Bioceramics, including hydroxyapatite (HA) and bioactive glasses (BGs), have been used for the regeneration of damaged and defective hard tissues. As a main inorganic component of bone, HA is known to form a direct contact with tissues without the formation of a fibrous intermediate layer. However, the use of its synthetic form has often led to a limited regeneration rate of bone defects, which is attributable to its slow mechanical strength and toughness. The glass ceramics made from glasses (BGs), have been used for the regeneration of damaged and defective hard tissues. However, BGs are known to sometimes exhibit a more rapid dissolution rate than that required for the remodeling of human hard tissues. As such, when the crystalline phases with predetermined composition and amount are directly added to the BG composition, the composite is expected to possess good bioactivity and mechanical properties.

With this in mind, the authors aimed to produce HA–BG composites. Attempts to produce HA–BG composites have been made by several researchers; however, no successful work has yet been reported. In most cases, the HA and glass react vigorously to form unwanted crystalline phases, which normally have unidentified biological properties. It has been almost impossible to densify HA–BG composites by the conventional sintering process without the undesirable occurrence of a thermal reaction and phase degradation.

The purpose of this study was to produce densified HA–BG composites retaining excellent bioactivity by means of the hot-pressing technique. Applying a pressure during sintering is believed to assist the densification of HA–BG by reducing the sintering temperature below the thermal reaction region. The effects of the hot pressing on the densification and thermal reaction in the HA–BG composites are examined, and the in vitro biological properties of the composites are addressed.

I. Introduction

Bioceramics, including hydroxyapatite (HA) and bioactive glasses (BGs), have been used for the regeneration of damaged and defective hard tissues. As a main inorganic component of bone, HA is known to form a direct contact with tissues without the formation of a fibrous intermediate layer. However, the use of its synthetic form has often led to a limited regeneration rate of bone defects, which is attributable to its slow degradation rate and low bioactivity as compared with the biological form of apatite. Previous works have proven that synthetic HA in a dense form remains almost intact over several years in vivo. Some improvement in the bioactivity of HA has been obtained by modifying the apatite structure with other ions.

BGs have also been extensively studied and are known to achieve direct apposition to bone through the newly formed apatite layer at the interface. They are more dissolveable and have higher bone-forming ability than synthetic HA, which is due to their initial rapid surface reaction with the body fluid. However, BGs are known to sometimes exhibit a more rapid dissolution rate than that required for the remodeling of human hard tissues. They are also known to possess extremely poor mechanical strength and toughness. The glass ceramics made from the BGs are thus often preferred for their mechanical benefits and lower dissolution rate, because of the crystalline phases present therein (mostly apatite and wollastonite). However, it is not easy to control exactly the type and amount of the phases formed in the glass ceramics. As such, when the crystalline phases with predetermined composition and amount are directly added to the BG composition, the composite is expected to possess good bioactivity and mechanical properties.

The purpose of this study was to produce densified HA–BG composites retaining excellent bioactivity by means of the hot-pressing technique. Applying a pressure during sintering is believed to assist the densification of HA–BG by reducing the sintering temperature below the thermal reaction region. The effects of the hot pressing on the densification and thermal reaction in the HA–BG composites are examined, and the in vitro biological properties of the composites are addressed.

II. Experimental Procedures

HA powder (Alfa Aesar, Ward Hill, MA) was used after calcination at 1200 °C, followed by crushing and sieving down to 220 μm. The BG (designated “53S” by the Hench group) with a composition of 53.9% SiO2, 22.6% Na2O, 21.8% CaO, and 1.7% P2O5 by mole) was fabricated from the precursors (SiO2, Na2HCO3, CaCO3, and P2O5) and then used after crushing and pulverization. The HA and BG powders were wet mixed in ethanol at various ratios (30, 40, and 50 wt%). The mixture powders were dried, sieved, and packed in a carbon mold, and then densified by hot pressing at 30 MPa under nitrogen at various temperatures (600–800 °C) for 1 h. For the purpose of comparison, the HA–BG mixture powders were also pressurelessly sintered at various temperatures (600–1000 °C) for 1 h.

The phase and morphology of the composites were characterized by X-ray diffraction (XRD; M18XHF-SRA, MAC Science Co., Yokohama, Japan) and scanning electron microscopy (SEM; JSM6360, JEOL, Tokyo, Japan), respectively. The density of the specimens was measured by the Archimedes method. The mechanical strength of the composites was observed using a four-point flexural configuration following the method described in our previous study.

The bioactivity of the composites was assessed by the formation of apatite on their surface in a simulated body fluid (SBF) following Kokubo’s recipe. The specimens (10 mm × 10 mm × 0.2 mm) were incubated in SBF at 37 °C for up to 14 days, and then their surface was evaluated with SEM. Moreover, the
in vitro cellular responses to the HA–BG composites were assessed in terms of the cell proliferation by the MTT method and the detection of the expressed osteoblastic phenotype, alkaline phosphatase (ALP). The cell culturing and assaying protocols are fully described in our previous study. Three specimens were tested for each condition, and the data were compared using ANOVA at a significance level of $p < 0.05$.

III. Results and Discussion

(1) HA–BG Dense Composite by Hot Pressing

By the normal sintering process, it was almost impossible to obtain dense HA–BG composites with the compositions used in this study (30%–50% BG). However, hot pressing the composites significantly improved their sinterability. Figure 1 shows the typical SEM morphologies of the HA–30% BG composites obtained either by normal pressureless sintering (Fig. 1(A)) or hot pressing (Fig. 1(B)) at 750°C. The pressureless-sintered composite was extremely porous, while the hot-pressed one was completely densified. This marked contrast in the morphology depending on the sintering technique was also observed for the other compositions (40% and 50% BG). The densities of the hot-pressed composites, as measured by the Archimedes method, were observed to almost reach the theoretical values when the hot-pressing temperatures of $\sim 700^\circ$C–$800^\circ$C were used (apparent porosity less than $\sim 5\%$). On the other hand, the pressureless-sintered specimens revealed apparent porosities as high as $\sim 35\%$–$40\%$ depending on the composition and sintering temperature. Even when the sintering temperature was increased, the enhancement in the densification was only minimal (porosities of $\sim 30\%$ were still observed above 1000°C). This poor sinterability has been described elsewhere as being one of the critical problems associated with HA–BG composites.

The poorly densified body could not be used effectively as replacements for hard tissues, due to its handling difficulties in surgical operations and mechanical breakdown under in vivo conditions. As an a priori index, the four-point flexural strength of the specimens was measured. The hot-pressed composites showed almost twice the strength ($\sim 60$ MPa) of the pressureless-sintered specimens ($\sim 30$ MPa). This improvement was due not only to the enhancement in the densification afforded by the hot pressing but also due to the composite effect of the two components (HA and BG). This is comprehensible when considering that the strength of pure HA in the dense form (used as a control) was $\sim 40$ MPa, which was only slightly higher than that of the HA–BG porous composite, while being much lower than that of the HA–BG dense composite. Based on these results, it is considered that the production of the HA–BG dense composites using the hot-pressing technique improves the mechanical performance of HA.

Of special consideration in the HA–BG composites is the thermal reaction between the two components (HA and BG) and the consequent phase degradation. Figure 2 shows the phase evolution of the specimens as analyzed by XRD. When the HA–30% BG specimen was pressurelessly sintered at 750°C, a small amount of the $\beta$-tricalcium phosphate ($\beta$-TCP) phase was detected (Fig. 2(A)). However, it should be noted that the specimen obtained at this temperature was extremely porous. As the sintering temperature was increased to 1000°C, the porosity was reduced slightly; however, many secondary phases (sodium calcium silicate, wollastonite, and $\beta$-TCP) were formed along with the consumption of the apatite phase (Fig. 2(B)). Similar trends were observed in the other compositions, although there was a slight difference in the contents of the secondary phases.

On the other hand, hot pressing the HA–30% BG composition made it possible to obtain a dense composite with only a slight formation of a secondary phase (sodium calcium silicate) (Fig. 2(C)). In practice, this newly formed phase has already been reported to be bioactive and developed as a bioactive crystalline phase in biomedical coatings. The bioactivity of the hot-pressed HA–BG will be discussed in the following section.

(2) In Vitro Biocompatibility of HA–BG

The biological properties of the densified HA–BG composites were investigated in terms of their apatite-forming ability and osteoblastic cellular responses in vitro. For these evaluations, only the densified specimens could be used, as the cells could not attach and proliferate on the pressurelessly sintered porous specimens. For this reason, densified pure HA was used as a control and the HA–30% BG specimen was tested as a representative example of the developed composites.

Figure 3 shows the change in the surface morphology of the samples after incubation in SBF for 14 days. On the surface of
the pure HA specimen, some regions became white, while most appeared to be intact (Fig. 3(A)). However, in the HA–BG composite, the whole surface was changed with the formation of granular deposits. A closer examination revealed that some apatitic crystals were deposited on the pure HA, but the degree of deposition was mild in most regions. On the other hand, the HA–BG composite showed more noticeable deposition of the crystallines. In practice, in the HA–BG composite, the precipitation of Ca–P was observed to be initiated within 3 days, while more than 10 days were required for the precipitation to begin.

Fig. 3. Change in the surface morphologies of (A, C) pure hydroxyapatite (HA) and (B, D) HA-30% bioactive glass hot-pressed composite after incubation of the samples in simulated body fluid for 14 days.

Fig. 4. Osteoblastic cellular responses to hydroxyapatite (HA)–30% bioactive glass (BG) composite in direct comparison with pure HA: (A) cell viability assessed by the MTT method and (B) alkaline phosphatase (ALP) activity. Statistical significance in the ALP level was observed with a $p$-value of 0.0147.
on pure HA. Although, there are some limitations associated with this in vitro test, the data suggest that the HA–BG composite has better bioactivity than pure HA, as the apatite-forming ability in SBF is generally used as a bioactivity index and, thus, is often used to predict the bone-forming ability in vivo.

Along with this SBF test, the osteoblastic cellular responses on the HA–BG composite were examined. The viability of the cells as quantified by the MTT method was observed to be comparable on both substrates (pure HA and HA–BG composite) after 3 days of culturing, as shown in Fig. 4(A). However, the cells on the composite were observed to possess a significantly higher level of osteoblastic activity (p<0.05), as assessed by their ALP expression (Fig. 4(B)). As ALP is a well-known phenotype of osteoblastic cells, it is generally regarded as an important measure of osteoblastic functional activity and subsequent bone forming ability. Based on the present findings, it is anticipated that the HA–BG composite may have a higher potential for the recruitment of osteoblastic cells and subsequent bone-forming process. Although the present in vitro studies are meaningful as a first step in the assessment of the biological potential of our newly developed material, these in vitro results could not be illustrated in direct extrapolation to the clinical performance. As such, more in-depth evaluations, particularly an in vivo animal study, are warranted in the near future.

IV. Summary and Conclusions

Biomedical composites constituted of HA and BG were produced by means of hot pressing. The HA–BG composites obtained by the conventional pressureless sintering were extremely porous and showed considerable thermal reaction and phase degradation. In contrast, the hot-pressed HA–BG composites were almost completely densified at ∼700–800 °C with minimal thermal reaction. The HA–BG composite produced in this study possessed better apatite-forming ability and osteoblastic activity in vitro than pure HA. The present observations suggest that the densified HA–BG composite may be potentially useful in the bone regeneration field.

References