Formation and Characterization of Bone-like Nanoscale Hydroxyapatite in Glass Bone Cement

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Glass based bone cement (GBC) was synthesized by mixing CaO-SiO_2-P_2O_5 based glass powder with ammonium phosphate liquid medium. Bone-like hydroxyapatite (HAP, $Ca_{10}(PO_4)_6(OH)_2$) was found to form after GBC was immersed in simulated body fluid (SBF). HAP crystal grew with an increasing time along c axle and reached about 200 nm in length after 30 days, however, the end plane granularity remained 30∼50 nm. The chemical composition, crystal structure and morphology of HAP formed from GBC were proved to have great resemblance with living HAP. It is believed that GBC was a desirable biomedical material with high bioactivity. Furthermore, the high compressive strength guaranteed the possibility of GBC in clinical application.

KEY WORDS: Bioactivity, Bone cement, Hydroxyapatite, Microstructure

1. Introduction

Bone cement, as a desirable repairing material for bone defects, has attracted great attention in biomedical field. Polymethyl methacrylate (PMMA), traditional bone cement, has recently been replaced by inorganic bone cement due to its lack of biocompatibility and the potential harmfulness of degraded products to human body[1]. Calcium phosphate cement (CPC) and glass based bone cement (GBC) have become the main substitutes for PMMA to act as bone defects repairing materials, because of their excellent biocompatibility and high mechanical strength. However, since the mechanical strength of CPC is lower than that of GBC, most recent researches are concentrated on the synthesis of bioactive glass powder with such resins as BIS-GMA and PMMA to get an even higher mechanical strength[2,3]. But few researches have been reported concerning the microstructure and bioactivity of pure glass bone cement. In the present research, a new type of bone cement based on CaO-SiO_2-P_2O_5 glass was developed by mixing the glass powder with an ammonium phosphate liquid medium. After being immersed in simulated body fluid (SBF) for different time intervals, the bone cement was characterized by using X-ray diffraction (XRD), and Fourier transform infrared (FTIR) spectroscopy. The morphology of the bone cement was also observed by scanning electron microscopy (SEM) and correlated with the properties measured.

2. Experimental

The composition of the glass powder used in this experiment was designed on consideration of a self-setting $Ca_3(PO_4)_2$ and $CaSiO_3$ crystals, which possesses a high mechanical strength. With respect to the preparation process and the properties of the final glass, glass with a nominal composition of CaO_2 45, SiO_2 35, and P_2O_5 20 by weight ratio was selected. Glass batch was melted in an SiC furnace at 1500°C for 4∼6 h. The melt was quenched into glass frit between stainless steel plates, then the frit was crushed and pulverized in an Al ball mill into fine powder (3 to 5 μm). Buffer solution at pH value of 7.4 was made by dissolving $(NH_4)_2HPO_4$ and $NH_4H_2PO_4$ of analytical reagent grade in deionized water at a certain ratio. The bone cement was set within 1 min by stirring the mixture of glass powder and the buffer solution homogeneously. After GBC hardened, it was immersed in SBF at 37°C. To keep it fresh, SBF was changed every 24 h. The hardened bone cement was subsequently measured and observed with different instruments.

The bone cement was analyzed by XRD (D/max 2550V, Rigaku Co., Tokyo, Japan) and FTIR (FTS-40 BIORAD, digilab division, Cambridge, MA). The morphology of hydroxyapatite (HAP, $Ca_{10}(PO_4)_6(OH)_2$) formed in GBC was observed with SEM (JSM-6700F, HITACHI, Japan). The effects of different immersion time on the formation and morphology of HAP were also monitored.

The dissolution rate of Ca^{2+} from GBC was measured in a traditional way with ethylenediamine tetraacetate (EDTA, China Medicine Inc., Shanghai, CN).

Compressive strength of GBC was measured using an Instron universal testing machine (Instron 1195, Instron Limited, Buckinghamshire, UK) at a crosshead speed of 0.5 mm/min. Rectangular beams (7 mm×7 mm×12 mm) were prepared and measured under wet conditions at room temperature after immersing in SBF at 37°C for 7, 15 and 30 days, respectively. For each time interval, at least 5 specimens were tested to get a mean value.

3. Results and Discussion

3.1 Phase composition of GBC

The peaks of 1040 cm^{-1} in Fig.1(a) corresponds to the existence of (SiO_4) group and 493 cm^{-1}, 550 cm^{-1} corresponds to the bending vibration of Si-O-Si and bending vibration of glassy P-O, bond respectively. It was concluded that the glass skeleton was composed of the network former coordination tetrahedral ([SiO_4], [PO_4]), while Ca cations acted as network modifiers, scattered between network gapse interstitials. The existence of large amount of network modifiers and the non-bridging P=O bonds lead to the decay of [SiO_4] and [PO_4], resulting in the phases rich in P and Si[4,5]. Furthermore, the larger field strength of P^{5+} (2.1) than that of Si^{4+} (1.5) results in the segregation of Ca^{2+} around P^{5+} to form a Ca-P phase. Because the bond between the P-rich phase and Si-rich phase is weak in the network, glass powder is easy to be

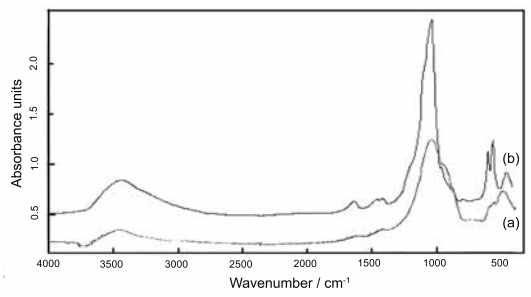

Fig.1 FTIR spectra of GBC samples of different immersion time (a) 7 days, (b) 30 days

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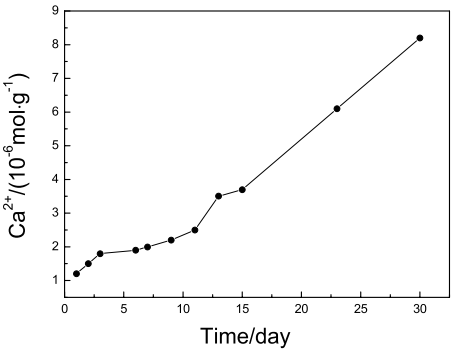

Fig.2 Changes of calcium content dissolved from GBC with time

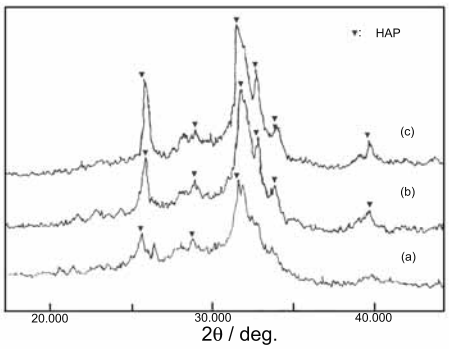

Fig.3 XRD patterns of GBC samples of different immersion time (a) 7 days, (b) 15 days, (c) 30 days

hydrated by fluid around it. In addition, a small particle size of glass powder (1∼5 μm), *i.e.*, a large surface area also contributes to the hydration of glass powder. Thus Ca^{2+} content dissolved from glass powder increased with a longer immersion time in SBF (See Fig.2). As more and more Ca^{2+} was dissolved from glass powder into fluid, the content of SiO_2 at glass powder surface increased. Meanwhile the glass network was easy to decompose with the hydration reaction of SBF and liquid medium. Therefore, the bonds of Si-O-SiO were broken and [Si-OH] groups were formed at the glass surface. The condensation and polymerization of the [Si-OH] groups resulted in a porous SiO_2-rich layer on the glass surface[6,7]. The peak of 464 cm^{-1} (See Fig.1(b)) corresponding to the metamorphosis vibration of O-Si-O indicates the appearance of the SiO_2-rich layer in GBC.

The peaks of 1420 cm^{-1} and 1450 cm^{-1} corresponded to the splitting and entrance of CO_3^{2-} groups into HA crystal. While the peaks of 493 cm^{-1} and 550 cm^{-1} disappeared after 7 days immersion, the peaks of 560 cm^{-1} and 602 cm^{-1} corresponding to the bending vibration of crystal P-O bond and 1080 cm^{-1} corresponding to the stretching vibration of P=O bond indicate the formation of hydroxy-carbonate apatite (HCA) in GBC[8].

The porous SiO_2-rich layer at the glass powder surface provided excellent nucleation sites for the formation of crystals. The continuous released Ca^{2+} ion incorporated with HPO_4^{2-} in SBF to form an amorphous $CaO\text{-}P_2O_5$-rich layer on the top of SiO_2-rich layer at the glass powder surface. As apatite has the lowest solubility in aqueous solution at 37°C[9], other calcium phosphate compounds formed in this environment were unstable and tended to be dissolved again. Hence, the solution was highly supersaturated with respect to apatite and a large number of HAP nuclei were formed at the SiO_2-rich layer. The dehydration, polymerization and crystallization of the $CaO\text{-}P_2O_5$-rich layer combined with the intermingle of CO_3^{2-} from SBF eventually resulted in the formation of HCA.

The XRD spectra (see Fig.3) shows that a longer immersion time in SBF decreased the amorphous glass phase in GBC while promoting the formation of HAP crystal.

Figure 3 shows that the peaks of HAP tended to become sharper and narrower over a longer period time.

Although a certain amount of HAP crystal was formed in GBC after 7 days, the lower intensity and wider shape of the peaks (see Fig.3(a)) indicate the formation of a fine crystal with less consummation. The crystal structure and size of HAP crystal tended to be improved with the proceeding of immersion (see Fig.3(a) and (b)). The formation of HAP from GBC was attributed to the hydration reaction of glass powder, which formed a porous SiO_2-rich layer. It was through this layer that Ca^{2+} and HPO_4^{2-} groups migrated and congregated, eventually leading to the formation of HAP crystal at a suitable temperature and pH value. As the amount of Ca^{2+} and HPO_4^{2-} dissolved from the glass powder increased with increasing immersion time, HAP crystal began to grow larger and to consummate, which can be seen from the narrowing and sharpening of the XRD peaks in Fig.3.

The living HAP crystal in human bone has been proved to be a needle-like crystallite, containing a small amount of CO_3^{2-} group, with the diameter of about 50 nm[10]. Through the analysis of XRD and FTIR spectra, it could be concluded that GBC has an excellent bioactivity due to the similarity of crystal phase composition and dopant of CO_3^{2-} with living HAP crystal.

3.2 Microstructure analysis of GBC

It has been asserted that the formation of bone-like HCA at the glass or glass ceramics surface is the premise of biomaterials that have an excellent bioactivity and can form a chemical bond with human bone[11,12]. Therefore the bioactivity of GBC should be judged by the formation of bone-like HCA after immersing in SBF.

In this experiment, block samples of GBC after different immersion time were observed by SEM to determine the morphology of HAC crystal. It could be seen from Fig.4(a), (b) and (c) that the surface of glass powder after different time distinguishes greatly. It is believed that the erosion of glass powder is intensified after a long period of immersion. The increment of hydration of glass powder eventually led to the change of the powder surface.

Although a small amount of fine particles were observed at the glass powder surface (see Fig.4(a)), a larger amount of glass phase still existed in GBC. It is judged by XRD spectrums (see Fig.3(a)) that these fine particles are HAP crystals. These HAP crystals are fine spherical particles with the size of 30∼50 nm (see Fig.4(d)). Meanwhile, the glass powder in GBC is connected through a $CaO\text{-}P_2O_5$-rich layer and the mechanical strength of GBC is relatively lower. A certain amount of pillar-like particles (see Fig.4(e)) were observed between the interfaces of GBC after immersing for 15 days. The particles were proved to be HAP crystals by XRD results (see Fig.3(b)). It was observed that after 15 days the spherical HAP crystal developed into pillar-like HAP crystals with the end plane granularity 30∼50 nm and the length of 100∼200 nm. The structure of GBC became even more compact after 30 days comparing with that of 15 days (see Fig.4(b) and (c)) owing to the formation of HAP crystals scattered between the interfaces of the glass powder (see Fig.4(f)). The length of the pillar-like HAP is increased to 200∼300 nm while the end plane granularity remains 30∼50 nm. These pillar-like HAP crystals filled into glass powder interfaces, reinforced the connection between the powder and reduced the number of cracks in GBC.

Figure 4 illustrates that HAP crystals formed from GBC are scattered between glass powder interfaces. The morphology of the crystal changed from the spherical particles of about 30∼50 nm (see Fig.4(d)) to pillar-like ones (see Fig.4(e) and (f)). The HAP crystals formed after 30 days is quite desirable, possessing a structure with the end plane granularity 30∼50 nm (see Fig.4(f)). From the crystallographic point of

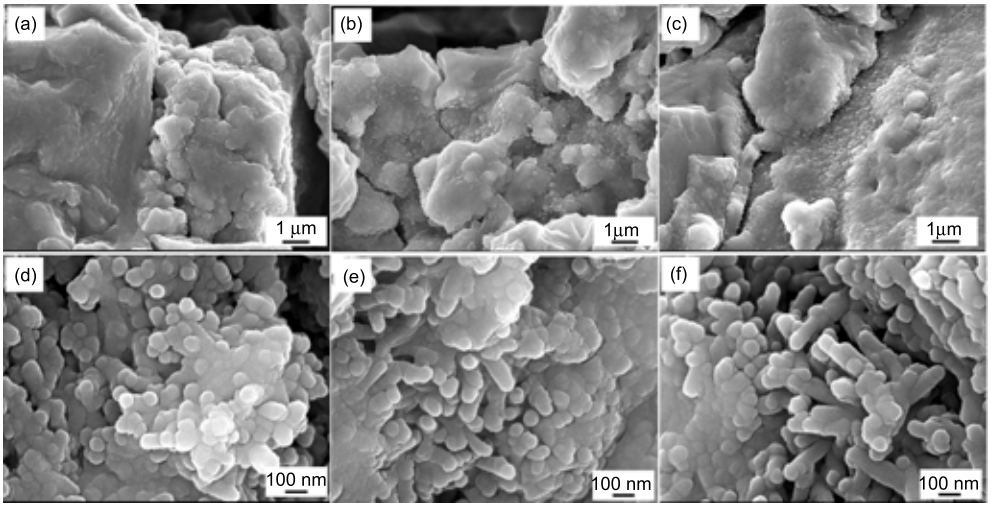

Fig.4 SEM of GBC samples obtained for different immersion time (a) 7 days, (b) 15 days, (c) 30 days, (d) 7 days, (e) 15 days, (f) 30 days

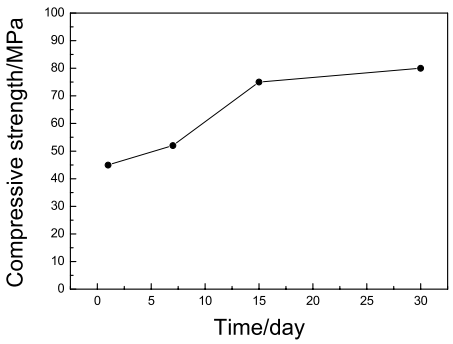

Fig.5 Compressive strength *vs* immersion time

view, the concentration of Ca^{2+} increased in the fluid between glass powder interfaces owing to the Ca^{2+} generated by the hydration reaction of SBF and liquid medium with the powder. This continuous dissolution of Ca^{2+} from glass powder to fluid led to the supersaturation with respect to Ca^{2+}. Thus apatite nuclei are prone to grow at the desirable nuclei sites provided by the porous SiO_2-rich layer due to its lowest solubility[6]. Once the apatite nuclei are formed at glass powder surface, they grew spontaneously by consuming Ca^{2+} and PO_4^{3-} supplied by SBF or dissolved from glass powder. Therefore, HCA, the most stable thermodynamically compound in aqueous solution, was formed through a series of composition and structure modification combined with the doped CO_3^{2-}. It could be observed from the Fig.4(a), (b) and (c) that the end plane granularity of HAP crystal did not change visibly although the length increased with increasing immersion time.

The HAP crystal formed from GBC is a pillar-like crystal with CO_3^{2-} doped in it. Therefore, it is clear that the HAP crystal is similar with living HAP crystal in crystallization, chemical composition and morphology. Thus this HAP crystal can be viewed as bone-like HAP crystal. GBC is sure to be a desirable biomaterial with an excellent bioactivity.

3.3 Analysis of compressive strength

The compressive strength of GBC increases with the immersion time (see Fig.5). This is owing to the increment of HAP formed between the hydrants of glass powder interfaces (see Fig.3). The inner structure of GBC changed gradually from an amorphous CaO-P_2O_5 layer to cross-linked HAP crystal network (see Fig.4(a) and (b)). Thus the cross-linking density of GBC structure is enhanced and the cracks between glass powder interfaces are reduced, resulting in the augment of compressive strength. Furthermore, with an increasing immersion time, HAP crystals formed from GBC began to grow larger, entangled with each other and filled into the gaps between the glass powders (see Fig.4(f)). Around HAP crystals, hydrated colloid layers were scattered. Therefore, the separated glass powder is connected through this colloid layer into a whole compact structure. The improvement of the inner structure of GBC, the entanglement of HAP crystals with each other and the reduction of gaps all eventually led to the enhancement of the compressive strength.

4. Conclusion

After immersing in SBF, bone-like hydroxyapatite crystal was formed from GBC. The formed HAP crystal is proved to be hydroxy-carbonate apatite with the end plane granularity about 30∼50 nm. It is clear that GBC is a desirable biomedical material with high bioactivity. Furthermore, the high compressive strength promises a nice future for the clinical application of GBC.

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