On the feasibility of phosphate glass and hydroxyapatite engineered coating on titanium

Hae-Won Kim, Eun-Jung Lee, In-Kook Jun, Hyoun-Ee Kim
School of Materials Science and Engineering, Seoul National University, Seoul, 151742, Korea

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Abstract: In this report, bioactive calcium phosphate (CaP) coatings were produced on titanium (Ti) by using phosphate-based glass (P-glass) and hydroxyapatite (HA), and their feasibility for hard tissue applications was addressed in vitro. P-glass and HA composite slurries were coated on Ti under mild heat treatment conditions to form a porous thick layer, and then the micropores were filled in with an HA sol–gel precursor to produce a dense layer. The resultant coating product was composed of HA and calcium phosphate glass ceramics, such as tricalcium phosphate (TCP) and calcium pyrophosphate (CPP). The coating layer had a thickness of approximately 30–40 μm and adhered to the Ti substrate tightly. The adhesion strength of the coating layer on Ti was as high as 30–33 MPa. The human osteoblastic cells cultured on the coatings produced by the combined method attached and proliferated favorably. Moreover, the cells on the coatings expressed significantly higher alkaline phosphatase activity than those on pure Ti, suggesting the stimulation of the osteoblastic activity on the coatings. On the basis of these observations, the engineered CaP coating layer is considered to be potentially applicable as a hard tissue-coating system on Ti-based implants. © 2005 Wiley Periodicals, Inc. J Biomed Mater Res 75A: 656 – 667, 2005

Key words: calcium phosphate coating; phosphate glass; hydroxyapatite sol–gel; titanium implant

INTRODUCTION

Bioceramic coatings on Ti-based metals have been developed primarily to find applications in orthopedic and dental implants. The bioactive coatings developed so far are mainly constituted of calcium phosphates (CaP) and glass/glass ceramics.1–9 CaP coatings, which mainly involve hydroxyapatite (HA) and tricalcium phosphate (TCP), are currently obtained by the plasma-spraying method,2–4 whereas glass and glass ceramics are welded on metals by heat-treating the glass slurries or frits above the glass-melting temperature.5–9 With regard to glass/glass ceramic coatings, most attempts have been made on silicate glasses.5–7 However, in this coating approach, the high processing temperature (≥800°C) required to melt the glass often caused thermal degradation of the metallic substrates. Therefore, the glass composition and processing conditions have often been limited, and special attention has been required.6

Phosphate glasses (P-glasses), whose chemical composition is compatible with that of human hard tissues, are also known to be bioactive and to induce enhanced cellular responses.10,11 Compared with silicate glasses, P-glasses possess a much lower melting temperature (usually 500–800°C depending on the composition), which facilitates their glazing on Ti-based substrates.8,9 Moreover, during the thermal treatment, a large amount of ceramic phases is produced because of the crystallization of the glasses. Although these glass and glass ceramic compositions are bioactive in bulk, the coating system needs careful consideration to avoid thermal mismatch and resultant cracking problems. Recently, the authors obtained dense and strong glass ceramic coatings on a zirconia substrate with use of P-glasses and HA composite slurries.12 The bioactive coating layer has proved to be favorable to enhance the in vitro osteoblast-like cell responses of the zirconia substrate. However, compared with the coating with pure glass, the glass-HA composite required much higher processing temperatures (>800°C) to densify the coating layer. Thus, the temperature range, even if safe for coating on zirconia, should be carefully considered in the case of coatings on Ti-based metals.
On the basis of these previous findings, in this study, the authors aimed to produce thick CaP coatings with a dense and crack-free morphology on a Ti substrate using P-glasses and HA. First, the P-glass and HA composite slurries were coated on Ti under mild thermal treatment conditions, at a temperature that is sufficiently low for the Ti substrate and thus is not high enough to densify the coating layer. The resulting porous layer was subsequently covered with the HA sol–gel precursor to produce a denser layer. The phase, morphology, and mechanical properties of the coating layers were investigated, and their biological properties were assessed in terms of in vitro dissolution and osteoblastic cellular responses.

**MATERIALS AND METHODS**

**Coatings**

P-glasses were prepared as described previously. Briefly, two glass compositions [xCaO-(55-x)Na2O-45P2O5, where x = 20 and 40 in mole %, and designated as 20CaO and 40CaO-glass, respectively] were obtained by using the calculated amounts of the precursors (P2O5, NaH2PO4, and CaCO3) with thermal treatment between 1100 and 1200°C for 1 h, followed by quenching. The resulting glasses were ground and sieved down to 25 μm to obtain fine glass powders. Glass-HA composite slurries were prepared from the glass and commercially available HA (Biotal) powders by mixing them in ethanol (powder/ethanol = 0.4 w/v, glass/HA = 1 w/w). For the purpose of comparison, pure glass slurries in ethanol were also prepared.

The HA sol was composed with slight modification from our previous studies. Triethyl phosphite [TEP; [PH(C2H5O)3], Aldrich, USA] of 1.8M was hydrolyzed for 24 h in ethanol with addition of distilled water ([OH]/[P] ~10). Separately, calcium nitrate [Ca(NO3)2·4H2O, Aldrich] of 3M was dissolved in ethanol. The Ca-containing solution was added slowly to the P-containing solution, and the mixture sol was aged at room temperature for 7 days, and then at 40°C for another 24 h. An HA sol (0.5M of Ca) with a lower concentration was also prepared by diluting the original HA sol in ethanol after stirring for 24 h.

As the substrate for coating, commercially pure Ti (cp-Ti, annealed; Goodfellow) was prepared in the form of a disk (6.13 mm × 1 mm) after polishing with diamond slurries down to 1 μm and cleaning with acetone and ethanol. First, the Ti disk was dip-coated with the glass and glass-HA slurries and dried at room temperature for 3 h and then at 80°C for another 24 h. After repeating the coating-drying process twice, the samples were heat-treated at 600°C for 1 h. The slurry-coated samples were placed in a flask and evacuated under a vacuum (~5 mm Hg) by using a rotary vacuum pump, and the HA sol was infiltrated into the coating layer using a syringe under vacuum. After infiltration with the sol–gel solution, the disks were taken out and immediately rotated by spinning them at 3000 rpm for 20 s with use of a spin coater. After drying at 80°C for 24 h, the samples were heat-treated at 500°C for 1 h in air. The coating-to-heat treatment process was repeated twice more with a thinner HA sol (0.5M). For the purpose of comparison, pure glass and glass-HA slurry coatings without the HA sol–gel treatment were also prepared. In Figure 1, the engineered coating design on Ti is schematically illustrated.

**Characterization and tests**

The phase of the coating layer was characterized with X-ray diffraction (XRD; PW4620, Philips). The surface and cross-sectional morphology of the coating layer was observed with field emission scanning electron microscopy (FESEM; JSM-6330F, JEOL). The coating composition was analyzed with energy dispersive spectroscopy (EDS) connected to the FESEM.

The adhesive property of the coating layer with respect to Ti substrate was evaluated by means of measuring the bonding strength using an adhesive testing apparatus (Sebastian V, Quad Group), as described previously. A stud was adhered to the coating layer with an epoxy by curing at 150°C for 1 h, and the stud was pulled until the coating layer failed, and then the adhesive strength was calculated from the maximum load recorded by taking the surface area into consideration. After the strength test, the detached surface was observed with FESEM to analyze a failure mode of each coating system. Ten samples were tested for each condition (n = 10).

The dissolution behavior of the coating layer was measured by immersing the coated disks in a physiological saline solution for up to 3 weeks at 37°C. At predetermined time periods, the samples were taken out, and the concentration of Ca and P dissolved from the layer was measured by using ICP-AES (Shimadzu). The removed samples were washed with distilled water/ethanol, and the surface was observed with SEM to detect any changes in the coating layer during the period of incubation. Dissolution tests were performed on three separate samples (n = 3).
In vitro cellular assays

A well-characterized human bone-derived cell line, HOS TE85, was used to assess the proliferation and phenotypic expression behavior of the cells on the coatings. The cells were preincubated in a complete culture medium containing 10% fetal bovine serum and then seeded at a density of 3 x 10^4 cells/mL on the samples, as well as on a Thermanox coverslip (NUNC, used as a control), and cultured for up to 10 days in an incubator humidified with 5% CO_2/95% air at 37°C. An MTT method was used to assess the viability of cells during attachment and growth stage. After 6 h and 3 days of culturing, MTT was added to each well and incubated at 37°C for 4 h. The blue formazan product was dissolved in an acidic solution, and the absorbance was measured at 570 nm with use of a spectrophotometer. The cell morphology was observed with SEM, after fixing the cells with 2.5% glutaraldehyde and dehydrating them with a series of graded ethanols (70, 90, and 100%) and critical point drying.

Alkaline phosphatase (ALP) activity was measured to assess the functional activity of the cells. After culturing for 10 days, the cells were detached with trypsin/EDTA solution, and the cell lysates were centrifuged and resuspended with 0.1% Triton X-100. The cell pellets were disrupted by alternating freezing-and-thawing processes. The ALP activity of the cells was measured colorimetrically by using p-nitrophenyl phosphate as a substrate (ALP kit 104, Sigma). The enzyme ALP expressed by the cells hydrolyzes the substrate to p-nitrophenol and, under alkaline conditions, the p-nitrophenol is converted to a yellow product. The absorbance of the product was measured at 410 nm by using a spectrophotometer. The ALP activity was calculated from a standard curve after being normalized to the total protein content (μmol p-nitrophenol/mg protein/h).

The in vitro tests were performed on six replicate samples for each condition (n = 6), and the statistical analysis was carried out by analysis of variance (ANOVA), at a significance level of p < 0.05.

RESULTS

Coating composition

Figure 2 shows the XRD patterns of the pure glass (A), glass/HA composite (B), and HA sol–gel-filled glass/HA composite (C) coatings on Ti. Data on the coatings with 20CaO-glass are representative shown. In the pure glass coating heat treated at 600°C, a large amount of glass-ceramics was produced because of the crystallization of the glass [Fig. 2(A)]. When HA was added to the glass of the same composition and coated, followed by heat treatment at 600°C, the glass crystallized to a mixed phase consisting mainly of calcium pyrophosphate (CPP) and tricalcium phosphate (TCP), whereas a large amount of HA.
remained [Fig. 2(B)]. Moreover, a level of amorphous calcium phosphate phase is also expected to remain, as deduced from the broadness of some crystalline peaks. The glass/HA composite coating was covered with the sol–gel HA and heat treated at 500°C. The coating product so obtained was similar to that obtained without the sol–gel HA treatment [Fig. 2(C)]. The trends in the coatings with 40CaO-glass were similar to those observed with the 20CaO-glass, except for a slight difference in the intensities of crystalline peaks (data not shown).

**Morphology**

Figure 3 shows the SEM morphologies of the pure glass coatings on Ti. Both of the pure glass coatings were dense but severely cracked [Fig. 3(A,B) for the 20CaO-glass and 40CaO-glass, respectively], suggesting that the pure glasses in these compositions are not favourable as a coating layer on Ti. The cross-sectional view of the pure 20CaO-glass showed more clearly the formation of cracks (indicated as arrows) across the coating layer [Fig. 3(C)].

In contrast to the pure glass coatings, the glass/HA composite coatings did not show any cracks on the surface but were not fully densified with a micro-porous structure, as shown in Figure 4(A,B). The composite coating with 20CaO-glass [Fig. 4(A)] appeared to be denser than that with 40CaO-glass [Fig. 4(B)], and this was deemed to the different thermal properties of the glass; in other words, the ternary glass used in this study normally has lower glass transition and melting points as the CaO content in the glass composition decreases. When the glass/HA porous coating layer was covered with the HA sol–gel precursor under vacuum and heat treated at 500°C, the pores on the surface were found to be covered almost completely, and the resultant surface appeared to be somewhat rough on the microscale [Fig. 4(C,D)]. The amount of the sol–gel HA filled into the pores is expected to be slightly higher on the composite coat-
ing with 40CaO-glass than with 20CaO-glass because of the initially higher level of porosity in the coating with 40CaO-glass. The polished cross-sectional view revealed that the sol–gel-treated composite coating layers were highly dense and adhered to the Ti substrate quite tightly [Fig. 4(E,F)]. The coating layer did not hold any cracks or delaminations.

The composition across the combined coating layer (with 40CaO-glass) was analyzed with EDS, as shown in Figure 5. The distribution of Ca and P varied depending on the position. The Ca/P ratios across the layer were in the range of approximately 0.8–1.7, reflecting the composition of the coating products consisting of various CaP ceramics (HA, TCP, and CPP) and glass.

**Adhesive property**

The adhesive strengths of the glass/HA coatings subjected to HA sol–gel treatment were measured with an adhesion testing apparatus, as shown in Figure 6(A). The strengths of the coatings were 25–40
physiological saline solution for periods of up to 3 weeks. Both Ca and P dissolved more in the coating obtained with the 20CaO-glass. For both coatings, the dissolution rate decreased with increasing incubation period, and the P dissolved more than the Ca. The SEM morphology of the coating surface after dissolution for 14 days is shown in Figure 7(B). The coating layer appeared to dissolve, revealing a surface morphology, which was different from the as-coated one [compare with Fig. 4(C)]. In particular, some crystals appeared to be precipitated (indicated as arrows). Closer examination revealed that the precipitates were calcium phosphate nanocrystallines, which usually form through the dissolution-reprecipitation process.

**Coating dissolution**

Figure 7(A) shows the dissolution behavior of the combined coatings on Ti after their immersion in MPa with mean values of 33 (with 20CaO-glass) and 30 MPa (with 40CaO-glass). There was no statistical significant difference in the strength value \((p > 0.05)\) between the combined coatings depending on the glass composition used. These values were similar to or higher than those reported for plasma-sprayed HA coatings,\(^{16–19}\) suggesting that the engineered coating layer adhered well to the Ti substrate. The failure surface of the coating on Ti (with 20CaO-glass) was observed with SEM, as shown in Figure 6(B). Significant amounts of coating fragments and epoxy residues were observed on the detached surface, suggesting that the failure mode consisted of failures at the coating/substrate interface, as well as within the coating layer and epoxy (each type of failure is indicated by arrows).

**Osteoblastic cell responses**

Figure 8 shows the SEM morphology of the HOS TE85 cells grown on the combined coatings after culturing for 3 days. The cells grew and spread well, with the membranes being in intimate contact with the coating surface, and this proved that the cells had good affinity to the engineered CaP coating layers.

Figure 9 shows the viability of the cells after culturing for 6 h and 3 days. Data on pure Ti and glass/HA composite coating without the HA sol–gel treatment were also presented. The initial cell attachment at 6 h was significantly higher on the glass/HA coatings, without or with the HA sol–gel treatment than that on pure Ti \((p < 0.05)\). With regard to the effect of the HA sol–gel treatment, no significant difference was observed between the two glass compositions in the initial attachment level. The cell viability at day 3 was significantly lower on both of the glass/HA coatings without HA sol–gel treatment \((p < 0.05)\) but was similar on those with HA sol–gel treatment, compared with that on pure Ti. In particular, the HA sol–gel treatment improved the cell viability at day 3 of the composite coatings (significantly different in the value for the 40CaO-glass; \(* p < 0.05\)).

Figure 10 shows the ALP activity of the cells on the combined coatings on Ti. The cells on the glass/HA coatings with HA sol–gel treatment exhibited significantly higher ALP expression levels than those on the coatings without the HA sol–gel layer \((p < 0.05)\) or those on pure Ti \((p < 0.05)\), suggesting that the osteoblastic functionality was significantly improved on the engineered glass-HA combined coatings. Regarding the effect of glass composition (20CaO and 40CaO-glasses), we could not observe any statistically significant difference in the cell viability and ALP activity for both coating groups (without and with HA sol–gel treatment).
DISCUSSION

CaP coatings supported on strong substrates have attracted a great deal of attention in the field of hard tissue implants. Currently, the plasma-spraying method is most commonly used in the production of thick HA coating layers (~30–150 µm) on Ti. On the other hand, bioactive layers have also been produced on Ti by heat treating glass frits and their slurries above the glass-melting temperature ($T_m$). In this coating process, one of the most important aspects to consider is the thermal mismatch between the glass/glass ceramics and the Ti substrate. Moreover, special care is needed during the thermal treatment to pre-
vent thermal degradation of Ti metal, because the glass melting point is usually above the temperature that the substrate can endure.

Two kinds of glasses have been studied for biomedical coatings on Ti-based metals, namely, silicate-based and phosphate-based glasses (P-glasses). However, based on the considerations raised above, the glass compositions have often been limited. Compared with silicate-glasses, P-glasses possess a much lower melting temperature. P-glasses in a ternary composition of $P_2O_5$–CaO–Na$_2$O have been reported to possess good bioactivity and to have melting points of approximately 500–800°C depending on the composition.\(^{10,11}\) However, although this glass composition is biologically favorable, the final coating product also needs to be considered, because P-glasses crystallize easily to form glass ceramics during thermal treatment.\(^{8,9,20,21}\)

Recently, the authors observed that when HA was combined with ternary P-glasses (45 mol% $P_2O_5$ with Na$_2$O and CaO) and heat treated to near the melting point, the final products were composed of biologically favorable phases, such as HA, TCP, and CPP,\(^{12}\) which were also observed in this study [Fig. 2(B)]. However, when HA was combined with the glass, the temperature at which a dense coating layer could be obtained was also likely to be increased. Although increasing the temperature was possible in the case of

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**Figure 7.** (A) Ca and P concentrations dissolved from the HA sol–gel-treated glass/HA coatings on Ti. Samples were diluted to 1/5 and assessed by using ICP-AES. (B) SEM morphology of the coating (with 20CaO-glass) after dissolution for 14 days. Precipitated products were enlarged: Scale bar = 10 μm (left) and 1 μm (right).
the coating on the zirconia ceramic (≥800°C), a limitation was encountered when coating on Ti. In practice, bioactive P-glass ceramics have been obtained without the addition of HA by using the composition of invert glasses (CaO/P₂O₅ > 2). However, in this case, the heat treatment temperature needs to be >800°C due to the high CaO-content and melting point, thereby resulting in significant interfacial reaction between the coating and Ti. Conclusively, obtaining a bioactive and dense calcium phosphate thick coating on Ti at a low fabrication temperature has been difficult to accomplish.

On the basis of these considerations, in this study, the authors circumvented the problem of producing a dense layer through a stepwise coating process, namely, coating the substrate initially with porous but bioactive glass/HA composite layer under mild thermal treatment conditions (600°C for 1 h) and subsequently covering this porous layer with the sol–gel HA to produce a denser coating structure. Without the pores in the microporous layer being filled in by the HA sol–gel, it is doubtful whether the glass/HA porous layer could be used for hard tissue implants because of its weak strength and the generation of coating debris. On the other hand, the HA sol–gel coating, although bioactive, cannot be used alone to produce such a thick layer (over tens of micrometers). Practically, the coating thickness has been limited only to several microns by the sol–gel approach. As such, the glass/HA slurry precoating is used to provide the thickness level (tens of micrometers) that is required for long-term use in orthopedic implants.

The HA sol–gel-filled glass/HA coatings consisted of calcium phosphate ceramics, such as HA, TCP, and CPP, and these phases are known to be biologically favourable. Moreover, the outer HA sol–gel layer, with its higher bioactivity and good cellular responses, is considered to offer favorable conditions for initial cellular responses.

The surface and cross-sectional morphology of the combined coating layer was very dense and did not hold any cracks or delaminations [Fig. 4(C–F)]. The coating layer proved to possess high mechanical properties, with bonding strengths of about 30–33 MPa,
which are above the minimum recommended values for the hard tissue implant coatings. The glass/HA composite coatings did not produce cracks during thermal treatment because of their porous structure, which effectively negated the thermal mismatch. Moreover, the HA sol–gel layer, infiltrated in the wet state, was observed to fill the pore channels uniformly and crystallized well, without generating any cracks or voids. However, regarding the mechanical feasibility of the coatings, further studies need to be performed to examine the change in strength, particularly under wet conditions over an extended period of time.

It can at least be said that, from the in vitro dissolution test performed herein, the combined coatings proved to dissolve moderately without any cracks or delaminations being created.

The engineered coating layer responded actively within physiological saline solution. The dissolution profile was not complicated, although several phases were mixed together, and the degree of dissolution was highly dependent on the glass composition [Fig. 7(A)], and this fact suggested that the dissolution rate can be controlled by changing the glass composition. Of special note was the precipitation of calcium phosphate crystals within the saline solution. Although the degree of precipitation was somewhat low compared with that previously reported for bioactive silicate glasses, the formation of apatite in a calcium-free medium is indicative of good bioactivity of the coatings.

Calcium ions, being absent in the initial solution, should be released from the coating layer to the level of supersaturation with respect to the precipitated products. This consideration needs more extensive study under conditions that are closer to the in vivo situation.

The biological feasibility of the combined coatings was well illustrated by the cellular responses in vitro. The cells grew and spread actively on both coatings with different glass compositions. Of special note was the ALP expression level, which was stimulated by the coatings. The ALP activity of the cells was significantly higher \((p < 0.05)\) on the glass/HA composite coatings than on pure Ti, and this was previously observed on a glass/HA coating on zirconia.\(^\text{13}\) Although the coating layer is more porous in this study, the ability of the bioactive glass/HA coatings to stimulate the ALP activity is considered to be preserved. Furthermore, after the HA sol–gel treatment, the ALP activity was further increased. The HA sol–gel layer is considered to be of importance in the expression of ALP by the cells, wherein both the dense morphology and the ionic release of the sol–gel layer are believed to play somewhat positive role. On the basis of the cellular responses, it is confirmed that the CaP coating layer was effectively engineered on the Ti substrate.

![Figure 9](image_url). Cell viability on the samples with culturing for 6 h and 3 days, as assessed by MTT method. Statistical significance was considered on all coatings with respect to pure Ti \((+p < 0.05)\) as well as on the HA sol–gel-treated glass/HA with respect to glass/HA without HA sol–gel at the equivalent glass composition \((^{*}p < 0.05)\). Mean ± 1 SD for n = 6.
This consideration, based on a series of in vitro experiments, requires further in vivo animal studies.

CONCLUSION

A bioactive calcium phosphate (CaP) layer was engineered on a Ti substrate under mild heat treatment conditions, through the combined use of phosphate glass/hydroxyapatite (HA) composite slurry and HA sol–gel precursor. The microporous layer obtained from the composite slurry was covered with the HA sol–gel to produce a dense and thick (~30–40 μm) layer. This coating layer adhered to the Ti substrate tightly with a bonding strength of approximately 30–33 MPa. The dissolution rate of the coatings was dependent on the glass composition initially used. Osteoblastic cells proliferated well and expressed significantly higher ALP levels on the engineered coatings than on pure Ti.

References


