Calcium phosphates and glass composite coatings on zirconia for enhanced biocompatibility

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Abstract

Calcium phosphates (CaP) and phosphate-based glass (P-glass, $x$CaO–(0.55–$x$)Na$_2$O–0.45P$_2$O$_5$ composition) composite coatings were obtained on a strong ZrO$_2$ to improve biocompatibility, the mechanical strength and biological activity. Hydroxyapatite (HA) and P-glass mixed powder slurries were coated on the ZrO$_2$ substrate, and subsequently heat-treated to obtain CaP- and P-glass composite coatings. The effects of glass composition ($x = 0.3, 0.4, 0.5$ mol), mixing ratio of glass to HA (30\%, 40\%, 50\% wt/wt), and heat treatment temperature (800\°C, 900\°C, 1000\°C) on the coating properties were investigated. After heat treatment, additional calcium phosphates, i.e., dicalcium phosphate (DCP) and tricalcium phosphate (TCP), were crystallized, resulting in the formation of triphasic calcium phosphates (HA–TCP–DCP) surrounded by a glass phase. The relative amounts of the crystalline phases varied with coating variables. The higher heat treatment temperature and glass amount, and the lower CaO content in the glass composition rendered the composite coating to retain the higher amounts of TCP and DCP while the initial HA decreased. These appearance of additional crystalline phases and reduction of HA amount were attributed to the combined effects, i.e., the melting-crystallization of P-glass and the reaction between glass liquid phase and HA powder during thermal treatment. As a result of the glass phase in the composite coatings, their microstructures became much denser when compared to the pure HA coating. In particular, a completely dense structure was obtained at coating conditions with large amount of glass addition (50 wt\%) at the glass composition of lower CaO content (0.3 mol CaO), and the following heat treatment above 800\°C for 2 h. As a result, the adhesion strengths of the composite coating layers were significantly improved when compared to the pure HA coating. The highest strength of the composite coating was $\sim 40$ MPa, an improvement of $\sim 80\%$ with respect to the pure HA coating. The composite coatings showed much higher dissolution rates than the pure HA coating due to the newly formed crystallines (TCP and DCP) and the remaining glass phase. The osteoblast-like cells grew and spread actively on the composite coating samples. The proliferation numbers and alkaline phosphate (ALP) activities of the cells on the composite coatings were improved by $\sim 30$–40\% when compared to Thermaonox control and ZrO$_2$ substrate, and were comparable to the pure HA coating. These findings suggested that the CaP and P-glass composites are potentially useful for hard tissue coating system, due to their morphological and mechanical integrity, enhanced bioactivity, and favorable responses to the osteoblast-like cells.

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1. Introduction

In hard tissue engineering, calcium phosphate (CaP) ceramics, such as hydroxyapatite [HA; Ca$_{10}$(PO$_4$)$_6$(OH)$_$_$_2$] and tricalcium phosphate [TCP, Ca$_3$(PO$_4$)$_2$], have attracted a great deal of attention due to their excellent biocompatibility and osteoconductivity [1–3]. Most clinical reports on the CaP ceramics proved their direct bonding to bone and complete osseointegration [4]. However, their poor mechanical properties, such as low strength and fracture toughness, limited wide applications in hard tissue implants [5]. A coating-substrate system, i.e., CaP layers formed on loadbearing materials, such as ceramics (ZrO$_2$ and Al$_2$O$_3$) and metals (Ti and its alloys), provides a solution to combining the mechanical and biological benefits [6–11].

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A great deal of effort has been exerted to develop a coating layer by means of physical, chemical, and electrochemical methods [6–11]. Among those, the methods using an aqueous system, such as sol–gel, powder-based slurry, and biomimetic solution, are efficient to form a uniform coating layer through complex shaped implant and porous bone scaffold.

Recently, the authors have developed HA coatings on ZrO₂ porous scaffold and Ti implants through sol–gel and slurry approaches [11–17]. Compared to sol-gel coating, the powder-based slurry system was observed to have the benefit of producing a thicker coating layer (≈ 30–50 μm) [13,14,16,17]. However, the processing temperature should be over 1200°C to consolidate the HA powders [14]. The resultant layer was relatively micro-porous with a bonding strength of ≈ 20–25 MPa with respect to ZrO₂ substrate [14]. However, for uses as load-bearing implants, a denser and stronger coating layer is recommended. In this respect, the glass phase was introduced to improve the HA coating properties from the morphological and mechanical viewpoints.

Glasses, especially phosphate-based glasses (P-glasses), offer great potential for usage as hard tissue implants due to their enhance bioactivity and similar chemical composition to the hard tissues [18–23]. They have a variety of dissolution rates depending on the structure and composition [18,19]. The solubility and release of appropriate ions were known to stimulate cell functions and bone formation [20,21]. In addition, small amounts of P-glass added into HA ceramic improved sinterability and mechanical properties of the dense body [22,23]. However, only a few studies were carried out regarding the coating system based on the P-glass [24,25] and P-glass/HA composites [26]. Ferraz et al. fabricated glass-HA composite coatings using a plasma-spraying technique, and showed favorable in vitro cellular responses [26]. Using pure P-glass, Kasuga et al. obtained CaP glass ceramics [27,28] and their coatings [24,25], in resultant compositions of Ca₃(PO₄)₂, Ca₃P₂O₇, and Ca(PO₃)₂, after thermal treatment, and investigated their bioactivity in a simulated body fluid. However, the resultant CaPs had relatively low Ca/P ratios compared to HA, and the dissolution rate of the glass ceramic was too high to form an apatite layer in a simulated body fluid even though a TiO₂-containing glass overcame this problem [24]. In the present approach, the authors used P-glass and HA composites as coating precursors, and the final coating is expected to maintain higher amount of HA phase, and thus be more acceptable in terms of dissolution and biological perspective. In comparison to the research on the silica-based glasses and their composites, only a few works were carried out on the P-glasses, especially on the coatings or composites with HA [24–29]. However, the P-glasses have the potential for use in hard tissue applications due to their compositional similarity and biological perspective.

In this study, we intend to fabricate the CaP ceramics and P-glass composite coatings on a ZrO₂ substrate. The ZrO₂ ceramic is widely used as a substrate in hard tissue applications due to its excellent strength and fracture toughness [30]. The previous works on the CaP–ZrO₂ composites and CaP-coated ZrO₂ porous scaffold have proved their good mechanical properties and cellular responses [31–34]. The present glass-aided CaP coating on a ZrO₂ disc is expected to give primary information on the advanced coating on a ZrO₂ porous scaffold for use as load-bearing bone scaffolds. The glass amount and composition, and heat treatment condition were varied and their effects on the coating properties were investigated. An emphasis was put on fabricating a dense coating layer, and its feasibility was compared with pure HA coating from mechanical and biological points of view.

2. Materials and methods

2.1. Glass preparation

Three different compositions of glasses \([x\text{CaO} – (0.55 – x)\text{Na₂O} – 0.45\text{P₂O₅}, \: x = 0.3, 0.4, 0.5]\) were prepared using \(\text{P}_2\text{O}_5\), \(\text{NaH}_2\text{PO}_4\), and \(\text{CaCO}_3\) as starting materials. The precursors calculated at stoichiometric glass compositions were weighed and placed in a Pt/10%Rh crucible type 71040 (Johnson Matthey), and thermally treated in a furnace between 1100°C and 1250°C. The composition and thermal history of the glasses are shown in Table 1. Especially for the glass with 50% CaO content, care was taken in the melting history by applying a stepwise temperature increase from 1100°C to 1250°C at 30 min interval to melt completely. The resulting glass melts were quenched on a steel plate and then ground and sieved down to 25 μm to obtain fine glass powders.

2.2. CaP-glass composite coatings

Commercially available HA powder (Plasma Biotal, UK) and the obtained glasses were used for making composite coating slurry. The specific surface area of the HA and glass powders, measured by Brunauer, Emmett, Teller (BET) method (ASAP2010, Micromeritics, USA), was 43.6 and 6.3 m²/g, respectively. The scanning electron microscopy (SEM) morphologies of each powder are presented in Fig. 1. The two powders were mixed at various ratios [Glass/(HA + Glass) = 30, 40, 50% wt/wt] in an ethanol solution for 24 h. The total powder amount (HA + Glass) was 40% w/v with respect to the ethanol. As a substrate for coating, a ZrO₂ disc was used. The discs were prepared after sintering a ZrO₂
powder (3 mol% Y$_2$O$_3$, Cerac) at 1400°C for 6 h. The ZrO$_2$ discs were dipped into the slurry and spin-coated for 10 s, and then dried for 30 min. The dipping-to-drying steps were repeated three times to obtain a uniform and thick coating layer. After drying for 3 h, the discs were heat-treated as follows; heating up to 700°C at 50°C/min, further to final temperatures (800°C, 900°C, and 1000°C) at 10°C/min for 2 h, and then cooling down to room temperature at 1°C/min. The thermal history was determined from differential thermal analysis (DTA, Setaram, UK) (Table 2). For the purpose of comparison, pure HA was also coated on ZrO$_2$, after heat treatment at 1250°C for 1 h, a condition for consolidation of HA particles [13,14].

### 2.3. Characterization

The phase change during heat treatment of the coating layer was analyzed using X-ray diffraction (XRD, Philips, Holland) at a scanning rate of 0.6° 2θ/min. The morphology and the composition of the coating layer were characterized using SEM (Cambridge Ltd., UK) and energy dispersive spectroscopy (EDS) attached to SEM, respectively.

### 2.4. Adhesion strength test

The adhesion strength of the coating layer was tested with an adhesion testing apparatus (Sebastian V, Quad Group, USA). A stud pre-coated 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 ~2 mm/min until the coating layer failed, and the bond strength was calculated from the maximum load recorded. For each condition, at least five samples were tested. After the bonding strength test, the failure surface of the specimen was examined with SEM and EDS.

### 2.5. Dissolution test

In order to observe the dissolution behaviors of the coating layer, each coating system was incubated in a
phosphate-buffered saline solution (PBS, tablet type, Aldrich, UK) at 37°C for up to 4 weeks. At predetermined periods of time, the sample was taken out and the ion concentrations in the solution were measured using Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES, Shimadzu, Japan). For each condition, three samples were tested.

2.6. In vitro cellular assessment

The HOS cells, derived from a human osteosarcoma, were used in this study. The cells were cultured in a humidified atmosphere of 5% CO₂ 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), 2 mM L-glutamine (Gibco), 50 IU/ml of penicillin and 50 mg/ml of streptomycin (Gibco). For sub-culture, the cells were washed with PBS (Gibco) and detached with trypsin-EDTA solution (0.25% trypsin, Gibco) with incubation at 37°C for 10 min. The cells were washed, centrifuged, and resuspended in the medium for further reseeding and growing tests.

The MTT assay 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/purple formazan product. The HOS cells were plated at a density of 1 × 10³ cells/well on the specimens and cultured for 6 h in 0.1 ml medium to allow the cells to attach (reference as proliferation). After that, the samples were placed into another plates and cultured for 2 and 5 days in 1.5 ml medium. The cell proliferation was evaluated following the MTT assay Kit CT020. The color product was measured at 570 nm (A570) using a spectrophotometer. Each test was performed in triplicate. The morphology of the proliferated cells was observed with SEM after fixation with glutaraldehyde (2.5%), dehydration with graded ethanol (70%, 90%, and 100%), and critical point drying.

For the assessment of alkaline phosphatase (ALP) activity, cells were cultured for 10 days. ALP activity

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**Fig. 2.** XRD patterns of the CaP-glass composite coatings on ZrO₂, obtained with variations in glass amount and composition, and heat treatment (HT) temperature: (A) 40 wt% glass and HT at 800°C, with different glass composition; (B) 50 wt% glass and HT at 800°C, with different glass composition; and (C) 50 wt% glass and 30 mol% CaO, at different HT temperatures. (∗) HA, (●) TCP, (■) DCP, and (□) ZrO₂.
was assayed by the detection of p-nitrophenol product from a p-nitrophenyl phosphate substrate, following the procedure in Sigma Kit 104. The absorbance was measured at 410 nm using a spectrophotometer, and ALP activity was calculated from a standard curve after normalizing to the total protein content.

3. Results

3.1. Phase and composition

Figs. 2A–C show the XRD patterns of the composite coatings on ZrO$_2$ obtained at different conditions. Fig. 2A shows the data on the composite coatings with glass amount of 40 wt% and heat-treated at 800°C for 2 h. For all glass compositions, three kinds of CaP phases, i.e., HA, tricalcium phosphate (TCP), and dicalcium phosphate (DCP), appeared. With increasing CaO content in the glass composition, the HA peaks increased while the TCP and DCP peaks decreased. Fig. 2B shows the data on the composite coatings with glass addition of 50 wt% and heat-treated at the same condition as Fig. 2A. The same CaP peaks were observed, and the trends with composition change were similar to those containing 40 wt% glass. However, compared to the 40 wt% glass coatings, the TCP and DCP phases were more apparent at the corresponding glass composition. Fig. 2C shows the data on the composite coatings after heat treatment at difference temperatures. The glass was 40 wt% in amount and had 40 mol% CaO in its composition. For all temperatures, the same coating phases were also formed. However, the increment in temperature increased the peak intensities of DCP while decreased those of HA, with little effect on TCP.

3.2. Morphology

Figs. 3A–C show the typical SEM morphologies of the CaP-glass composite coatings on ZrO$_2$, after heat treatment at 800°C for 2 h in air. The amount and composition of the glass were 50 wt% and 30 mol% CaO, respectively. The composite coating surface was highly dense and rough (Figs. 3A and B). At high magnification, the layer was observed to consist of lots of faceted crystals (area ‘C’ with sizes of several microns and the surrounding glassy phases (area ‘G’). The EDS composition analysis of the areas showed that higher amounts of Ca were detected within area ‘C’; the Ca/P ratios were in the range of about 1–1.6 in area ‘C’ but much less (0.6–0.7) in area ‘G’. The variation in Ca/P within area ‘C’ was due to the mixed crystalline phases (HA, TCP, and DCP). The compositional ranges observed were quite comparable to estimated Ca/P ratios of crystallines (1–1.7) and initial glass (0.43). The cross-sectional view showed the formation of a highly dense and homogeneous coating layer on ZrO$_2$ substrate.

![Fig. 3. SEM morphologies of the CaP-glass composite coating on ZrO$_2$: (A,B) surface and (C) cross-sectional view. The glass amount and composition used were 50 wt% and 30 mol% CaO, respectively, and the HT temperature was 800°C.](image-url)
The layer had a thickness of approximately 30–40 μm, and appeared to tightly adhere to the substrate. There were no cracks or delaminations within the coating layer and at the coating/substrate interface.

When the pure HA was coated on the ZrO$_2$ substrate without the addition of glass, the coating structure was quite porous (Figs. 4A and B), in marked contrast to the CaP-glass composite coatings.

### 3.3. Mechanical properties

The adhesion strengths of the CaP-glass composite coatings were evaluated using an adhesion test fixture. Fig. 5 shows the adhesion strength of the coating layer with respect to ZrO$_2$ substrate after heat treatment at different temperatures. The amount and composition of the glass used were 50 wt% and 30 mol% CaO, respectively. The test groups were chosen for their maintaining dense morphologies and offering different coating compositions. Pure HA coating heat-treated at 1250°C was also tested for comparison. The strengths of the composite coatings were as high as 35–40 MPa at all heat treatment temperatures, showing considerable improvements by 60–80% when compared to the pure HA coating (~22 MPa).

Figs. 6A and B show the detached surface of the composite coating heat-treated at 1000°C for 2 h, after adhesion strength test. In the composite coating, there was a mixed failure mode, i.e., within the coating (area ‘C’), at the coating/substrate interface (area ‘I’), and within an epoxy (area ‘E’) (Fig. 6A), as was confirmed by the EDS composition analysis. The relatively large failure areas related with coating and epoxy confirmed that the coating/substrate interface was quite strong. Such a fact was well illustrated at the magnified scale, showing that the coating fragment adhered to the ZrO$_2$ substrate quite firmly (Fig. 6B). However, the pure HA coating was detached quite clearly with only a slight trace on the substrate, as was previously observed [13].

### 3.4. In vitro solubility

The in vitro dissolution behaviors of the composite coatings after heat treatment at different temperatures were investigated after immersion in a PBS solution for periods up to 4 weeks. The amount and composition of the glass used were 50 wt% and 30 mol% CaO, respectively. For the purpose of comparison, the pure HA coating was also tested. Fig. 7 shows the concentration of cations released from the coating layers. All the composite coatings exhibited much higher Ca$^{2+}$ ion releases when compared to the pure HA coating. Among
the composite coatings, the sample heat-treated at higher temperature appeared to release higher Ca\(^{2+}\) ions, however, little significant difference was observed in the Na\(^{+}\) ion release. The release profiles of the cations (Ca\(^{2+}\) and Na\(^{+}\)) from the composite coatings were relatively high at short periods and the rates were slowed down with increasing the time.

### 3.5. In vitro cell responses

The osteoblast-like cellular responses to the composite coatings were assessed in terms of cell proliferation and differentiation. The morphologies of the HOS cells cultured on the CaP-glass composites for 2 and 5 days are shown in Figs. 8A and B, respectively. The morphology on pure HA after culturing for 2 days was compared in Fig. 8C. The amount and composition of the glass used were 50 wt% and 30 mol% CaO, respectively, and the heat treatment was carried out at 1000°C for 2 h. When cultured on the composite surface for 2 days, the cells spread and grew well (Fig. 8A). After culture for 5 days, much larger number of cells grew actively on the surface (Fig. 8B). Especially, the coating surface at 5 days culture appeared to be pitted and rough, presumably due to the selective dissolution of the surface. Compared to the composite coating, the cells were slightly contracted on the rough and micro-porous HA surface (Fig. 8C).

The cell proliferation was evaluated using an MTT assay, and is shown in Fig. 9. Data were represented as relative values to the initial cell density (at 6 h) after culturing for 2 and 5 days. Two types of composite coatings were used, with difference only in their heat treatment temperatures (800°C and 1000°C), while with
the same amount and composition of glass (50 wt% and 30 mol% CaO, respectively). Pure HA coating on ZrO₂, and bare-ZrO₂ substrate were tested for comparison. The cells on the composite coatings proliferated favorably, showing considerable increments with culture time. All the samples tested showed similar cell proliferation behavior.

The cell differentiation was assessed in terms of the ALP activity of the cells, as shown in Fig. 10. The ALP activities on the composite coatings were much higher than those on Thermanox control or ZrO₂ substrate, and even comparable to those on pure HA coating. There was little significant difference between the composite coating samples.

4. Discussion

The CaP coatings on a ZrO₂ substrate were introduced to enhance biocompatibility in terms of
bioactivity and mechanical strength for use as hard tissue implants. Previously, pure HA and its composites with other CaP ceramics (HA–TCP and HA-FA) were coated on a ZrO2 scaffold using a powder slurry method [13–17]. The HA coating layer had favorable cellular responses in terms of proliferation and differentiation. However, since the HA coating layer was highly porous, a question might be raised on its feasible usage as a load-bearing part due to the possibility of weakening of the coating layer. Moreover, the high processing temperature (>1200°C) used to consolidate the HA powders decreased the bioactivity of the HA layer [14,15].

Practically, when compared to human hard tissue, the synthetic HA is known to have much higher crystallinity and lower solubility. The bioactivity needs to be increased in order to improve the bone-forming ability and shorten the osseointegration period. Practically, the HA–TCP biphasic and glass-reinforced HA composite ceramics were reported to improve the cellular responses compared to pure HA, due to their having enhanced bioactivities. Recently, the strength and bioactivity of the slurry coating layer were somewhat enhanced by over-coating a sol–gel-derived HA layer on the slurry coating layer [15]. Based on those findings, the current research was focused on fabricating a more bio-compatible coating system in terms of mechanically and biologically. The added glass was expected to enhance bioactivity and aid solidification of the HA particles.

In the fabrication of phosphate-based glasses (P-glasses), the CaO–P2O5 binary glass was difficult to obtain especially at high CaO content (Ca/P > 0.6) due to high processing temperature and crystallization [24,25]. Therefore, glass modifiers such as Na2O, MgO, TiO2, and CaF2 should be added to certain amounts [24,25,29]. In this study, the ternary glass CaO–P2O5–Na2O with difference in CaO/Na2O ratio at a fixed P2O5 (45mol%) was chosen for its predictable thermal and dissolution properties depending on the composition and the well-established fabrication conditions [18–22].

The coating process was carried out to optimize the effect of glass addition, i.e., the glass amount and composition, and thermal treatment conditions were carefully controlled. The addition of glasses and further heat treatment resulted in the formation of additional CaPs, such as DCP and TCP. Noticeably, their appearances differed according to the glass amount, composition, and heat treatment temperature. When the glass amount was high, the CaO content was low, and the temperature was elevated, the amounts of DCP and TCP phases increased, and between them the DCP increase was more manifest.

At this point, it is postulated that there would be material transfer between HA and glass at elevated temperatures. This was possible when the glass was above melting point and became liquid phase. At the interface between the glass and HA, the new crystallites, mainly DCP and TCP, were formed during cooling. The differences in chemical potential of Ca2+ and PO43− ions between the glass and HA should be the main driving force for mass transfer. When considering the relative Ca/P ratios among the glass and CaP crystallines, the phenomena would be well understood. The Ca/P ratios of the glasses used (0.43–0.71) were much lower that that of the HA (1.67), and the DCP and TCP phases had Ca/P ratios about in the middle, i.e., 1 and 1.5, respectively. In fact, these CaP crystalline phases were reported to form from the P-glass single phase. However, in the P-glass ceramics, instead of DCP or TCP, others with lower Ca/P ratios (much less than 1), such as 4CaO·3P2O5 and Ca(PO3)2, were readily observed. Compared to the pure glass coating, the composite coatings maintained the HA initial phase to a considerable amount, and only crystallines with relatively higher Ca/P ratios were observed. Since the biocompatibility of HA and TCP crystals were well guaranteed, and the DCP was also observed to be bioactive [29], the composite coatings were preferred to the glass single coating. The existence of HA should play a role in the formation of the crystalline phases by means of interacting with the glass. In reality, the appearance of the new phases and the decrease of the HA phase suggested that the crystalline phases were not just from the glass composition alone.

These material transfer and crystallization phenomena were reflected in the phases depending on glass amount and composition, and processing temperature. The increase of glass amount made it easier for materials to transfer, and consequently produced a higher amount of TCP and DCP by consuming the HA phase. The temperature increment also had a similar effect on the crystallization. The glass with lower CaO content also affected similarly since the lower CaO glass had the lower Tg and Tm (Table 2), and thus was less viscous. After all, the lower Ca/P ratio acted as increasing the TCP and DCP phases instead of HA.

This dynamic coating process aided by the glass liquid phase rendered the coating structure to be highly dense (Fig. 3), being in marked contrast to the HA single coating (Fig. 4). Of special to note was the significant improvement in the coating strength caused by the dense structure. The value as high as 40MPa was quite noticeable in the CaP coating system. Kasuga et al. also obtained a strong glass coating on a Ti-alloy, assisted by a reaction between coating and substrate [27,28]. The Ti-based metals and CaP ceramics are known to react readily relatively at low temperatures (<800°C). However, compared to Ti, the ZrO2, a substrate used in this study, has high thermal stability to CaP, since the reaction temperature is usually over 1100°C [13,14,31,32]. Hence in this system, there should be little reaction between the coating and substrate, if any, at an atomic scale within the temperature range covered in...
this study (800–1000°C). Unlike the Ti-based metals, the ZrO2 ceramic substrate can play a positive role in obtaining a good bonding with CaP layer without the assistance of interfacial reactions. Such a postulation was understood from the previous work, reporting higher bonding strength of HA sol–gel layer deposited on ZrO2 rather than on Ti substrate [11,12]. In which, the HA layer also bonded tightly without the occurrence of chemical reactions. In order to investigate the substrate effects in detail, the HA-glass composite coating on Ti is currently underway. Another point to be highlighted is the effect of HA powder component on the coating integrity. The composite coating was obtained via partial reactions between the glass and HA powder. During the process, the coating layer could become much denser by the glass liquid phase. However, the HA powder itself should not be consolidated due to the low processing temperature and loose powder compaction. Therefore, there should be some pores at a nano-scale in the region that the glass and HA did not react, and this should act to relieve the thermal mismatch between the coating and substrate. Practically, such a phenomenon is impossible in the glass single coating. Although residual pores exist, compared to pure HA coating, the glass-aided composite coating was highly dense and retained considerably enhanced coating integrity. Such a high bonding ability of the composite coating to ZrO2 can facilitate the potential uses of ZrO2 ceramic in load-bearing hard tissue applications.

Along with the mechanical strength, cellular responses to the composite coatings were observed in terms of in vitro cell proliferation and differentiation. The cells grew and spread actively on the composite coatings. More importantly, the cells on the composite coatings expressed higher ALP activities when compared to ZrO2 substrate, and even comparable to pure HA coating. At this point, the cellular responses relating to the dissolution behaviors should be addressed. Practically, the composite coatings had higher bioactivity compared to pure HA coating (Fig. 7). Within the culture medium, the composite coating surface became much rougher when cultured for 5 days (Fig. 8B). The newly formed TCP and DCP as well as the remaining glass phases should increase the dissolution of the coating layers. Therefore, compared to HA, the composite coatings were more dynamic in both chemical and physical status in responding to the cells, and those things could affect the in vitro cellular properties [33,34]. However, there were little significant differences between pure HA and composite coatings in terms of proliferation and ALP expression levels. The only difference was the cell growth morphology; cell membranes were a bit flattened in the composite coatings, and this phenomenon was previously observed in the more bioactive sol–gel coating samples [15]. The concurrent changes in chemical and physical status might alter the cell behaviors [35,36]. Further studies on the coating system remain as to in vitro tests by human primary cells and in vivo performance by anima test.

5. Concluding remarks

Hydroxyapatite and phosphate glass composites were coated on a ZrO2 substrate to improve biocompatibility. After heat treatment over 800°C, triphasic calcium phosphates (HA–TCP–DCP) and glassy phases were obtained. The coating layer was highly dense and bonded to ZrO2 substrate tightly. The adhesion strength of the coating layers was as high as ~40 MPa, with an improvement of ~80% compared to that of pure HA coating. The osteoblast-like cells responded to the composite coating quite actively, and their proliferation and ALP activities were improved as compared to bare ZrO2 substrate, and were comparable to the pure HA coating. The HA-glass composite coating on a ZrO2 substrate finds high potential for use as hard tissue implants from its morphological integrity, improved mechanical strength and cellular responses.

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