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In Vitro Bioactive Characteristics of Borate-Based Glasses with Controllable Degradation Behavior

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Silicate-based bioactive glasses undergo incomplete conversion to a calcium phosphate material after *in vivo* implantation, which severely limits their biomedical application. In this communication, novel borate-based glasses with controllable degradation behavior were developed and their bioactive potential was investigated *in vitro*. When immersed in a 0.02M K$_2$HPO$_4$ solution at 37°C, these glasses reacted to form a carbonate-substituted hydroxyapatite (c-HA) on their surfaces, indicating their bioactive potential. The conversion rate to c-HA was controlled by adjusting the B$_2$O$_3$/SiO$_2$ ratio in the glass composition. The results indicate the potential application of the borate-based bioactive glass as scaffold materials for bone tissue engineering.

I. Introduction

TISSUE engineering has shown great promise for creating biological alternatives to implants. A key part of the approach is the use of porous scaffolds that serve as templates for cell seeding and tissue regeneration.[1] In recent years, scaffold materials have been the subject of numerous investigations because they can significantly affect the cell seeding and growth both *in vitro* and *in vivo*. Many types of biomaterials, including ceramics and polymers, have been developed recently and some of them are now used for repairing and reconstructing diseased or damaged bones or tissue.[2,3] However, stoichiometric hydroxyapatite (HA) and 45S5 (silicate) bioactive glass are absorbed slowly or undergo incomplete conversion into a bone-like material after *in vivo* implantation, which severely limits their use in biomedical applications.[4] Bone regeneration applications require gradual resorption of the implanted biomaterials and concurrent replacement of the biomaterials by host bone. It is therefore desirable to develop materials with sufficient bioactivity and controllable degradation behavior to meet these requirements for a wide range of bone repair applications or for use as scaffolds for bone tissue engineering.

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Some silica-free borate glasses have been shown to possess low chemical durability, and to convert rapidly to calcium phosphate in physiological media.[5] However, it is expected that a controlled degradation rate would be required to match the growth rate of bone *in vivo*. The objective of the present work was to control the degradation rate of a borate glass through modification of the glass composition. A series of glass compositions with different B$_2$O$_3$/SiO$_2$ molar ratios were prepared by replacing part of B$_2$O$_3$ in the borate glass with SiO$_2$. The *in vitro* bioactive characteristics of the borate-based glasses were studied by determining the rate of formation of a calcium phosphate layer on the glass surfaces upon immersion in a dilate solution of phosphate ions. [Correction added after publication 13 December 2006: physiological media was corrected to a dilute solution of phosphate ions]

II. Experimental Procedure

Glass composition was selected as 6Na$_2$O · 8K$_2$O · 8MgO · 22CaO · 18xB$_2$O$_3$ · (54–18x)SiO$_2$ · 2P$_2$O$_5$ mol%. When $x = 0$, 1, 2, 3, the glass was designated as 0B, 1B, 2B, and 3B, respectively. Glass samples of each composition were prepared by melting reagent-grade chemicals in a platinum crucible at 1100°–1300°C and quenching between cold stainless-steel plates. The glasses were crushed and sieved to give particles with sizes in the range of 150–300 μm.

The assessment of *in vitro* bioactivity was carried out by statically immersing the glass particles (1 g) in 0.02M K$_2$HPO$_4$ solution (100 cm³) with a starting pH value of 7.0 at 37°C for different times up to 2400 hours. [Correction added after publication 13 December 2006: 24:00 was corrected to 2400] To simplify and quicken the reaction of glass particles with the solution, in this experiment the diluted phosphate solution was used instead of simulated body fluid (SBF). Whereas the concentration of PO$_4^{3-}$ ions in the solution was ~20 times that of the SBF, it was chosen to ensure that sufficient PO$_4^{3-}$ ions were available to react with all the calcium ions of the glass to form HA. After each immersion, the samples were removed from the phosphate solution, washed with deionized water, dried at 90°C, and the weight loss was measured, and then the samples were placed in the original solution again, as described in detail by Huang *et al*.[6] The pH value of the solution was determined each time after removing the sample from the solution for the weight loss measurement.

The formation of apatite-like layers on the surfaces of the glass particles without any treatment was determined using X-ray diffraction (XRD; Model D/max 2550 v, Rigaku, Tokyo,

Japan), Fourier transform infrared spectroscopy (FTIR; Model 1760-X. Perkin Elmer Corp., Norwalk, CT), and scanning electron microscopy (SEM; S-4700, Hitachi, Pleasanton, CA). XRD was performed using $CuK\alpha$ radiation ($\lambda = 0.15406$ nm) at a scanning rate of $1.8°/min$ in the 2θ range of $10°$–$80°$ according to the standard powder technique. FTIR was performed in the wavenumber range of 400–2000 cm^{-1} on disks prepared from a mixture of 2 mg of reacted powder (selected from the reacted glass surface) and 150 mg of high-purity KBr, so that the reacted product had a high concentration in the sample and the sensitivity of FTIR was increased.

III. Results and Discussion

Figure 1(a) shows the fractional weight loss ($\Delta W/W_0$) versus reaction time (t) for the four glass samples during immersion in the phosphate solution ($\Delta W = W_0 - W$, where W_0 is the initial mass of the glass and W is the mass at time t). For each glass, the weight loss increased with immersion time. The reaction rate and the maximum measured weight loss increased with increasing $B_2O_3:SiO_2$ ratio of the glasses. For the 3B sample, the measured weight loss was $>50\%$ after a reaction for 2400 hours, whereas it was only $<10\%$ for the 0B and the 1B samples. After 2400 hours of immersion, the weight loss curve of the 3B sample still showed a strong tendency to increase with time, implying that the dissolution and conversion reactions would apparently continue until the glass was almost fully reacted to form a calcium phosphate material. [Correction added after publication 13 December 2006: in the two preceding sentences, 24:00 was corrected to 2400] The remarkable difference in the reaction rate between silicate-based glass and borate-based glass is due to the difference in the glass network structure. It is well known that the main structural units of the borate glass network are $[BO_3]$ trihedron or chains of $[BO_3]$ triangles.[7] Because of its threefold coordination number, B cannot fully form a three-dimensional network structure when compared with Si. The borate glass, therefore, has a lower chemical durability, and hence a faster dissolution rate.

Figure 1(b) shows the variation in the pH of the phosphate solution as a function of immersion time for the four glass samples. The pH increased rapidly with time, eventually reaching a nearly constant value. Such an increase in the pH could favor the *in vitro* formation of HA. The pH value of the solution increased more rapidly with increasing B_2O_3 content of the glass, showing the same trend as the weight loss data. Upon immersion of the glasses into the phosphate solution, the dissolution and conversion reactions that led to weight loss of the glasses were also the same reactions that controlled the pH of the solution, so it may be expected that the weight loss and pH variation would show approximately the same tendency.[6] The
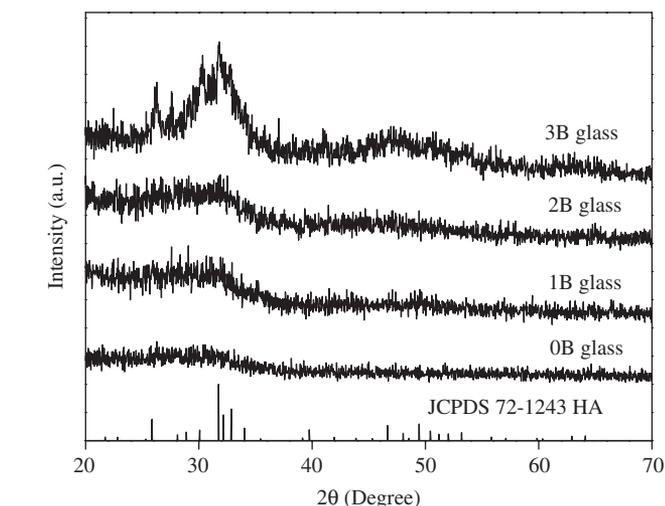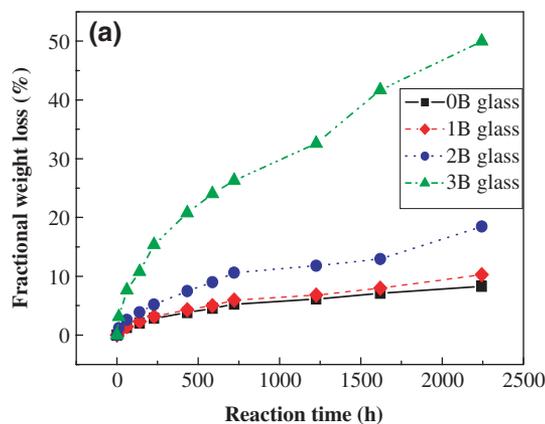

Fig. 2. X-ray diffraction patterns of the four glasses after immersion for 2400 hours in 0.02M K_2HPO_4 solution at 37°C. [Correction added after publication 13 December 2006: 24:00 was corrected to 2400] For comparison, the peaks corresponding to a standard hydroxyapatite (HA) are also shown.

increase in the pH resulted from differences in the acidity of BO_3^{3-}, HBO_3^{2-} or SiO_4^{4-}, $HSiO_4^{3-}$ ions, and the basicity of the alkali ions when the glass dissolved into the solution, accompanied by weight loss. [Correction added after publication 13 December 2006: in the preceding sentence, HO_3^{2-} was corrected to HBO_3^{2-}] A higher weight loss of the glass sample produced a higher pH of the phosphate solution. In Fig. 1(a), the weight loss was calculated from the number of released ions from glass, whereas in Fig. 1(b), the pH value was measured on the logarithm of the concentration of OH^- ion in the solution. The pH rise curves, therefore, were more similar than the weight loss curve for all the samples containing borate.

XRD patterns of the four glass samples after immersion in the phosphate solution for 2400 hours are shown in Fig. 2. [Correction added after publication 13 December 2006: 24:00 was corrected to 2400] The spectrum of a standard HA is also shown in this figure for comparison. For the 3B glass, the major peaks in the pattern corresponded to those of a standard HA, $Ca_{10}(PO_4)_6(OH)_2$ (JCPDS 72–1243), indicating the formation of HA during the reaction. The peaks for the 0B, 1B, and 2B glasses were not sufficiently prominent to be detected by the powder diffraction technique, presumably because this technique was not sensitive to the small amount of HA on the surfaces of the glass particles. This speculation was confirmed by the fact that for a glass with a composition similar to that of the 0B glass, and for reaction conditions similar to those used in the
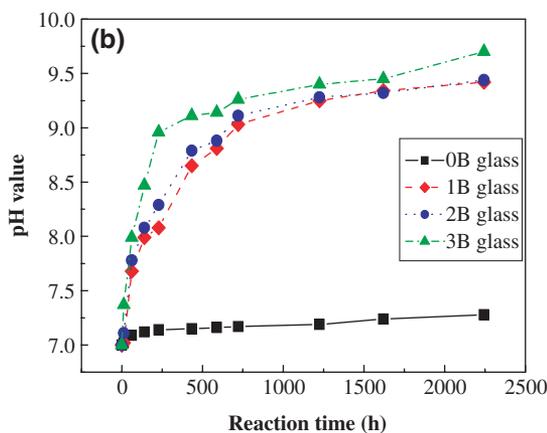

Fig. 1. Fractional weight loss (a) and pH values (b) versus reaction time for particles of the four glasses during immersion in 0.02M K_2HPO_4 solution at 37°C.
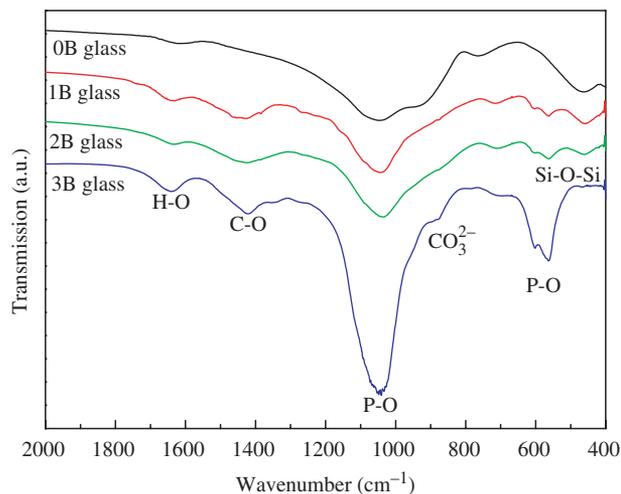
Fig. 3. Fourier transform infrared spectroscopic spectra of the four glasses after immersion for 2400 hours in 0.02M K$_2$HPO$_4$ solution at 37°C. [Correction added after publication 13 December 2006: 24:00 was corrected to 2400]

present work, the formation of HA was detected by the thin-film XRD technique.[8] With increasing B$_2$O$_3$:SiO$_2$ molar ratio, the increase in the strongest intensity corresponding to the (211) HA reflection was observed in the XRD patterns, suggesting that the borate-based glass possessed a higher bioactivity than that of silicate-based glass. For all cases, it is clear that the diffraction peaks of the reacted glasses were broader and less intense than those of the standard HA, which was an indication that the as-formed HA was poorly crystallized or that the crystallite size of the HA was on a nanometer scale, or a combination of both.

Infrared spectroscopy proved to be more sensitive than the powder XRD technique for monitoring the formation of HA on the surfaces of the glass particles, as the concentration of HA in the FTIR sample was higher than that in the powder XRD sample. Figure 3 shows FTIR spectra for the four glass samples after immersion for 2400 hours in 0.02M K$_2$HPO$_4$ solution. [Correction added after publication 13 December 2006: 24:00 was corrected to 2400] The resonances at 560 and 605 cm^{-1}, corresponding to P–O symmetric stretching vibration in PO$_4^{3-}$ groups in the HA lattice,[9] were observed for all four glasses, providing further indication for the formation of an HA layer on the surface of the glass particles soaked in phosphate solution. The resonances at 1415 and 866 cm^{-1} were attributed to C–O in the CO$_3^{2-}$ function group.[10] The FTIR spectra therefore indicated that the surfaces of the glasses were covered by a carbonate-substituted HA (c-HA). The FTIR spectra in Fig. 3 also showed a resonance at 440 cm^{-1}, attributed to the Si–O–Si bending vibration in the SiO$_4^{4-}$ group,[11] found in the products of 0B, 1B, and 2B samples, but not in the 3B sample, indicating that the 0B, 1B, and 2B glasses were only partially converted to HA and that residual SiO$_2$ or a soluble SiO$_2$-rich gel layer remained in the reaction products.

SEM micrographs (Fig. 4) of the 0B and 3B glasses after immersion for 2400 hours in the phosphate solution indicate that HA layers fully covered the surfaces of both glasses. [Correction added after publication 13 December 2006: 24:00 was corrected to 2400] The as-formed c-HA layer on the 0B glass was highly porous (Fig. 4(b)), with the crystals having plate-like shapes. The c-HA layer on the 3B glass surface appeared to have a denser microstructure (Fig. 4(d)), consisting of rod-like crystals with a length of ∼50 nm.

It has been suggested that the formation of an HA layer *in vitro* is indicative of a biomaterial's bioactive potential *in vivo*.[12] The as-prepared borate-based glasses have been demonstrated to have the ability to convert to c-HA on their surfaces when immersed in the phosphate solution, which is an indication of the bioactive character of this novel glass, and its expected bone-bonding behavior *in vivo*.
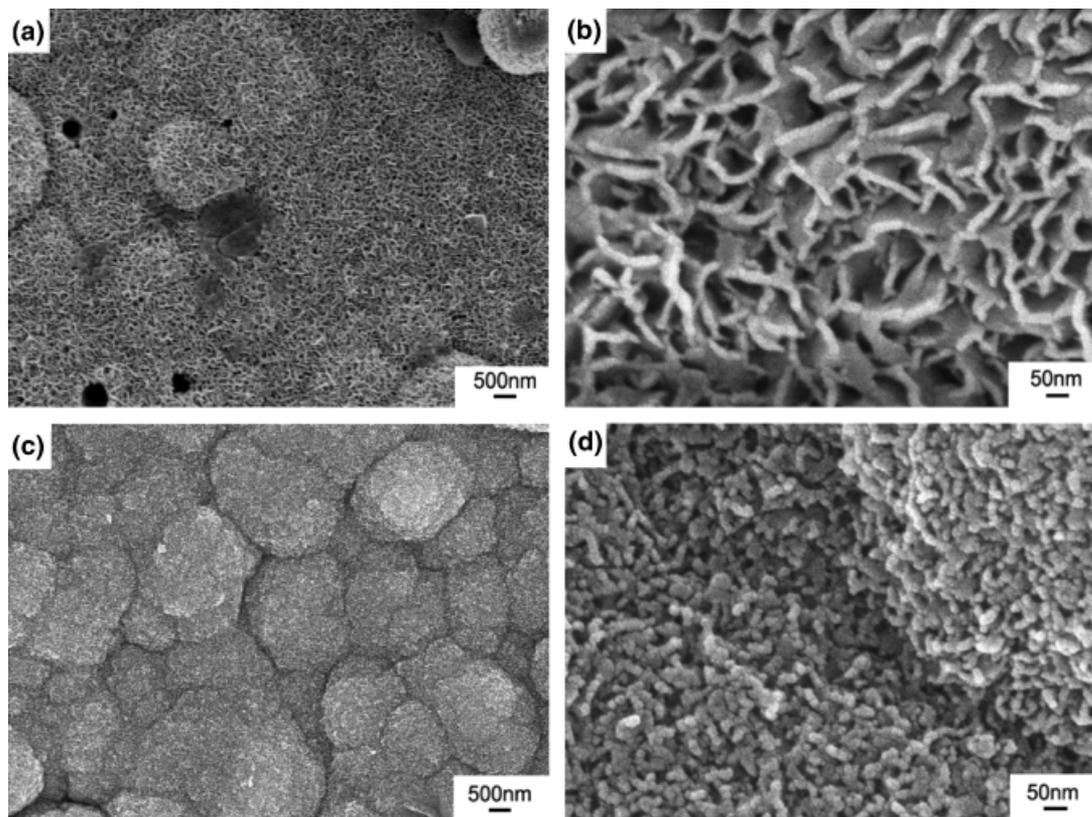

Fig. 4. Scanning electron micrographs of the surfaces of the 0B glass (a), (b), and the 3B glass (c), (d), after the glass particles were immersed for 2400 hours in 0.02M K$_2$HPO$_4$ solution at 37°C. [Correction added after publication 13 December 2006: 24:00 was corrected to 2400]

The mechanisms of HA layer formation in silicate-based bioactive glasses have been described by Hench.[12] The conversion of borate glasses to c-HA is believed to follow a set of dissolution–precipitation reactions similar in nature to those in the 45S5 bioactive glass system, but without the formation of a SiO_2-rich layer.[6] Essentially, all ions dissolve from the glass into the solution, and at the same time the B–O network structure of the glass is attacked by phosphate solution. Within all possible compounds formed, only HA has the lowest solubility. Immediately, the PO_4^{3-} ions from the solution react with Ca^{2+} located on the glass surface, leading to nucleation of HA. The continuation of the dissolution–precipitation reactions leads to thickening of the HA layer from the surface of the glass particle inward. For the borate glass, 3B, the process continues until the glass is completely converted to c-HA. However, for the silicate 0B and borosilicate glasses (1B and 2B), the reaction effectively stops before complete conversion to c-HA, and hence residual SiO_2 or a soluble SiO_2-rich gel layer is left in the reaction products, as found in the FTIR spectra.

IV. Conclusion

Novel borate-based bioactive glasses with controllable biodegradation behaviors were developed. The borate, borosilicate, and silicate bioactive glasses reacted and converted to c-HA on their surfaces when immersed in a $0.02M$ K_2HPO_4 solution at 37°C, regardless of the B_2O_3 content of the glass. The conversion rate to c-HA was dependent on the B_2O_3:SiO_2 ratio of the glass. A higher B_2O_3 content brought about a conversion to c-HA. Particles of a borate glass were fully converted to c-HA, whereas the silicate and borosilicate glasses were only partially converted to c-HA.

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