Hydroxyapatite and Titania Sol–Gel Composite Coatings on Titanium for Hard Tissue Implants; Mechanical and in Vitro Biological Performance

Hae-Won Kim,1,2 Hyoun-Ee Kim,1 Vehid Salih,2 Jonathan C. Knowles2

1 School of Materials Science and Engineering, Seoul National University, Seoul, 151-742, Korea
2 Biomaterials and Tissue Engineering, Eastman Dental Institute, University College London, 256 Gray’s Inn Road, London WC1X 8LD, UK

Received 18 November 2003; revised 23 February 2004; accepted 25 February 2004
Published online 12 October 2004 in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/jbm.b.30073

Abstract: Hydroxyapatite (HA) composites with titania (TiO2) up to 30 mol % were coated on a titanium (Ti) substrate by a sol–gel route, and the mechanical and biological properties of the coating systems were evaluated. Using polymeric precursors, highly stable HA and TiO2 sols were prepared prior to making composite sols and coatings. Coatings were produced under a controlled spinning and heat treatment process. Pure phases of HA and TiO2 were well developed on the composites after heat treatment above 450°C. The HA–TiO2 composite coating layers were homogeneous and highly dense with a thickness of about 800–900 nm. The adhesion strength of the coating layers with respect to Ti substrate increased with increasing the TiO2 addition. The highest strength obtained was as high as 56 MPa, with an improvement of about 50% when compared to pure HA (37 MPa). The osteoblast-like cells grew and spread actively on all the composite coatings. The proliferation and alkaline phosphatase (ALP) activity of the cells grown on the composite coatings were much higher than those on bare Ti, and even comparable to those on pure HA coating. Notably, the HA–20% TiO2 composite coating showed a significantly higher proliferation and ALP expression compared to bare Ti (p < 0.05). These findings suggest that the sol–gel-derived HA–TiO2 composite coatings possess excellent properties for hard tissue applications from the mechanical and biological perspective. © 2004 Wiley Periodicals, Inc. J Biomed Mater Res Part B: Appl Biomater 72B: 1–8, 2005

Keywords: hydroxyapatite (HA); titania (TiO2); sol–gel; composite coating; titanium (Ti); adhesion strength; cellular response

INTRODUCTION

Titanium (Ti) and its alloys have long been recognized as dental and orthopedic implant materials. To improve the implant–tissue osseointegration, considerable effort has been exerted to modify the Ti surface structure both physically and chemically.1–3 Among the methods, hydroxyapatite [HA, Ca10(PO4)6(OH)2] coatings on Ti have shown good fixation to the host bone and increased bone ingrowth to the implants.4,5 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.5 Traditionally, HA coatings were carried out by a plasma-spraying method. However, the plasma-sprayed HA coating layer is known to be inhomogeneous in structure and have low bonding strength (20–30 MPa) due to the high-temperature processing and resultant thick coating layer.7,8 Thin HA films (less than several μm) obtained by physical vapor deposition and sol–gel methods were reported to have high purity and relatively higher strength (~40 MPa).9–13 However, the authors observed that the sol–gel-derived HA layer on a Ti substrate had a limited bonding strength due to the weak bonding capability between HA ceramic and Ti metal.10 To improve the bonding ability, a TiO2 layer was inserted between HA and Ti, to find out if a significant improvement in the bonding strength could be achieved due to the high chemical affinity of TiO2 with respect to both HA and Ti.10 Based on previous work, in this study, we proposed to deposit HA–TiO2 composite coatings on a Ti substrate. Practically, the HA–TiO2 composite coating system has been tried by a few other groups.14–17 The layers were reported to have improved mechanical properties or in vitro cellular responses.16,17 However, in most cases, the composite coat-
ings were made by a plasma-spraying method, or else the HA powder was mixed with TiO\textsubscript{2} sol. In such cases, the resultant layer was thick, rough, and inhomogeneous. Practically, there have been no reports on the HA–TiO\textsubscript{2} composite film produced by using sol–gel precursors of both HA and TiO\textsubscript{2}. The sol–gel approach was favored due to the chemical homogeneity and fine grain size of the coating layer, as well as the low crystallization temperature and mass producibility of the processing. In preliminary works, we observed that the HA and TiO\textsubscript{2} sols easily reacted to form a Ca–Ti compound (CaTiO\textsubscript{3}); otherwise, the HA and TiO\textsubscript{2} phases were poorly crystallized. To overcome these problems, the coating sols should be stabilized and well controlled. Recently, the authors prepared the HA and TiO\textsubscript{2} sols separately, with each sol possessing chemical and thermal stability. Using both sols, in the present study, we fabricated HA–TiO\textsubscript{2} composite coatings on a Ti substrate, and investigated their mechanical and biological performance.

**MATERIALS AND METHODS**

**Preparation of HA and TiO\textsubscript{2} Composite Coatings**

Calcium nitrate tetrahydrate (Ca(NO\textsubscript{3})\textsubscript{2}·4H\textsubscript{2}O, Aldrich, UK) and triethyl phosphate (PO\textsubscript{3}(CH\textsubscript{2}H\textsubscript{2})\textsubscript{3}, Aldrich, UK) of 2 M were hydrolyzed in a separate beaker containing ethanol (purity >99.7%, BDH, UK) and distilled water (DW) for 24 h. Both solutions were mixed at a Ca/P ratio of 1.67, and the mixture was stirred vigorously for 30 min. Ammonium hydroxide (NH\textsubscript{4}OH, BDH, UK) was added stepwise up to 6% to the mixture. The amount was considered from preliminary study to improve the gelation and polymerization process. The mixtures were stirred slowly at room temperature in ambient conditions for 24 h, and further aged at 60°C for 72 h to produce an HA sol. The state of gelation of HA sol during hydrolysis and aging was monitored using Fourier-transformed-infrared (FTIR, PerkinElmer, UK) spectroscopy. To produce a TiO\textsubscript{2} sol, titanium propoxide (Ti(OCH\textsubscript{2}CH\textsubscript{2}CH\textsubscript{3})\textsubscript{4}, Aldrich, UK) of 1 M was hydrolyzed within an ethanol-based solution, containing diethanolamine [(HOCH\textsubscript{2}CH\textsubscript{2})\textsubscript{2}NH, Aldrich, UK] and distilled water, and stirred at room temperature for 24 h and further at 50°C for 72 h. The molar ratios of the diethanolamine/Ti and water/Ti were 1 and 2, respectively. The prepared HA and TiO\textsubscript{2} sols were mixed together at various ratios (10, 20, 30, and 40 mol %), and the mixtures were stirred vigorously for 6 h and slowly for a further 3 h at 40°C, to finally obtain HA–TiO\textsubscript{2} composite sols. For the purpose of comparison, pure HA and TiO\textsubscript{2} sols were prepared without mixing.

As a coating substrate, commercially pure Ti (c.p. Ti, grade II) disc was prepared after polishing with silicon carbide paper (#1500 grit), and then cleaning in acetone and ethanol. The Ti substrate was dipped into the composite sols and then spin-coated at 2000 rpm for 10 s. After drying at 80°C for 24 h, samples were heat treated at various temperatures (450, 500, and 550°C) for 2 h in air at a heating and cooling rate of 1°C/min. For the purpose of comparison, pure HA and TiO\textsubscript{2} coatings were also prepared at the same coating and heat treatment conditions. Furthermore, sol–gel composite powders were also prepared after drying the sols at 80°C for 3 days and heat treating the dried gels under the same conditions as for the coatings.

**Characterization and Adhesion Strength Test**

The phase change during heat treatment was characterized using X-ray diffraction (XRD, Philips, Holland) at a scanning rate of 0.6° 2θ/min, and the peaks for HA and TiO\textsubscript{2} were indexed based on JCPDS reference file. The surface and cross-sectional morphologies of the coating layer were observed using field emission scanning electron microscopy (FESEM, JEOL, Japan). The coating thickness was measured using SEM, and the average value was taken from arbitrary five areas. Roughness of the coating layer was measured using a surface profiler (Proscan 1000, UK) by laser scanning the surface within an area of 200 × 200 μm at an interval of 1 μm. Roughness parameters, such as Ra (average height above center line), Rq (root mean square of Ra), and Rz (average of the highest peaks and the lowest valleys on five measurement lengths), were obtained. Three different areas were scanned, and five sections for each area were measured to determine the mean ± standard deviations (n = 15).

The adhesion strength of the coating layer was measured using an adhesion test apparatus (Sebastian V, Quad Group, USA). A stud precoated by the manufacturer using an epoxy of a proprietary composition was adhered to the coating layer by curing the epoxy at 150°C for 1 h. The stud with a diameter of 2.69 mm was pulled at a loading rate of 5 mm/min until the coating layer failed, and the adhesion strength was determined from the maximum load recorded and divided by the surface area. Six specimens were tested for each condition, and data were represented as mean ± SD (n = 6). The fractured surface was observed with scanning electron microscopy (SEM, Philips, USA). The atomic composition attached to the stud was analyzed with energy dispersive spectroscopy (EDS) connected to the SEM to interpret the failure mechanism associated. Moreover, the area of each failure mode was quantified using optical microscopy and image analysis.

**In Vitro Cellular Responses**

Human osteosarcoma HOS (TE-85) cells were cultured in a humidified atmosphere of 5% CO\textsubscript{2} in air at 37°C. The culture medium consisted of 10 mL of Dulbecco’s modified Eagle’s medium (DMEM, Gibco, UK) supplemented with 10% fetal calf serum (FCS, Gibco, UK), 2 mM l-glutamine, 50 IU/mL of penicillin and 50 μg/mL of streptomycin. After culture for 3 days until confluence, the cells were washed with phosphate-buffered saline (PBS, Gibco, UK), detached with trypsin-EDTA solution (0.25% trypsin) at 37°C for 10 min, and centrifuged and resuspended for further reseeding and growing tests.
The MTT method was used to assess the cell proliferation. MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) is a yellow substrate, which is converted, by living cells, to dark blue formazan product. The HOS cells were plated at a density of $1 \times 10^4$ cells/well on the specimens placed in 24-well plate and cultured for 6 h in 0.1 mL medium to allow the cells to attach ($t = 6$ h, used as an initial cell density for further proliferation test). Thereafter, the samples were placed into new plates and cultured for up to 7 days in 1.5 mL medium. The cell proliferation was evaluated using the MTT assay Kit (CT020, Sigma, UK). The color product was measured at 570 nm using a spectrophotometer. Cell growth morphology was observed by SEM at an accelerating voltage of 15 kV, after fixing, dehydrating, critical point drying, and gold coating the cells.

Cell functionality was assessed by measuring the alkaline phosphatase (ALP) activity. The ALP was recognized as a marker for osteogenic or osteoblastic cell activity undergoing sequential differentiation in the bone formation process. The ALP activity of the cultured cells was measured colorimetrically using p-nitrophenyl phosphate substrate (ALP kit
104-LL, Sigma, UK). This substrate is hydrolyzed by the enzyme ALP to p-nitrophenol and inorganic phosphate. Under alkaline conditions, the p-nitrophenol was converted to a yellow product and its absorbance was subsequently measured at 410 nm using a spectrophotometer. The ALP activity was calculated from a standard curve after normalizing to the total protein content, which was measured with a commercial kit (DC protein assay kit; BioRad, Hercules, CA). The MTT and ALP tests were performed in triplicate for each condition, and data were represented as mean ± SD. For statistical analysis, multiple groups of data were compared by analysis of variance (ANOVA) followed by the Boneferroni correction test using SPSS v.11.0 (SPSS Inc., USA). A p value of less than 0.05 was considered significant.

**RESULTS**

**Composite Phase**

Figure 1 shows the XRD patterns of the HA–20% TiO2 composite coatings on Ti (A) and powders (B), respectively, after heat treatment at various temperatures for 2 h in air. After heat treating the coating layer at 450°C, small apatite peaks were observed [Figure 1(A)]. When the heat treatment temperature increased to 500 and 550°C, the HA peak intensities increased. Moreover, TiO2 phases (both anatase and rutile) were observed. These phase evolutions were clearly observed from XRD analysis of the composite powders [Figure 1(B)]. Characteristic HA and TiO2 peaks were well developed for all heat treatment temperatures. In particular, the TiO2 rutile phase evolved above 500°C. There were no other TiO2 phases observed above 500°C. The HA–TiO2 composite coatings lay between those of HA (37 MPa) and TiO2 (70 MPa), and with increasing TiO2 content, the strength increased. The highest strength was approximately 56 MPa with 30% TiO2 addition, and this value was an improvement of approximately 50% with respect to pure HA coating.

**Coating Morphology**

Figure 2(A–D) represents the SEM morphologies of the HA–TiO2 composite coatings on Ti after heat treatment at 500°C for 2 h in air. For other HA–TiO2 composite coatings, the HA and TiO2 phases observed were similar with only a difference in their peak intensities (data not shown here).

**Adhesion Strength**

To observe the mechanical properties of the composite coating layers, the adhesion strength was measured and is shown in Figure 3. Pure HA and TiO2 coatings were also tested for the purpose of comparison. 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. The highest strength was approximately 56 MPa with 30% TiO2 addition, and this value was an improvement of approximately 50% with respect to pure HA coating.

**TABLE I. Surface Roughness Parameters of the Samples**

<table>
<thead>
<tr>
<th>Materials</th>
<th>Polished Ti</th>
<th>Pure HA</th>
<th>HA + 10% TiO2</th>
<th>HA + 20% TiO2</th>
<th>HA + 30% TiO2</th>
<th>Pure TiO2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ra (μm)</td>
<td>0.88 (±0.06)</td>
<td>0.59 (±0.04)</td>
<td>0.60 (±0.04)</td>
<td>0.64 (±0.06)</td>
<td>0.69 (±0.05)</td>
<td>0.57 (±0.05)</td>
</tr>
<tr>
<td>Rq (μm)</td>
<td>1.09 (±0.08)</td>
<td>0.76 (±0.06)</td>
<td>0.81 (±0.06)</td>
<td>0.83 (±0.05)</td>
<td>0.86 (±0.05)</td>
<td>0.73 (±0.07)</td>
</tr>
<tr>
<td>Rz (μm)</td>
<td>5.01 (±0.34)</td>
<td>3.54 (±0.25)</td>
<td>3.78 (±0.24)</td>
<td>3.90 (±0.21)</td>
<td>4.46 (±0.29)</td>
<td>3.13 (±0.21)</td>
</tr>
</tbody>
</table>

**TABLE II. Thickness of the Coatings after Heat Treatment at 500°C for 2 h in Air**

<table>
<thead>
<tr>
<th>Coating type</th>
<th>Pure HA</th>
<th>HA + 10% TiO2</th>
<th>HA + 20% TiO2</th>
<th>HA + 30% TiO2</th>
<th>Pure TiO2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coating thickness (nm)</td>
<td>860 (±80)</td>
<td>880 (±100)</td>
<td>810 (±70)</td>
<td>800 (±80)</td>
<td>510 (±20)</td>
</tr>
</tbody>
</table>
TiO₂ composite coating, the HA coating had a much larger area related to coating failure (area “C”). The coating failure area was quantified using image analysis (Table III). The HA–TiO₂ composite coatings had values between those of pure HA and TiO₂, and the area decreased with increasing TiO₂ addition. These observations suggest that the composite coating layers sustained applied load to a much higher degree until epoxy-related failure occurred, being contrary to the predominant coating failure over epoxy failure as observed in the pure HA.

Cellular Responses

The cellular responses to each coating system were assessed in terms of cell proliferation and ALP activity. Figure 5 shows the SEM morphologies of the HOS cells grown on the bare Ti (A,B) and HA–20% TiO₂ (C,D) coating samples after culturing for 5 days. On both surfaces, the HOS cells spread and grew actively, almost completely covering the coating surface. For the other coating samples, the cell growth morphologies were similar (data not shown here). The proliferation of the HOS cells was quantified by MTT assay, as shown in Figure 6. For all samples, the cells proliferated actively with culture time. The proliferation rates were similar on all the coatings and bare Ti. The SEM and MTT results implied that the coating systems had favorable and comparable cell viability without exhibiting any cytotoxicity. Figure 7 shows the alkaline phosphatase (ALP) activity of the cells after culturing for 7 days. The cells on all the coating samples exhibited higher ALP expression levels compared to those on bare Ti. In particular, the HA–20% TiO₂ composite coating showed significantly ($p < 0.05$) higher value when compared to bare Ti, in terms of ALP expression by the HOS cells.

DISCUSSION

In this study, a composite coating system, consisting of HA and TiO₂, was proposed to improve the biocompatibility of a
Ti implant. To obtain a thin and homogeneous coating layer, the sol–gel method was utilized. Special care was taken to prepare both HA and TiO$_2$ sols prior to producing mixed sols. Practically, when the initial sols were unstable structurally and chemically, it was impossible to obtain a composite coating with pure phases. In such cases, additional phases, such as CaTiO$_3$, tricalcium phosphate (TCP), and CaO were readily observed from a reaction between HA and TiO$_2$ at elevated temperatures, or else, the HA and TiO$_2$ phases were poorly crystallized. Compared to the pure HA and TiO$_2$ coatings, the composite coating would be restricted in the crystallization, because each phase can act as a barrier to crystallization for another phase by limiting atomic arrangement. From preliminary studies, it was concluded that these were attributed to the instability and immaturity of both initial sols and their high reactivity. In this study, the well-controlled aging conditions introduced for both sols enabled the composite coating to retain a higher stability and maturity. In particular, the addition of small amounts of NH$_4$OH ($<6\%$) to the HA sol improved the gelation and polymerization of the HA structure, as was observed by an enhanced apatitic structure development and viscosity increase with NH$_4$OH addition. Moreover, the aging time and temperature of the mixed sols were optimized to minimize the reaction between HA and TiO$_2$, that is, aging over 24 h and heat treatment over 600°C started to produce CaTiO$_3$ phase. Under the controlled processing conditions, the composite coatings retained a mixture of pure phases of HA and TiO$_2$ rutile and anatase (Figure 1). The coating layer obtained was thin (thickness of about 800–900 nm), dense, and uniform, and

<table>
<thead>
<tr>
<th>Coating type</th>
<th>Pure HA</th>
<th>HA + 10% TiO$_2$</th>
<th>HA + 20% TiO$_2$</th>
<th>HA + 30% TiO$_2$</th>
<th>Pure TiO$_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Area related to coating failure (%)</td>
<td>64.8 (±13.3)</td>
<td>54.2 (±6.6)</td>
<td>31.0 (±12.0)</td>
<td>23.4 (±13.1)</td>
<td>13.1 (±5.2)</td>
</tr>
</tbody>
</table>

All the coatings were heat treated at 500°C for 2 h in air.

Figure 5. SEM morphologies of the HOS cells growing on bare Ti substrate (A,B) and HA-20% TiO$_2$ coating on Ti (C,D) after culture for 5 days. The composite coating was heat treated at 500°C for 2 h in air.
bonded tightly to the Ti substrate with TiO additions up to about 30% (Figure 2). Those phase and morphological features in the sol–gel-derived composite coating were in marked contrast to the reported ones obtained by a plasma-spraying method or using an HA powder precursor. However, the higher addition of TiO2 (over 40 mol %) was observed to render the morphology quite porous and rough during heat treatment (data not shown). This was believed to be attributable to the high content of an organic additive, mainly the diethanolamine ([Ti]/[diethanolamine] = 1), which was added to preserve the stability of TiO2 sol during hydrolysis. Thus, when producing a composite containing higher amounts of TiO2, alternative precursors need to be selected.

The HA–TiO2 composite coatings had higher bonding strengths than the pure HA, and increasing the TiO2 addition increased the strength. With 30% TiO2 addition, the improvement was as high as 50% (56 MPa) with respect to pure HA coating (37 MPa). The strength of the pure TiO2 coating was as high as 70 MPa, which was comparable to the strength of the bonding epoxy. Clearly, the TiO2 component played a role in improving the bonding capability of the composite coatings. As reported previously, compared to HA, the TiO2 layer has a higher chemical affinity to the Ti substrate, and this contributed to the improvement of the coating/substrate interfacial strength. Furthermore, the superior mechanical integrity of TiO2 to HA should account for the increased strength value because the coating failure occurred, not only at the interface, but also within the coating layer. At this point, one thing to be addressed is the thermal mismatch between the coating and substrate. With regard to the coefficient of thermal expansion (CTE), the addition of TiO2 (8.3 × 10^{-6}) would be favorable to relieve thermal mismatch between HA (14.6 × 10^{-6}) and Ti (8.6 × 10^{-6}), and consequently, for the coating strength.

The mechanically strong composite coatings exhibited favorable in vitro cellular responses (Figures 5–7). The osteoblast-like HOS cells on the composite coatings proliferated actively and typically expressed high levels of ALP activity. The ALP levels expressed by the cells on the composite coatings were higher than those of cells grown on bare Ti substrate, and comparable to those on pure HA coating. In particular, the cells on HA–20% TiO2 coating appeared to show a higher ALP level than those on pure HA coating, even though the difference was small. Previously, the authors observed slightly lower ALP level in cells grown on the pure TiO2 coating compared to the pure HA coating. Moreover, from a series of ALP experiments on HA-associated materials, such as those created by sol–gel coating, powder slurry coating, and bulk systems, it has been observed that the pure HA composition induced the highest ALP activity by osteoblast-like cells (MG63 and HOS). When bioinert ceramics, such as ZrO2 and Al2O3, were added to HA, ALP activity was reduced even though these composites exhibited improved mechanical properties. Interestingly, in the present study, the composite coating, especially the HA–20% TiO2 enhanced ALP expression by HOS cells compared to the pure HA coating. The cellular responses may be complicated by alterations in surface status both physically and chemically, as a result of the addition of TiO2 to HA.

Clearly, in this study, the sol–gel composite coatings of HA–TiO2 have proven to be favorable in terms of mechanical strength and in vitro cellular responses. Still, the rationale for the higher osteoblastic cellular response to the HA–20% TiO2 composite could not be clearly elucidated. The change in degree of mixing, homogeneity, and grain size, as well as different chemical status could be attributable to this. When the structure was well homogenized and consisted of small-sized grains, the materials were often reported to have favorable

Figure 6. Proliferation rate of the HOS cells on the composite coatings on Ti after culture for up to 5 days. Data were represented relative to the initial cell density at t = 6 h. Bare Ti substrate and pure HA coating on Ti were also tested for comparison. All coatings were heat treated at 500°C for 2 h in air (mean ± SD, n = 3).

Figure 7. Alkaline phosphatase activity of the HOS cells on the composite coatings on Ti after culture for 7 days. Bare Ti substrate and pure HA coating on Ti were also tested for comparison. All coatings were heat treated at 500°C for 2 h in air (mean ± SD, n = 3).
cellular behaviors. Currently, transmission electron microscopy and X-ray photoelectron spectroscopy studies are in progress to examine the nanoscale structure and chemical composition of the sol–gel composites. Furthermore, in addition to the ALP assay, the analyses of the secretion of bone-associated proteins, such as osteocalcin, bone-sialo protein, and collagen I, need to be conducted to confirm the state of cell differentiation and functional activity on the composite coatings.

CONCLUSIONS

Hydroxyapatite (HA) and titania (TiO₂) composite coatings were obtained on a Ti substrate by means of a sol–gel process. After heat treatment above 450°C, the coatings retained well-developed HA and TiO₂ pure phases. The coating morphology was thin (<1 µm) and highly dense, and tightly bonded to the Ti substrate. The adhesion strength of the composite coatings increased with increasing TiO₂ addition up to 30%. The strength obtained in HA–30% TiO₂ (56 MPa) was improved by about 50% compared to the value for the pure HA coating (37 MPa). The osteoblast-like HOS cells on all the composite coatings spread and grew actively, and proliferated similarly to those on bare Ti and pure HA coating. The alkaline phosphatase (ALP) activity expressed by the cells on the composite coatings exhibited higher levels compared to that on bare Ti, and similar or slightly higher (in HA–20% TiO₂ case) when compared to that on HA coating.

REFERENCES