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Preparation and Characterization of Nano Bioactive Glass based on the $CaO–P_2O_5–SiO_2$ System

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ABSTRACT

Nano Bioactive glass material of the type $CaO–P_2O_5–SiO_2$ was obtained by the sol-gel processing method. The obtained material was characterized by X-ray powder diffraction (XRD) and surface electron microscopy. The bioactivity was examined *in vitro* with respect to the ability of a hydroxyapatite layer to form on the surface as a result of contact with simulated body fluid (SBF). The XRD studies were conducted before and after contact of the material with SBF.

Keywords: bioactive material, bioactivity, sol gel processing
Abbreviations: BG, bioactive glass; **HCA**, hydroxylcarbonate apatite

INTRODUCTION

Glasses and glass-ceramic materials based on the SiO_2-CaO-P_2O_5 system constitute an important group of materials that have found wide application in medicine as bone implants (Oki *et al*. 2004; Balamurugan *et al*. 2007). The prerequisite for glasses and glass-ceramics to bond to living bone is the formation of a layer of biologically active hydroxylcarbonate apatite (HCA) on the surface of these biomaterials when they are exposed to physiological fluids (Yan *et al*. 2006). Hench has described a sequence of five reactions that result in the formation of an HCA apatite layer on the surface of these bioactive glasses (BGs). These reactions are summarized on **Table 1**. The first reactions are the ion exchange between the alkali in the glass and water. This is followed by a breakdown of the silica network, forming silanol bonds that repolymerize to form a hydrated, high surface-area, silica-rich layer. This silica-rich surface attracts organic molecules (proteins, mucopolysaccharides, and collagen) and facilitates the formation of the HCA layer on the glass (Hench and LaTorre 1993; Zhong and Greenspan 2000; Saravanapavan *et al*. 2003).

The behavior of BGs in the formation of new bone tissue depends on the chemical composition and textural properties (pore size and volume) (Saravanapavan and Hench 2001; Sepulveda *et al*. 2002).

BGs can be formed either from the traditional melt-quenching or by the modern sol-gel method (Balamurugan *et al*. 2007). Sol-gel processing, an alternative to traditional melt processing of glasses, involves the synthesis of a solution (sol), typically composed of metal organic and metal salt precursors followed by the formation of a gel by chemical reaction or aggregation, and lastly thermal treatment for drying, organic removal, and sometimes crystallization (Olding *et al*. 2001).

Compared with conventional melt-processed BGs, sol-gel BGs are processed at lower temperatures and have better compositional control (Saboori *et al*. 2009).

Additionally, sol-gel BGs are more easily created with the combination of bioactivity and bio-degradability. Results from both *in vitro* studies in a cellular simulated body fluid (SBF) (Li *et al*. 1992; Pereira *et al*. 1994; Sepulveda *et al*. 2002) and *in vivo* tests in rabbit models (Hamadouche *et al*. 2000; Wheeler *et al*. 2000; Hamadouche *et al*. 2001) demonstrate that sol-gel derived BGs are more bioactive (as represented by the induction time of apatite) and degradable (as represented by the amount of residual glass) than BGs made by conventional melt processes. Sol-gel BGs also have other potential applications, such as delivery of drugs or biological molecules (Santos *et al*. 1999; Livage *et al*. 2001; Radin *et al*. 2001) and as particulate fillers for *in situ* tissue regeneration (Hench and Polak 2002).

It has also been proved that an increase in the growth rate of apatite-like layer as well as the wider bioactivity were observed depending on the compositional range used for the preparation of bioglass by sol-gel method (Vallet-Regi and Ramila 2001; Vallet-Regi *et al*. 2003).

The objective of the present study was to synthesize a SiO_2-CaO- P_2O_5 bioactive glass system through sol-gel synthesis and to study its *in vitro* bioactivity in SBF.

Table 1 Reaction stages of bioactive glass.

Stage 1	Rapid exchange of cations such as Na^+ or Ca^{2+} with H^+ or H_3O^+ from solution, Si—O—Na^+ + H^+ + OH^- → Si—OH + $Na^+_{(solution)}$
Stage 2	Loss of soluble silica in the form of si$(OH)_4$ to thr solution resulting from breakage of Si—O—Si bonds and formation of Si—OH (silanols) at the glass solution interface. 2 (Si—O—Si) + H_2O = Si—OH + OH—Si
Stage 3	Condensation and repolymerization of a SiO_2-rich layer on the surface depleted in alkali and alkaline earth cations. Si—OH + OH—Si = —Si—O—Si— + H_2O
Stage 4	Migration of Ca^{2+} and PO_4^{3-} group to the surface forming CaO— PO_4^{3-} clusters on the top of the SiO_2-rich layer, followed by growth of the amorphous CaP
Stage 5	Crystallization of the amorphous CaP by incorporation of OH^-, CO_3^{2-} anions from solution to form a hydroxyl-carbonate apatite layer.

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Research Note

MATERIALS AND METHODS

In the first step, the solution was prepared as follows: 13.33 g (0.064 mol) of tetraethyl orthosilicate (TEOS; Merck, Germany) was added into 30 mL of 0.1 M nitric acid (Merck); the mixing process was allowed to be continued for 30 min for the acid hydrolysis of TEOS to proceed almost to completion. The following reagents were added in sequence. About 45 min have to be given to each reagent to react thoroughly: 0.91 g (0.005 mol) triethyl phosphate (TEP; Merck), 7.32g (0.031 mol) of calcium nitrate tetrahydrate (Merck). To allow completion of the hydrolysis reaction, mixing was continued for 1 h after the last addition. The solution was cast in a cylindrical teflon container and kept sealed for 10 days at room temperature to allow the hydrolysis and polycondensation reactions take place until the gel was formed. The gel was kept in a sealed container and heated at 70°C for 3 days. To get rid of the water a small hole was contrived in the lid to allow the leakage of gases while heating the gel to 120°C for 3 days to remove all the water. The dried gel was then heated at 1000°C in oven for 24 h to stabilize the glass and eliminate residual nitrate.

X-ray diffraction (XRD) analysis

For X-ray diffraction analysis, prepared bioglass were ground and powdered. The resulting powders were analyzed with Philips PW 3710. This instrument works with voltage and current settings of 30 kV and 25 mA, respectively and uses Cu–Kα radiation (1.540510 Å). For qualitative analysis, XRD diagrams were recorded in the interval $20° \leq 2\theta \leq 50°$ at a scan speed of 2°/min.

Scanning electron microscopy (SEM)

The samples were coated with a thin layer of gold (Au) by sputtering (EMITECH K450X, England) and then the microstructure of them were observed on a scanning electron microscope (SEM; VEGA TESCAN 2XMU) that operated at the acceleration voltage of 15 kV.

In vitro studies in simulated body fluid (SBF)

In vitro studies were carried out by soaking the samples (listed in **Table 2**) in SBF at 37°C for intervals 0 (without soaking in SBF), 7 and 14 days. After soaking, the powder was filtered, rinsed with doubly distilled water, and dried in an oven at 120°C for 12 h before analysis by XRD and SEM.

Table 2 Sample codes in relation with incubation time in SBF.

Sample code	Time of incubation in SBF
BG-0D	0 day (without soaking in SBF)
BG-7D	7 days
BG-14D	14 days

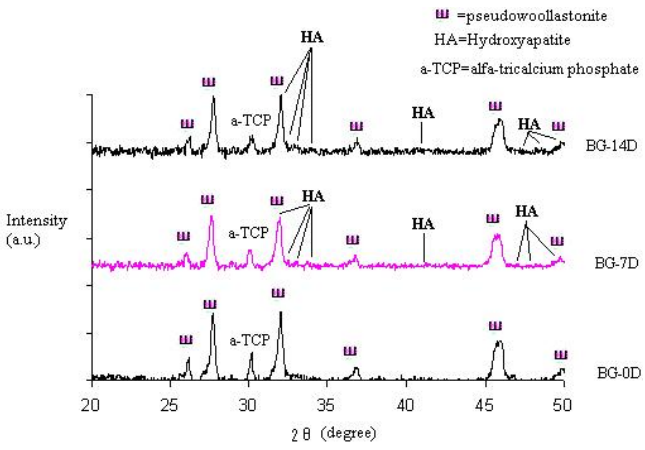

Fig. 1 XRD patterns of samples BG-0D, BG-7D and BG-14D.

RESULTS AND DISCUSSION

Fig. 1 shows the XRD patterns of the samples BG-0D, Bg-7D and BG-14D. It can be seen from these patterns that prepared bioglass has been partially crystallized. The main phase which is formed is Wollastonite (Pseudowollastonite, JCPDS No.: 19-0248). It has been reported that Pseudowollastonite is a bioactive material, and its *in vitro* and *in vivo* tests have been investigated (De Aza *et al*. 1999, 2000, 2004; Sarmento *et al*. 2004). Pseudowollastonite is a bioactive ceramic material that induces direct bone growth (Yang and Prewitt 1999; Fernández-Pradas *et al*. 2002). The presence of α-TCP phase is reasonable due to the existence of phosphate and silicate groups in bioglass structure and the high temperature (1000°C) which is used in the process.

After soaking in SBF, prepared samples were analysed by XRD. It is quite clear that after soaking in SBF, hydroxyapatite has been formed and it can be detected in the XRD patterns of BG-7D and BG-14D (**Fig. 1**).

The amount of formed hydroxyapatite was measured by comparison between the XRD patterns of samples BG-7D and BG-14D. The intensity of two formed phases (hydroxyapatite and pseudowollastonite) was chosen in the specific angle. The angle was $2\theta = 36.80$ for wollastonite and $2\theta = 40.17$ for hydroxyapatite. These peaks can be considered characteristic peaks for these two phases. **Fig. 2** shows that the amount of the ratio of formed hydroxyapatite to pseudowollastonite is increasing with the time of incubation in SBF.

Furthermore, SEM results support the XRD patterns and the formed hydroxyapatite can be seen on the surface of the samples. **Fig. 3** shows SEM pictures of BG-0D. **Fig. 4** shows SEM pictures for BG-14D. These two pictures can be used for comparison. Therefore, hydroxyapatite particles can be detected on the surface as expected. Also, it is interesting to see that the particle size ranges from 200 nm to >1 μm. It can be related to the method from which the bioglass has been synthesized. As sol-gel method increases the surface energy of the particle of the bioglass, the bioactivity will be increased and each of the bioglass particles is an active element to produce hydroxyapatite on the surface and the nano-sized hydroxyapatite particle can be detected in the SEM pictures.

CONCLUSION

Sol-gel derived bioglass with the composition SiO_2-P_2O_5-CaO shows bioactivity due to the formation of hydroxyapatite layer on its surface after soaking in SBF. Synthesized bioglass partially crystallized due to the thermal treatment up to 1000°C. Because of the sol-gel method, nano-sized particles of hydroxyapatite have been formed on the surface of the bioglass samples.

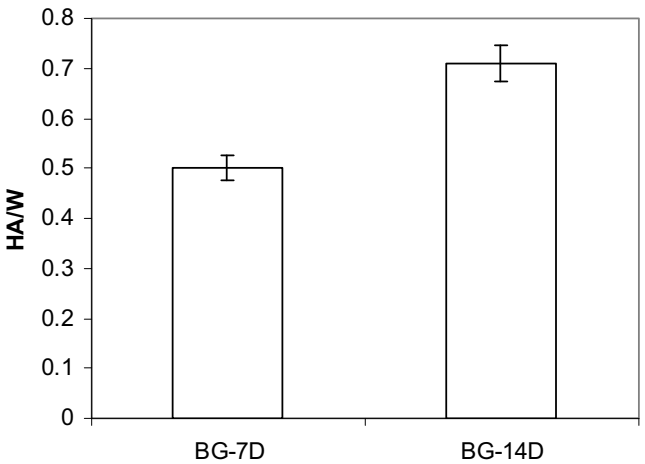

Fig. 2 Ratio of hydroxyapatite to pseudowollastonite for the samples BG-7D and BG-14D. Values represent mean ± Standard Error (SE). *n* = 3.

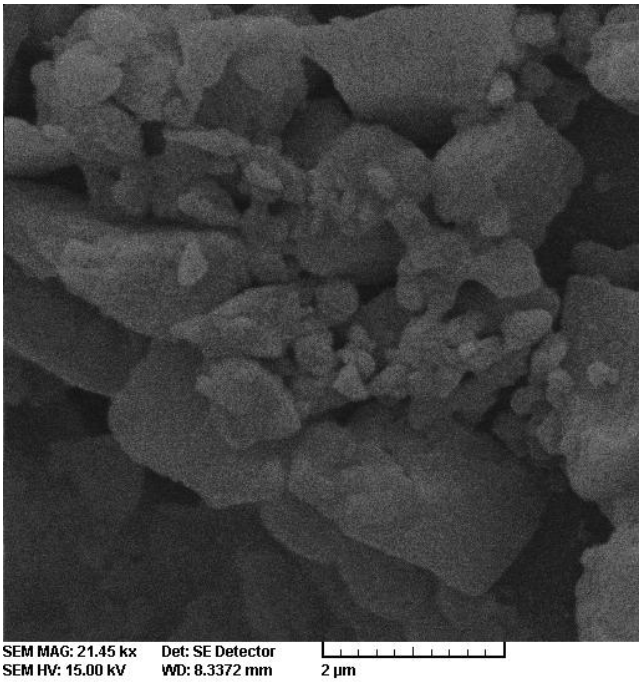

Fig. 3 SEM of bioactive glass before soaking in SBF (BG-0D).

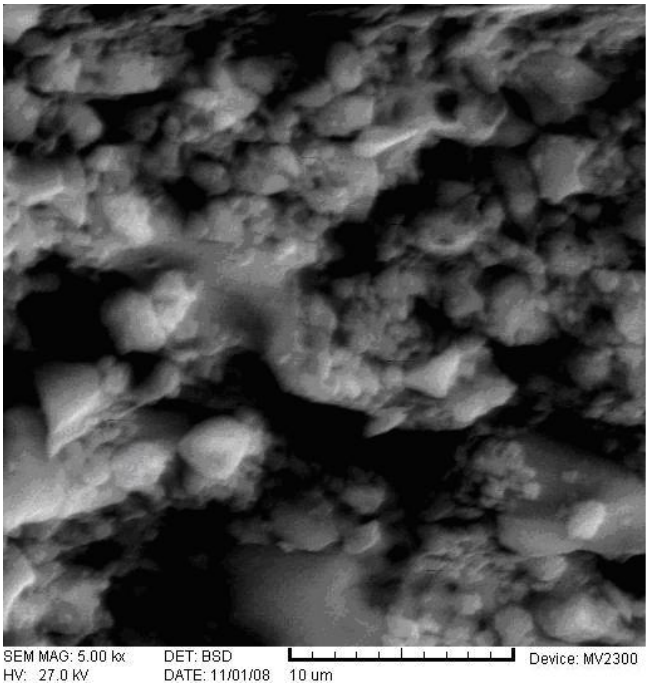

Fig. 4 SEM of bioactive glass after soaking in SBF for 14 days (BG-14D).

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