A hydroxyapatite/fluorapatite (HA/FA) double layer was coated on ZrO2 by a powder slurry method. The FA layer between the HA layer and ZrO2 substrate was effective in suppressing the reaction between HA and ZrO2. The rheological properties of the slurry and the thermal treatment conditions were optimized. Addition of small amounts of tri-ethyl phosphate dispersant and polyvinylbutyral binder (up to 5 wt%) was effective in reducing the slurry viscosity, even at high loadings of HA powder. Each layer (HA, FA) was deposited on ZrO2 repeatedly to induce a uniform layer, and the final heat treatment was carried out above 1200°C to consolidate the coating layer. During this process, without the FA-intermediate layer, a severe reaction between HA and ZrO2 occurred to form tri-calcium phosphate and CaZrO3 products. However, the presence of the FA layer between HA and ZrO2 effectively suppressed the reaction up to 1300°C. The obtained HA/FA double coating layer was micro-porous and relatively rough, but was firmly adhered to the ZrO2 substrate, having a bonding strength of approximately 25 MPa after heat treatment above 1200°C. The osteoblast-like cells cultured on the HA/FA coating layer spread and proliferated favorably, having a cell proliferation rate comparable to that of a plastic control and HA bulk ceramic.

I. Introduction

Because of its excellent biocompatibility, hydroxyapatite [HA, Ca10(PO4)6(OH)2] ceramic has been used in orthopedics and dental surgeries in the form of bulk ceramics as well as powders or granules. However, the low mechanical properties of bulk HA restricted its wider applications in the load-bearing compartments. Therefore, HA coatings on metals (Ti, Ti-based alloys, and stainless steel) or bioinert ceramics (ZrO2 and Al2O3) have been introduced to achieve a high mechanical strength as well as a suitable biocompatibility of the system.

The currently used coating methods, such as plasma spraying, sol-gel synthesis, physical vapor deposition, and immersion technique, have their own advantages as well as shortcomings. The coating layer by the plasma-spraying technique is thick (~50–200 μm) and relatively dense; however, phase instability and non-uniformity are some of its weaknesses. The films synthesized by sol-gel and physical vapor deposition methods are relatively uniform and the fabrication method is simple; however, the layer is too thin (~1 μm) to be applied for long-term usage. Apatite layers obtained by an immersion technique were quite porous and weak, raising concerns about the longevity and stability of the layer. In this respect, relatively thick coating layers (>10 μm) retaining high chemical and thermal stability are necessary for long-term biological applications. Moreover, a simple and low-cost fabrication method is required for real applications. A previous study proposed that a thick HA coating might be fabricated by the HA powder slurry by tailoring the slurry conditions and thermal treatment history. The most important considerations were the properties of the coating slurry containing polymeric additives and the thermal treatment conditions, such as the burnout of polymers and the densification of the coating layer.

Many attempts to fabricate HA composite with ZrO2 revealed that an extensive reaction occurred between them. The reaction products (z- or ß-tricalcium phosphate (TCP) and Ca-ZrO3) were found to be undesirable for mechanical properties as well as for biological stability. Therefore, in an HA/ZrO2 coating system, the degradation of the HA coating layer remains a primary concern. Recently, fluorine (F)-substituted HA composites with ZrO2 were successfully fabricated without reaction problems. The excellent chemical stability of fluorapatite [FA, Ca10(PO4)6F2] with respect to ZrO2 has effectively prevented the decomposition reaction. In practice, FA has attracted attention because of its slow bio-resorption as well as its comparable bone fixation and ingrowth compared with HA.

In this study, an FA layer was coated between the HA layer and ZrO2 substrate to improve the integrity and stability of the HA coating on ZrO2. The fabrication method of the HA/FA double-layer coating on ZrO2 is addressed in relation to slurry properties as well as coating and thermal treatment conditions. The mechanical and biological performances of the coating system are briefly assessed in terms of bonding strength and in vitro cell proliferation behavior, respectively.

II. Experimental Procedures

(1) Preparation of Coating Slurry

A commercially available HA powder (Alfa Aesar, Ward Hill, MA) was used to make an HA slurry for coating. The HA powder was mixed with a controlled amount of tri-ethyl phosphate (TEP, PO(C2H5O)3, Aldrich, St. Louis, MO) in 100 mL ethanol, and the mixture was stirred vigorously with a magnetic spin bar. After stirring for 24 h, polyvinylbutyral (PV, Aldrich) was added slowly to the slurry and the mixture was stirred again for 48 h. TEP and PV were used as a dispersant and a binder, respectively, and the amounts were changed from 0.5 to 10 wt% with respect to HA. The amount of HA powder was varied from 10 to 50 wt% relative to the ethanol.

The FA powder was fabricated using TCP (Ca10(PO4)3, Merck Co., Darmstadt, Germany) and calcium fluoride (CaF2, Aldrich) by reacting them at 1000°C for 3 h, as follows:

$$3\text{Ca}_3(\text{PO}_4)_2 + \text{CaF}_2 = \text{Ca}_{10}(\text{PO}_4)_6\text{F}_2$$
Procedures for preparing the FA slurry were the same as the case of HA as described above.

(2) HA/FA Coating on ZrO2
The substrate (ZrO2) was prepared as a disk shape after molding and sintering a high-purity ZrO2 powder (with 3 mol% Y2O3, Cerac Inc., Milwaukee, WI) at 1400°C for 3 h. To fabricate an HA/FA double coating layer on ZrO2, the FA layer was coated first, and then HA was coated on the FA layer. After cleaning and drying, the ZrO2 disk was immersed into the FA slurry at a dipping/pulling speed of 0.5 mm/min, and then dried at room temperature and subsequently at 80°C for 3 h. The dried sample was heat treated at 800°C for 3 h and further at various temperatures (1000°C–1300°C at 100°C intervals) for 3 h to prepare an FA-intermediate layer onto ZrO2. Binder burnout temperature was selected as 800°C based on thermal analysis data, and a slow heating rate (2°C/min) was adopted up to 800°C to avoid an abrupt burning out of the polymeric precursors. A relatively thick and uniform FA layer was obtained by repeating this coating process. The FA-coated sample was dipped into the HA slurry and dried, heated under the same conditions, and then the procedure was repeated four times. The coating cycle of each layer was changed to obtain an optimum coating structure. For the purpose of comparison, an HA-coated ZrO2 disk without an FA layer was also prepared.

(3) Characterization and Bonding Strength Test
The rheological properties of the HA slurries were investigated at a constant temperature of 25°C using a rheometer (Rheostress 600; Thermo Haake Co., Karlsruhe, Germany). The viscosity of the slurries was determined at various shear rates (from 0.1 to 10 s⁻¹) for 60 s. To avoid solvent evaporation, silicone oil was embedded around the slurry after molding, and the viscosity was measured as a function of time at a fixed shear rate. The effects of the additions of dispersant (TEP; 0.5–10 wt%) and binder (PVB; 0.5–10 wt%) as well as the loadings of HA powder (10–50 wt%) on the viscosity were investigated.

To observe the sintering behaviors of the HA and FA powders, each powder was compacted separately in a metal die at a low pressure (~0.5 MPa), further pressed isostatically at 150 MPa, and then sintered at temperatures of 900°C–1400°C for 1 h in air. The density of the sintered body was measured by the Archimedes method. The phase and the morphology of the coating layer were evaluated with an X-ray diffractometer (XRD) and a scanning electron microscope (SEM), respectively.

The adhesion strength of the coating layer was tested with an adhesion testing apparatus (Sebastian V, Quad Group, Spokane, WA). A study, 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 2.69 mm diameter stud was pulled at a loading rate of ~2 mm/min until the failure of the coating layer, and the bonding strength was determined from the maximum load recorded.

(4) Cell Proliferation
For an assessment of the proliferation behavior of cells on the coating system, an MG63 cell line was pre-incubated in flasks containing Dulbecco’s modified Eagle’s medium (DMEM, Life Technologies Inc., Rockville, MD) supplemented with 10% fetal bovine serum (FBS, Life Technologies Inc.). Then, the cells were plated at densities of 5 × 10³ and 3 × 10⁴ cells/mL for proliferation rate calculation and cell morphology observation, respectively, on 24-well plates containing fabricated coating specimens and a cell culture dish (Thermanox; NUNC, Rochester, NY) as a control. After culturing for 3 days in an incubator humidified with 5% CO₂/95% air at 37°C, the cells were detached with a trypsin-EDTA solution, and the living cells were counted using a hemocytometer (Superior Co., Lauda, Germany). Each set of tests was performed in triplicate, and the data were normalized by taking the surface area into consideration.

To observe the morphology of the growing cells, SEM was performed after fixing the cells with glutaraldehyde (2.5%), dehydrating with a graded series of ethanol (70%, 90%, and 100%), and critical point drying in CO₂.

III. Results

(1) Rheological Properties of Slurries for Coating
The viscosities of the HA slurry, with and without the addition of a TEP dispersant, with respect to the shear rate are shown in Fig. 1. The loading of HA powder was fixed at 30 wt% relative to the ethanol. In pure HA slurry, the viscosity exhibited a typical shear-thinning behavior. A small addition of TEP (0.5 wt%) decreased the viscosity of the HA slurry sharply in the 0–10 s⁻¹ range, illustrating the effectiveness of TEP as a dispersant. On increasing the TEP content up to 5 wt%, the viscosity continued to decrease at all shear rates tested. However, there was little, if any, change in viscosity with further additions, suggesting the appropriate amount of TEP addition to be ~5 wt%.

The effect of PVB binder addition on the HA slurry is shown in Fig. 2. The loading of HA powder and the TEP was fixed to 30 and 5 wt%, respectively. When the shear rate was low (0.1 s⁻¹), small additions of PVB (up to 5 wt%) were effective in decreasing the viscosity, while further additions gradually increased the value. When the shear rate was 1 s⁻¹, a similar trend was observed, even though the degree of decrease in viscosity was not so large. At a high shear rate (10 s⁻¹), the viscosity remained constant up to 5 wt% and increased at higher additions of PVB. This result clearly indicates that only small amounts of PVB are effective in reducing the viscosity of the slurry.

Figure 3 shows the viscosity of the HA slurry as a function of the HA powder loading into ethanol at a constant shear rate of 1 s⁻¹. The added amounts of TEP and/or PVB were 5 wt% relative to the HA powders. Regardless of the TEP and/or PVB addition, the viscosity of HA slurries increased with increasing HA loadings. In the pure HA slurry, the viscosity increased sharply at HA loadings of ~30–50 wt%. However, the addition of 5 wt% TEP suppressed any significant increase in the viscosity even at HA powder loadings up to 50 wt%. In the slurry containing both TEP and PVB of 5 wt%, the increase in viscosity was much slower than that in pure HA slurry, but slightly higher than that in slurry containing TEP only.

(2) Consolidation and Stability of Coating Layer
To determine the thermal treatment temperatures, the sintering behaviors of HA and FA were monitored first, as shown in

![Fig. 1. Viscosity change of hydroxyapatite (HA) slurry with and without various additions of triethyl phosphate, presented with respect to shear rates from 0.1 to 10 s⁻¹. Loading of HA powder was fixed at 30 wt% relative to ethanol.](image-url)
Fig. 4. For both HA and FA, the densities increased with sintering temperature, reaching nearly theoretical values above 1200°C. These data indicated that the final thermal treatment temperature of the HA and FA slurry coating should be between 1200°C and 1300°C in order to obtain a dense and strong coating layer.

When HA was coated on ZrO₂ without the FA-intermediate layer, high-temperature heating resulted in a severe decomposition of HA, as manifested by Figs. 5(A)–(D). There was little decomposition of HA after heat treating at 1000°C (Fig. 5(A)). However, as the temperature increased to 1100°C, a considerable degree of HA decomposition occurred, with a large amount of β-TCP being produced (Fig. 5(B)). Further increases in temperature up to 1200°C and 1300°C resulted in the formation of CaZrO₃ and/or α-TCP, as shown in Figs. 5(C) and (D), respectively.

In contrast to the active decomposition of HA, when the FA layer was present between HA and ZrO₂, the decomposition of HA was effectively suppressed or reduced, as shown in Figs. 6(A)–(D). No decomposition was detected by the XRD analysis after heat treatment at 1000°C and 1100°C, as shown in Figs. 6(A) and (B), respectively. Only a trace of β-TCP at 1200°C (Fig. 6(C)), and somewhat more at 1300°C (Fig. 6(D)), was observed. However, when compared with the direct coating of HA on ZrO₂, the degree of decomposition above 1200°C is negligible.

(3) Structure and Bonding Strength of the Coating Layer
Figures 7(A)–(C) show the microstructures of FA coating layers on ZrO₂ at different heat-treatment temperatures and coating cycles. After heat treating at 1100°C (Fig. 7(A)), the FA particles were not fully consolidated apparently due to the insufficient sintering temperature, as was noted in Fig. 4. However, after
heating at 1200°C (Fig. 7(B)), the FA particles were consolidated to form a network while parts of the ZrO2 substrate surface were still uncovered with the coating layer. By repeating the coating process twice, the substrate was fully covered with the FA coating layer (Fig. 7(C)).

Figures 8(A)–(C) show the morphologies of HA coating onto the FA pre-coated ZrO2. After single coating and heat treatment at 1200°C, the HA layer covered the substrate only partially, as shown in Fig. 8(A). By repeating the coating cycle once more, the HA layer uniformly covered the whole surface of the FA-coated ZrO2 (Fig. 8(B)). The cross-sectional morphology of the HA/FA double layer on ZrO2 is shown in Fig. 8(C). The thicknesses of the HA and FA layers were about 20–30 and 5–10 μm, respectively, confirming the formation of a relatively thick coating layer, which might be useful for long-term applications. Moreover, the two layers were bonded quite tightly and no cracks or delamination were observed between the layers.

The bonding strength of the HA/FA double layer on ZrO2 with respect to the final heat-treatment temperature is shown in Fig. 9. The strength was quite low (<10 MPa) at temperatures up to 1150°C, but increased sharply at 1200°C (22 MPa). The strength remained high up to ~1300°C, but decreased slightly at 1400°C.

4. Cellular Response

As a preliminary biological test of the HA/FA coating on ZrO2, the in vitro cell proliferation behavior was evaluated using osteoblast-like MG63 cells. The morphology of the growing cells on the HA/FA-coated ZrO2 after culturing for 3 days is shown in Figs. 10(A) and (B). The MG63 cells were flattened and intimately adhered to the rough coating surface. The membranes spread in an elongated shape. Some spherical cells appeared to undergo a cell division (arrowed).

The number of cells proliferated on the coating system was about the same as those on the culture dish or HA bulk ceramic, as shown in Fig. 11. Such a comparable proliferation rate indicates the favorable cell viability of the coating layers.

IV. Discussion

In this study, we addressed the processing conditions, mechanical properties, and biological performance of a relatively thick HA coating layer onto ZrO2 by using a powder slurry method. In the process, understanding and controlling of the slurry characteristics, as well as of the thermal treatment behavior, were found to be essential. Generally, ceramic powders in the slurry are known to be agglomerated due to van der Waals attraction between particles, and the use of a polymeric dispersant is recommended.18–22 The addition of a small amount (5 wt%) of TEP as a dispersing agent considerably decreased the viscosity of the slurry (Fig. 1). This decrease in viscosity implies a less agglomerated state of the HA powder, and this was possible by the adsorption and electrostatic stabilization mechanism induced by the TEP. The TEP additive, due to its polarity, can easily adsorb onto the HA, which surely have a bunch of adsorption sites, consisting of ionic groups.23,24 When adsorbed onto the surface of HA powders, the TEP is expected to exert electrostatic stabilization rather than steric hindrance as the TEP has a degree of polarity and short ethyl groups at the end.25

Along with the dispersant, the addition of a PVB binder is needed for stable thermal treatment because during the early
stage of heat treatment, the PVB sustains the initial HA coating shape and structure, and also prevents the powder from detaching off the substrate. The appropriate amount of PVB appeared to be less than 5 wt%, a range within which the viscosity did not increase significantly for all shear rates tested (Fig. 2). Unlike TEP, the PVB is non-ionic and consists of long polymeric chains; hence, higher additions of PVB should increase the viscosity due to the decrease in adsorption efficiency to the HA particles, i.e., the excess PVB chains would interact with each other and be deleterious to the slurry property. For this reason, the PVB is well known as a binder, normally increasing the viscosity of ceramic slurries.\textsuperscript{25,26} However, at small additions, it could also reduce the viscosity, by adsorbing onto the HA surface efficiently and rendering the HA particles to be in steric repulsion. The adsorption of the PVB (non-ionic long chain) on HA would be different from that of TEP (ionic and short molecular); the PVB would adsorb mainly by an electrostatic energy, while an ionic interaction exerts to a high degree in the TEP adsorption. Hence, the TEP adsorption would be more efficient and strong as compared with PVB.

Fig. 8. Scanning electron micrograph morphologies of hydroxyapatite coating layers on fluorapatite pre-coated ZrO\textsubscript{2} after a final heat treatment at 1200 °C: (A) surface morphology with a single coating, (B) surface morphology with cycled coating twice, and (C) cross-sectional morphology with cycled coating twice.

Fig. 9. Bonding strengths of an HA/FA double layer coated on ZrO\textsubscript{2} presented as a function of final heat-treatment temperature.

Fig. 10. Scanning electron micrograph surface morphologies of MG63 cells proliferated on an HA/FA double-coated layer on ZrO\textsubscript{2} after culture for 3 days: (A) at low magnification and (B) at high magnification. Micrographs show the cross-sectional view at a low tilting angle. The arrow indicates the cell undergoing division.
The solid loading is also important to the quality of the final product as a higher loading generally leads to a higher packing density and minimizes the shrinkage of the coating layer at elevated temperatures. However, this is true only when the suspension is well dispersed without agglomerates or sedimentation.\(^{21}\) Even at high HA loadings (40–50\%), the viscosity of the TEP-containing slurry did not increase much, in contrast to the pure HA slurry, in which the viscosity increased drastically at loadings above 30\% \((\text{Fig. } 3)\). Based on the rheological property observations, a well-dispersed HA suspension containing polymeric precursors was prepared for further coating processes.

With viscosity-controlled slurries, the next step to obtain a coated specimen consisted of binder burnout and final consolidation of the coating layer. The binder burnout was performed at a relatively slow heating rate \((2 \, \text{C}/\text{min}) \) below 800\(^\circ\)C so as not to destroy the coating structure due to an abrupt polymer evaporation. After this, a relatively higher heating rate was used to avoid HA dehydration or decomposition above 800\(^\circ\)C. From the powder sintering data, it was confirmed that a temperature of at least 1200\(^\circ\)C was necessary to consolidate the HA and FA particles \((\text{Fig. } 4)\). In fact, the data in \text{Fig. } 4 are not directly related to the coating density as the full densification of the coating layer would be somewhat limited by the low initial packing density and the underlying \(\text{ZrO}_2\) substrate. However, the data would give important information at least on the sintering phenomenon occurring locally. Practically, when heat treated above 1200\(^\circ\)C, a considerable necking and sintering of the powders were observed to occur on the porous coating layer.

When there was no FA layer between the HA layer and substrate, heat treatment above 1200\(^\circ\)C caused a severe reaction between HA and \(\text{ZrO}_2\) to form TCP (\(\alpha\)- and/or \(\beta\)-) and \(\text{CaZrO}_3\) \((\text{Fig. } 5)\). The TCP formed at the interface is undesirable due to its possible bioresorption prior to HA dissolution and subsequent detachment of the coating layer from the substrate. To overcome this problem, an FA thin layer was inserted into the HA/\(\text{ZrO}_2\) interface and was observed to suppress the HA decomposition effectively \((\text{Fig. } 6)\). The higher chemical stability of FA with respect to \(\text{ZrO}_2\) was responsible for the prevention of a decomposition reaction.

To form the double-layered HA/FA coating uniformly, each coating step \((\text{for both HA and FA})\) was performed repeatedly. The single coating of each layer resulted in an uneven coverage, while the whole surface was covered uniformly after repeated coating \((\text{Figs. } 7 \text{ and } 8)\). Within the HA/FA double coating layer, there were no cracks or delamination, presumably due not only to the relatively micro-porous structure that alleviates thermal mismatch but also due to the composition and crystallographic similarity between HA and FA \((\text{Fig. } 8\text{C})\). In practice, the measured bonding strength of the HA/FA coating to \(\text{ZrO}_2\) substrate was as high as \(\sim 22\)–\(25\) MPa after heat treatment above 1200\(^\circ\)C. Such values are comparable to those obtained by plasma-sprayed coating, confirming the possible application to load-bearing parts.\(^7\) As expected, the bonding strength data behaved similarly with the sintering data \((\text{see Figs. } 9 \text{ and } 4)\), illustrating that the consolidation of the coating layer is crucial to obtain a strong coating layer. The slight decrease in strength at 1400\(^\circ\)C was deemed to be due to the degradation of the FA-intermediate layer to TCP and \(\text{CaZrO}_3\). At this point, a question might be raised on the ingress of the epoxy through the porous coating layer, and its influence on the bonding strength. The epoxy might be infiltrated into the coating layer to some extent, but it should not ingress so deep into the coating layer as to affect the final strength. Such problems are usually raised in thin and porous coatings. The SEM observation of the failure surface revealed that failures occurred both within the coating layer and at the coating/substrate interface, and there was no epoxy residue detected on the failure surface.\(^7\) Such a fact confirmed that the bonding strength was dominated by both the interface interlocking and the coating layer integrity. Hence, if there were epoxy ingress deep into the coating layer or even to the interface, and if the epoxy would affect the coating integrity or interface strength, the failure would occur mostly at the interface or within the coating accompanied by an epoxy residue. Therefore, there should be little, if any, effect of epoxy on the final coating strength.

The biological properties of the HA/FA coating system were investigated by the proliferation behavior of MG63 osteoblast-like cells. The cells grew, spread, and proliferated actively on the specimen, confirming that the HA/FA-coated \(\text{ZrO}_2\) system has no cytotoxic effect on the osteoblastic cells and retains a reasonable cytocompatibility. Moreover, similar proliferation of coating sample to the Thermanox control confirmed its reasonable cell viability.\(^3\) In many reports, the physical status of the surface, such as roughness, morphology, and porosity, is known to affect the cell attachment, proliferation, and differentiation.\(^31\),\(^32\) However, in this study, the relatively rough HA coating surface appeared to have no significant influence on the cell response at least at a proliferation level, as confirmed by the similar cell proliferation rate with the smooth HA bulk material. Along with the proliferation, the cells should further undergo a differentiation step for bone formation. Previously, the authors had observed that in a porous scaffold system, the alkaline phosphate (ALP) activity was expressed to a higher degree on the HA/FA-coated \(\text{ZrO}_2\) when compared with a pure \(\text{ZrO}_2\) scaffold, and suggested its improved biocompatibility. Although there would be morphological differences between the scaffold and disk on which the cells grow, the results would be applicable to this disk system.

V. Conclusions

A double layer, composed of outer HA and inner FA layers, was coated on \(\text{ZrO}_2\) by a powder slurry method. Slurry and coating parameters were controlled to obtain an optimal coating condition. Appropriate additions of TEP dispersant and PVB binder effectively reduced the viscosity of the slurry containing the HA powder up to 50 wt\%. Through repeated coatings and heat treatment above 1200\(^\circ\)C, a uniform HA/FA double layer was deposited on \(\text{ZrO}_2\). The FA-intermediate layer completely prevented the reaction between HA and \(\text{ZrO}_2\) up to 1300\(^\circ\)C. The bonding strength of the double layer was approximately 25 MPa after heat treatment above 1200\(^\circ\)C. The MG63 cells cultured on the coating system spread, grew, and proliferated favorably, demonstrating a comparable cell viability of the specimen compared with HA bulk ceramic.

References


