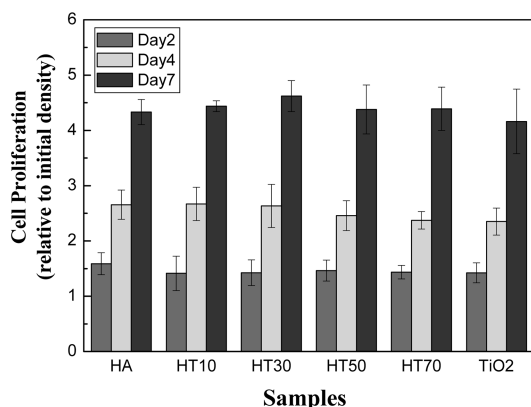


Figure 8. Proliferation of the MG63 cell on coatings Ti after culture for 2, 4, and 7 days.

DISCUSSION

The XRD patterns of the PH and PT coating layers had the characteristic peaks of the pure HA and TiO₂ phase with anatase, respectively. In the Figure 1(b)-(e), the composite coatings had the representative peaks of the HA and TiO₂ anatase phase. No other peaks were produced in the composite coatings suggesting a high chemical and thermal stability of both

HA and TiO₂ phases.⁹⁾ When the TiO₂ amounts adding into the HA sol increased, the morphology of the composite coatings became rougher. This was believed to be attributable to the high content of an organic additive, mainly the diethanolamine, which was added to preserve the stability of TiO₂ sol during hydrolysis. Thus, evaporating of organic compounds contained in TiO₂ sols causes the surface to be rougher. In the same reason, roughness of coating surface increased with the increase in TiO₂ contents. The roughness of HA/TiO₂ coatings with more than 30 mol % TiO₂ sols have significant difference ($p < 0.05$) compared to PH coatings. In Figure 3, cross-section images of the coatings on Ti substrate clearly depict the formation of a highly dense and homogeneous structure. Moreover, there was no delamination at the interface, suggesting a tight bond between the film and the substrate. In addition, the thickness of all coatings was less than 1.5 μm . The thickness of the coatings affects both its resorption and mechanical properties.¹⁰⁾ A thicker coating usually exhibits poorer mechanical properties. A thinner coating (<50 μm) exhibits significantly higher shear strength than a thick coating, and results to avoid fatigue failure while still providing reasonable coating bioresorption and consistent bone growth.¹⁰⁾

The adhesion strength between coating and substrate slightly increased with increase of TiO₂ contents. Among the composite coatings, the critical load of HT70 coatings was higher than that of HT10 and HT30 ($p < 0.05$). The addition of the TiO₂ into HA had improved the bonding strength of HA coating on Ti substrate. Kim et al. reported that the strength values of all the composite coatings lay between those of HA (37 MPa) and TiO₂ (70 MPa), and with increasing TiO₂ content, the strength increased.¹¹⁾ The highest strength was approximately 56 MPa with 30 mol % TiO₂ addition, and this value was an improvement of approximately 50% with respect to pure HA coatings. Liu et al. reported that the bonding strength of wollastonite/TiO₂ composite coatings increased with the increase in TiO₂ contents.¹²⁾ Such an improvement was to be expected, given the dense and uniform coating structure, as well as the tight bonding of the TiO₂ to both the HA and the Ti substrate. The favorable chemical affinity of TiO₂ with respect to HA as well as to Ti, i.e. its tight bonding to both HA and Ti, greatly contributed to the observed improvement in bonding strength.

The formation of the apatite layer can be simulated in an in vitro environment by using a simulated body fluid (SBF). Figure 6 shows SEM images of the coating surfaces on the Ti substrate after immersion in the SBF for 7 days. Calcium phosphate deposits were observed on the film surfaces of samples after the soaking for 1 week. The process and kinetics of apatite formation on HA could be affected by bulk factors such as density and surface area as well as by surface factors such as composition and structure.¹³⁾

The effect of surface properties of biomaterials on the cellular responses has been studied extensively.¹⁴⁾ Based on these

studies, the physical properties (surface roughness and morphology) and chemical status (crystallinity and solubility) affected cellular responses *in vitro*, such as cell attachment, proliferation, and differentiation as well as *in vivo*. Moreover, the topography and morphological feature affected cell attachment and proliferation. In particular, osteoblast-like cells exhibit roughness-dependent phenotypic characteristics. They tend to attach more readily to surfaces with a rougher microtopography. The initial cell attachment on the films is quantified in Figure 7 after culturing for 6 h. With respect to the cell attachment on the coatings for 6 h, cell numbers attached on all the composite coatings except HT70 have no statistical difference, compared to that on HA coatings ($p > 0.05$). Therefore, in this study, HT70 coatings which had slightly rougher surface than other composite surfaces had the most excellent attachment of MG63 cells.

Spreading is an important step for essential biological properties of the cell such as proliferation. Cell proliferation is reported to be effected various factors such as roughness, pH, crystallinity and chemical environment.¹⁴⁾ It has been reported that the rough surfaces of Ti/Ti-alloy induced enhanced initial cell attachment, whereas the proliferation and differentiation of the cells on them were affected differently depending on the cell types and roughness level. Linkcks et al. have shown that MG63 cells on the smooth surface had high proliferation rates but ALPase and osteocalcin production were low.¹⁵⁾ Ramirez et al. reported that there were no significant differences between the proliferation of osteoblastic cells on the materials which had various chemical compositions of HA and TiO₂.¹⁶⁾ In addition, the higher cell attachment on the rough film was attributable to the higher degree of binding sites for serum proteins.¹⁴⁾ Because the osteoblast cells are anchorage-dependent, the adhesion proteins play a crucial role in cell attachment. Practically, some functional proteins are known to bind to HA crystal quite selectively. Regarding the protein effect and adhesion mechanism, further study remains by investigating the binding of specific adhesion molecules on the HA bulk ceramics. The proliferation levels on the films with culturing for up to 7 days is shown in Figure 8, as assessed by an MTT method. The cells on all samples proliferated actively with culture period. Statistically analysis revealed that there were no significant differences between the proliferation of osteoblastic cells on the various samples ($p > 0.05$). Despite the enhanced cell attachment on the rough film surface, the subsequent cell behaviors were not mirrored in the proliferation. The continuously dissolving film, resulting chemical environment and morphological difference with culture period would complicate the cellular responses at later stage.

CONCLUSION

In this study, we fabricated HA/TiO₂ hybrid coatings on Ti

by sol-gel method to combine advantages of both materials: the adhesion strength of the titanium dioxide on the substrate and the bioactivity of the HA. The composite coatings on Ti showed the characteristic peaks of pure HA and TiO₂ with anatase and rutile structures, without secondary phases. When the TiO₂ amounts adding into the HA sol increased, the surface of the composite coatings became slightly rougher. The Ca/P ratio of the composite coatings was similar to that of HA, 1.67. It means that the coatings consisted of amorphous phases of the HA. The cross-section images of the coatings on Ti substrate clearly depict the formation of a highly dense and homogeneous structure. The critical load between coatings and substrates slightly increased when the amount of TiO₂ added into HA sol increased to 30, 50, and 70 mol%, respectively. The favorable chemical affinity of TiO₂ with respect to HA as well as to Ti, i.e. its tight bonding to both HA and Ti, greatly contributed to the observed improvement in bonding strength. After the soaking for 1 week, calcium phosphate deposits were observed on the film surfaces. The *in vitro* cellular responses to the sol-gel-derived coatings on Ti substrate were assessed in terms of cell attachment and proliferation. HT70 coatings had the most excellent attachment of MG63 cells as cells. The cells on all samples proliferated actively with culture period. Statistically analysis revealed that there were no significant differences between the proliferation of osteoblastic cells on the various materials ($p > 0.05$).

From above results, we convinced that TiO₂ enhanced the bond strength between HA coatings and Ti substrates. Especially, HT70 composite coating had the excellent strength as well as bioactivity. In addition, the coating had the most excellent attachment of MG63 cells due to its rough surface.

ACKNOWLEDGEMENT

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

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