Synthesis and Characterization of Bioactive Quaternary Silicate Gel-glasses

T.T.Swe1, H.Mohamad1, K.A. Shariff1, A.F.M.Noor1, K.Ishikawa2, A.A.Thant3

1 School of Materials and Mineral Resources Engineering, Universiti Sains Malaysia, Nibong Tebal, Penang, Malaysia
2 Faculty of Dental Science, Kyushu University, Japan
3 Department of Physics, University of Yangon, Yangon, Myanmar

Email: hasmaliza@usm.my

Abstract. Bioactive glasses and glass-ceramics were applied in bone fix applications, dental applications as well as in tissue engineering. In the current study, a new quaternary silicate bioglass S50P4 (50%SiO₂–24.5%Na₂O–21.5%CaO–4%P₂O₅) with different aging time (3, 5 and 7 days) was prepared by sol-gel method. These synthesized glasses were analyzed using X-ray powder diffraction (XRD), Scanning electron microscopy (SEM) and Fourier transform infrared spectroscopy (FTIR). Whereas the bioactivity of the optimized samples were observed through in-vitro test using Hank’s Balanced Salt Solution (HBSS) at different soaking time (7 and 14 days). XRD results indicate that the crystalline patterns in gel-derived glass aged for 3 days exhibit stronger peaks compare to samples aged for 5 and 7 days. This could be attributed to the difference of water content inside the gels. Moreover, the peak at 2θ~33° became sharper and more intense for longer soaking times corresponds to the apatite structure. Besides, in-vitro test shows, the formation of hydroxyl carbonated apatite layer (HCA) with finer grains.

1. Introduction

Bioactive silicate glasses can be used as bone substitutes in orthopedic and dental applications as well as in the field of bone tissue engineering, in the form of granules, scaffolds, porous or dense powders, and coatings[1]. Bioactive glasses are synthetic materials, which show highly positive interactions with hard and soft tissues [2]. The first bioactive glass 45S5, known worldwide for its trademark Bioglass®, was invented by Professor L.L.Hench at the University of Florida in 1969 [3].

The production of 45S5 bioactive glass has traditionally been carried out through melting technology. In the melting process, precursors were mixed homogeneously and melted at a high temperature (>1300°C). The product was then quenched to form amorphous glass. However, the high temperature can induce the volatilization of phosphate components, which may change the composition of the glass. However, the sol-gel method can be used for the fabrication of both glassy and ceramic materials [4]. The primary advantage of the sol-gel technique is the potential of gaining high purity, homogeneity materials and lower processing temperatures than the traditional melting method [5].
Generally, the sol-gel processes include hydrolysis, polycondensation, gelation, aging, drying, and stabilization. In the sol-gel processes, the precursors are catalyzed and dissolved in the solvent to form a sol. The sol gradually becomes a gel containing both a liquid and a solid phase. To strengthen the network, an aging process is necessary. Removal of the remaining liquid (solvent) phase requires a drying process. Finally, a thermal treatment (stabilization) is often carried out to enhance the mechanical properties and to improve the structural stability [5]. In this study, the new glass composition S50P4 is designed based on the assumption of L.L Hench [3] and synthesized by so-gel method in tend to get greater surface area for the purpose of better bioactivity. The effect of aging time on the structure of gel-derived S50P4 bioglass was studied by Fourier-transform infrared spectroscopy (FTIR) and X-ray diffraction (XRD) techniques. The bioactivity of the glass was evaluated by immersing the samples in HBSS for 7 and 14 days to study the formation of apatite layer on the glass surface.

2. Materials and Method

2.1 Gel Preparation

The S50P4 bioglass with the composition (50%SiO₂–24.5%Na₂O–21.5%CaO–4%P₂O₅) in wt. % was prepared by sol-gel method. The main raw materials used were tetraethyl orthosilicate (TEOS, Si(OC₂H₅)₄, Fluka), triethyl phosphate (TEP, (OP(OC₂H₅)₃), Merck), sodium nitrate ((NaNO₃), Emsure) and calcium nitrate tetrahydrate ((Ca(NO₃)₂.4H₂O), Emsure) as precursors of SiO₂, P₂O₅, Na₂O and CaO respectively. Samples with different aging times (3, 5 and 7 days) were prepared according to the ratio of SiO₂, P₂O₅, Na₂O and CaO stated for the composition.

Briefly, the required amount of TEOS (37 mL) was poured into 1M dilute nitric acid solution and stirred for 1 h at room temperature for acid hydrolysis. Then TEP (2 mL), calcium nitrate (18.09 g) and sodium nitrate (13 g) were added into the TEOS with constant stirring for 45 min for each reagent to react completely. The mixture was then continuously stirred for another 1 h to complete the hydrolysis reaction. The solution was kept and sealed at the ambient condition with different aging periods (3, 5 and 7 days) to homogenize and re-precipitation. The resulting gel was aged at 60°C for 2 days and dried at 100°C for 2 days to eliminate the adsorbed water from the pores and finally, calcined at 700°C for 5 h to eliminate the residual nitrate and organic substances.

2.1.1. X-ray Diffraction (XRD). X-ray diffraction was used to analyse the glass powders using an XRD Bruker DX 8 operating with CuKa radiation (λ = 1.5406 Å). The diffraction patterns were obtained in the 2θ range from 20° to 80° in a fixed counting time of 1 second. The obtained data were analysed using X’Pert HighScore Plus Software for phase analyzation.

2.1.2. Fourier Transform Infrared Spectroscopy (FTIR). Surface structural of glass powders was determined using FTIR (PERKINELMER SPECTRUM ONE). Transmission technique was implied with KBr powder used as a reference. The weight ratio of sample to KBr was set at 1:100. The mixture of KBr and glass powder was pressed into pellet form using hand press. Sample with lower thickness has good transparency which provides a relatively short path length for the beam. Spectra were collected in the region between wavenumber 4000 and 400 cm⁻¹ with a resolution 4 cm⁻¹.

2.1.3. Field Emission Scanning Electron Microscope (FESEM). The microstructure of glass powders was characterized using thermal field emission gun scanning electron microscope (FESEM). FESEM was used to study the surface morphology and topography of the glass before and after soaking in Hank’s Balanced Salt Solution (HBSS). In this study, Zeiss Supra 35VP Field Emission Scanning Electron Microscope (FESEM) was used. Because of the poor electrical conductivity of the samples, their surfaces were coated with a thin layer of gold before the test.
3. Results and Discussion

3.1 X-ray Diffraction (XRD)

The diffraction patterns of gel-derived S50P4 bioglass aged under different periods and stabilized at 700°C are shown in figure 1. Results show the formation of several crystalline sodium calcium silicate phases together with an amorphous structure of the bioactive glass. Among them, the most interesting phase is combeite (Na$_2$Ca$_2$Si$_3$O$_9$) because it was significantly influence the bioactivity. As pointed out by Peitl et al., combeite (Na$_2$Ca$_2$Si$_3$O$_9$) is highly bioactive [6]. The formation of the combeite phase was associated with the heat treatment performed at 700 °C during the drying step in sol-gel synthesis [7]. However, the structure of the glass still exhibit amorphous up to 7 days aged (figure 1). It can be seen that the S50P4 bioglass aged 3 days was crystalline, however by increasing of aging time, the formation of amorphous structure increased where the sharpness of peaks decreased. This phenomenon was believed due to the dehydration of the mixture during [8]. The formation of amorphous structure proved that the sol-gel method could prepare glasses with aging time up to 7 days. However, in the current study, combeite phase was only detected in glass powder aged 5 days which is optimized for in-vitro analysis by soaking in HBSS.

![XRD patterns of the glass samples produced at different aging time (3, 5 and 7 days)](image)

**Figure 1.** XRD patterns of the glass samples produced at different aging time (3, 5 and 7 days)

The XRD patterns of the 5 days aged bioglass after immersion in HBSS for 7 days and 14 days was shown in figure 2. The formation of an apatite layer during the immersion in HBSS was proved by the XRD analysis. As discussed in the previous part, before immersion in HBSS, the XRD spectrum shows the partially amorphous structure of Na$_2$Ca$_2$Si$_3$O$_9$ (Combeite) by a broad peak between 30° and 35°. After 7 days of soaking in HBSS, the formation of apatite layer on the glass surface can be detected as shown in figure 2. With increasing soaking times up to 14 days, the new peaks can be observed at around 26°, 33° and 49° assigned to the apatite reflections according to the standard JCPDS (09-0506). The peak at 2θ~33° became sharper and more intense with increasing the soaking times up to 14 days, it happens due to the reflection of (2 1 1) plane of apatite.
3.2 Fourier Transform Infrared Spectroscopy (FTIR)

The FTIR spectra of the bioglass prepared at different aging times (3, 5 and 7 days) were compared in figure 3. The main characteristic bands ranging from 400 to 1400 cm$^{-1}$ were related to the silicate network group vibrations with different bonding arrangements silicate. While the spectra from 1400 to 4000 cm$^{-1}$ clearly consists of vibrations refer to water or hydroxyl groups [9]. No obvious distinction was seen among these samples, which means their compositions were not affected by increasing the aging time. The FTIR spectra reveal Si-O-Si bending (467 and 526 cm$^{-1}$), Si-O-Si stretching (symmetric) (884 cm$^{-1}$) and Si-O-Si stretching (asymmetric) (1040 cm$^{-1}$) bands, which were known and accepted to be mainly characteristic of silicate network [9]. This might be attributed to the presence of major SiO$_2$ as a basic building constituent. The wavenumbers 619 cm$^{-1}$ was due to the presence of sodium calcium silicate (Na$_2$Ca$_2$Si$_3$O$_9$) crystalline phase [10]. The peak at 1384 cm$^{-1}$ was due to the vibration of nitrate (NO$_3^-$) [11] which was appeared only in 3 and 7 days aged. The existence of the peak at 1648 cm$^{-1}$ band indicates there is water absorption reaction take placed at the glass interface [12]. The bands at the 800 - 1200 cm$^{-1}$ were assigned to the stretching vibration of the SiO$_4$ tetrahedral with the different number of bridging oxygen atoms and the peak at 1400-1530 cm$^{-1}$ corresponds to carbonate (CO$_3^{2-}$) groups. The presence of carbonate was attributed to a carbonation process of the material due to the atmospheric CO$_2$ as a consequence of the high calcium content [11].
The Fourier transform infrared (FTIR) transmittance spectra of bioactive glasses after soaking in HBSS for different period of time (7 days and 14 days) are shown in figure 4. Before soaking, the FTIR pattern shows Si-O-Si bending (467 and 526 cm\(^{-1}\)), Si-O-Si stretching (symmetric) (884 cm\(^{-1}\)) and Si-O-Si stretching (asymmetric) (1040 cm\(^{-1}\)) bands. After HBSS treatment, it was noticed that some peaks at 526 cm\(^{-1}\) (Si-O-Si bending) and 1412 cm\(^{-1}\) (C-O stretch) are disappeared. The peaks of O-H bending at 1643 and 3454 cm\(^{-1}\) became more intense. Evolving of P - O bending (610 - 600 cm\(^{-1}\)) bands indicates the formation of hydroxyl carbonate apatite (HCA) layer on the glass surface. However, the C - O stretching (~1429 cm\(^{-1}\)) and P - O stretching (1200 - 910 cm\(^{-1}\)) bands were attributed due to hydroxyl carbonated apatite (HCA) layer. These results confirmed that the formation of apatite layer on the surface of glass powder was carbonated apatite, which is similar in composition and structure to the bone apatite and was also found on bioactive glasses [13].

![FTIR patterns for S50P4 before and after soaking at different periods in HBSS](image)

**Figure 4.** FTIR patterns for S50P4 before and after soaking at different periods in HBSS

### 3.3 Scanning Electron Microscopy (SEM)

The microstructure of 5 days aged gel-derived S50P4 glasses before and after soaking in HBSS were illustrated in figure 5. The gel-derived glasses were exhibited porous structure on the surface of all glasses. Pores are necessary for tissue formation because they allow migration and proliferation of cells [14]. The porous structure shows the typical morphology of gel-derived glasses fabricated in acid-catalysed conditions [14]. Figure 5 (b) and (c) shows the surface morphology of the glass specimen after soaking in HBSS for 7 days and 14 days respectively. Comparison with the unreacted sample figure 5(a), there was an evidence of apatite layer appeared on the glass surface in figure 5(b) and (c). The images displayed the formation of the apatite particles over the entire surface of the samples, as a result of the action and reaction between the HBSS and glass particles. This pattern was typical of HCA precipitated on the glass surface. In figure 5(c), the precipitation of apatite crystals can be seen as homogenously covered the glass particles. The formation of apatite layer was confirmed by not only XRD results but also FTIR patterns.
Figure 5. SEM images for 5 days aged S50P4 (a) before and after soaking in HBSS at different periods (b) 7 days and (c) 14 days

4. Conclusion
In the present study, the effect of aging time on the structure of gel-derived S50P4 glass and in-vitro bioactivity test with HBSS were investigated. The crystallinity of the bioglass was reduced with increasing aging time but combeite (Na$_2$Ca$_2$Si$_3$O$_9$) phase was only detected after 5 days aged. Before soaking in HBSS, the glass powder was agglomerated as the particle size is reduced in tend to get greater surface area for the purpose of better bioactivity. The precipitation of apatite crystals was homogeneously covered the glass particles after soaked for 14 days.

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