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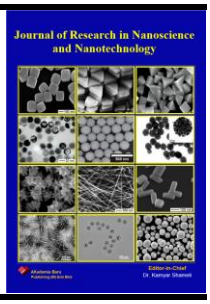

Development of Bioactive Glass Ceramics Based on SiO_2-CaO-Na_2O-P_2O_5 System using Limestone and Silica Sand as Starting Materials

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ABSTRACT

Malaysia has an abundance of mineral deposits, but their utilisation in the production of high-end products, particularly in the biomedical applications are still lacking. This study aimed to identify the potential of limestone and silica sand for the development of bioactive glass ceramic. The glass ceramic composition was developed from the parent glass composition of SiO_2 (55 wt.%), Na_2CO_3 (10 wt.%), $CaCO_3$ (35 wt.%), and P_2O_5 (3 wt.%). To produce the glass ceramic, the glass powder was compacted and heated to 600 °C for nucleation, followed by sintering temperatures of 850, 950, and 1000°C for 60 minutes. X-ray diffraction (XRD) analysis, Vickers hardness, and density measurements were performed to characterise the glass ceramic. An *in vitro* bioactivity test of glass ceramic was examined by immersion in simulated body fluid (SBF) for 1, 3, 7, and 14 days, and the sample was characterised by FESEM-EDX. The XRD analysis of a glass ceramic pellet sintered at 950°C revealed the presence of high crystallisation of wollastonite and nepheline. It also exhibits a high density and Vickers hardness, with values of 3.10 g/cm^3 and 4.60 GPa, respectively. After 14 days of immersion in SBF, the FESEM-EDX analysis indicated the formation of an apatite layer with a Ca/P range of 1.54 to 1.81. Thus, this study demonstrated that local limestone and silica sand have enormous potential for use in the development of bioactive glass ceramics that can be employed as implant materials and in dental applications.

Keywords:
Bioactive glass ceramic, bioactivity, wollastonite, limestone, silica sand

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1. Introduction

A bioactive material is a biomaterial that possesses a specific biological response at its interface, resulting in the formation of a bone-like apatite layer between the material and the body tissues [1]. Bioactive materials are typically derived from glass and glass ceramics such as Bioglass®, apatite-wollastonite glass ceramic, hydroxyapatite (HA) and tri-calcium phosphate (TCP) have received significant attention in biomedical implants and devices [2,3].

Most bioactive glass and glass ceramics are based on silicate glass. Hench [1] introduced the first bioactive glass, known as Bioglass® 45S5, which contains 45 wt.% SiO_2, 24.5 wt.% Na_2O, 24.5 wt.% CaO, and 6 wt.% P_2O_5. Bioglass® 45S5 is developed from a soda lime silicate (SLS) ternary glass system composed of phosphorus pentoxide (P_2O_5) to act as a nucleating agent. It was reported that the presence of phosphate promotes apatite formation [4, 5]. Bioactive glass has numerous therapeutic applications particularly in the regeneration of hard tissues in craniofacial, maxillofacial, and other disciplines of dentistry and cancer treatment as well [6-8]. Their highly reactive surface, high biocompatibility, and antimicrobial properties have made them ideal biomaterials in various fields of medicine and dentistry. Despite its excellent characteristics, one of the primary limitations of bioactive glass is their poor mechanical strength and toughness, which limit its use in load-bearing applications [9]. One of the most effective ways to enhance the mechanical properties of the bioactive glass is through heat treatment by partial crystallisation. This technique has been observed to have an effect on their microstructure, mechanical properties, and biological activity [3,4].

Most researchers have recently expressed an interest in developing bioactive glass and glass ceramics from glass waste, agriculture, and biowaste [10-12]. Unfortunately, only a few studies have been published on the utilisation of mineral resources like limestone and silica sand in the fabrication of bioactive glass ceramics. Malaysia has an abundance of limestone and silica sand deposits, which are widely found in Perak and Sarawak. Limestone is composed of calcium carbonate ($CaCO_3$) and is mainly used in the construction industry and the manufacturing of cement, and pharmaceutical products. According to the Department of Mineral and Geoscience Malaysia (JMG), there are an estimated 27 billion metric tonnes of untapped limestone reserves worth RM304 billion. While silica sand has been extensively utilised in the glass melting and chemical industries. It is typically mined from natural sand deposits and tailing dumps in alluvial tin mining areas. The reserve of silica sand was estimated to be 368 million metric tonnes, with values of RM 21.4 billion [13].

Taking advantage of the existing and abundant Malaysian mineral deposits, this study offers an alternative material for developing bioactive glass ceramics by utilising limestone and silica sand as starting materials. As a consequence, the limestone and silica sand will be characterised, and a glass composition based on a SiO_2-CaO-Na_2O-P_2O_5 ternary glass system will be designed. The bioactive glass ceramic was then fabricated and evaluated for physical, mechanical, and bioactivity properties.

2. Materials and Methods

2.1 Raw Materials and Characterization

Limestone from Keramat Pulai, Perak, and silica sand from Bintulu, Sarawak, were used as starting materials in the development of bioactive glass ceramic. Both mineral samples were sun-dried to remove the moisture before being pulverised to obtain particle sizes that passed through a 45 μm sieve. For the preparation of simulated body fluid (SBF) solution, sodium chloride (NaCl), sodium hydrogen carbonate ($NaHCO_3$), potassium chloride (KCl), calcium chloride ($CaCl_2$), and tris (hydroxymethyl) aminomethane ($(CH_2OH)_3CNH_2$) were supplied from Fisher Scientific and other salts such as sodium sulphate (Na_2SO_4), di-potassium hydrogen phosphate trihydrate ($K_2HPO_4.3H_2O$), magnesium chloride hexahydrate ($MgCl_2.6H_2O$) and hydrochloric acid (HCl) were procured from Merck®. The chemical composition, mineral phase, and heavy metal element content were determined using X-ray fluorescence spectroscopy (XRF-1800, Shimadzu, Japan), X-ray diffraction (D8 Advanced, Bruker, Germany), and inductively coupled plasma optical emission (Model Optima 5300 OV, Perkin Elmer, USA), respectively. Figure 1 depicts the limestone and silica sand obtained from the quarry.

(a) (b)

Fig. 1. Starting material of (a) limestone and (b) silica sand

2.2 Glass and Glass Ceramic Preparation

In this study, the glass melting technique was chosen to prepare the glass. The glass composition was developed using limestone, silica sand and reagent grades of phosphorus pentoxide (P_2O_5) and sodium carbonate (Na_2CO_3). According to CaO and Hench [14], composition in region A of the SiO_2-Na_2O-CaO (SLS) ternary phase diagram in Figure 2 forms a bond with bone and is defined as a bioactive bone bonding boundary. Hence, a glass composition of SiO_2 (55 wt.%), Na_2CO_3 (10 wt.%), and, $CaCO_3$ (35 wt.%) was designed on the basis of the diagram. 3 wt.% of P_2O_5 was fixed and added on top of this 100% glass composition to accelerate the nucleating formation. All the raw materials were mixed in the plastic bottle using a Heidolph Reax 2 test tube shaker for 4 hours to attain a homogenous mixture. The mixture was then melted in a furnace at 1450 °C with a heating rate of 10 °C/min and poured into a preheated stainless steel mould. The molten glass is then annealed and cooled to ambient temperature in a furnace. The bulk glass formed was further crushed and ball milled to produce glass powder.

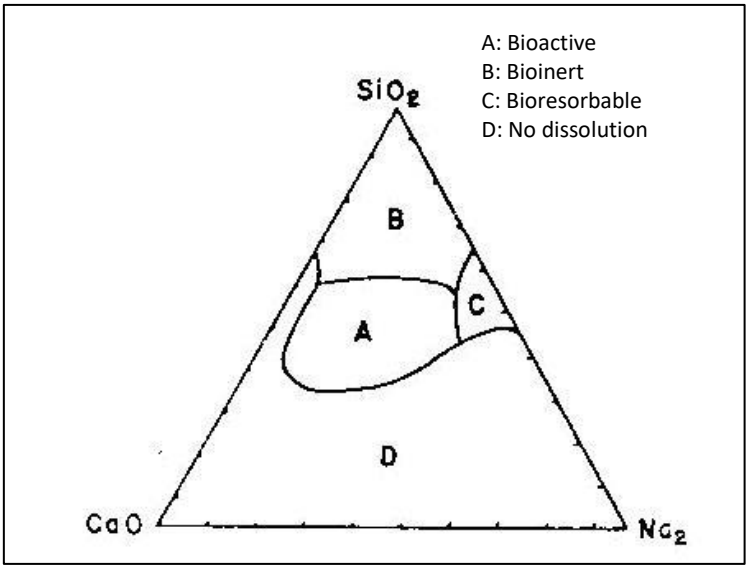


Fig. 2. The SiO_2-Na_2O-CaO (SLS) ternary phase diagram

In order to form glass ceramic, a glass powder formed in a pellet was subjected to a controlled heating process with the nucleation temperature at 600 °C for 60 minutes, followed by sintering to temperatures of 850, 950, and 1000 °C with a heating rate of 5 °C/min and immersion for 60 minutes. The sintered glass ceramic pellet was analysed using XRD to identify the phase, Vickers hardness (Vickers Hardness Tester MMT-3, Matsuzawa, Japan) to measure the hardness, and density. Figure 3 displays a schematic diagram of the process flow of bioactive glass ceramic preparation.

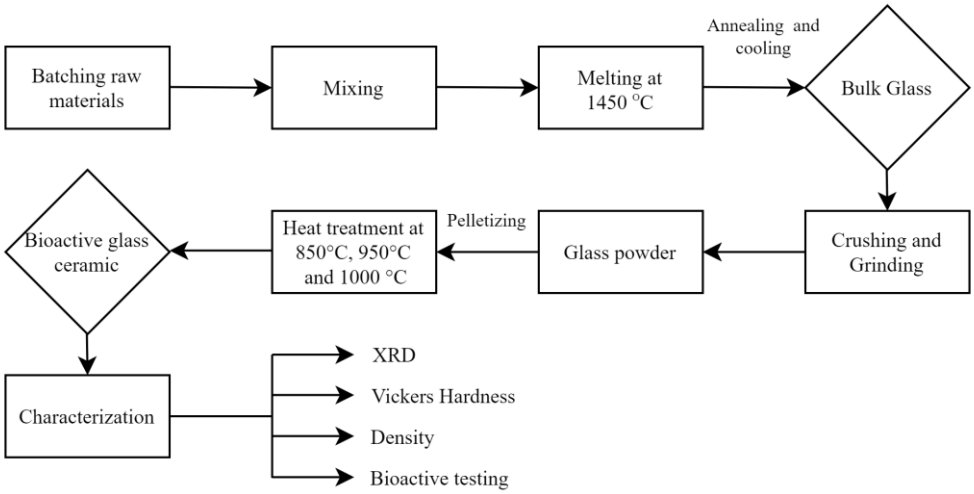


Fig. 3. Flow chart of bioactive glass ceramic preparation

2.3 Bioactivity Testing

To assess the bioactivity of glass ceramic, an in vitro test using simulated body fluid (SBF) in which the ion concentrations were closely near human blood plasma was employed to examine the formation of apatite on the surface of the glass ceramic pellet. The SBF solution was prepared according to the method established by Kokubo and Takadama, [15] in a 1 litre polyethylene bottle. The glass ceramic pellet was immersed in the SBF solution at a temperature of 36.5 °C for 1, 3, 7, 14 days and a pH of 7.3–7.4. After immersion, the samples were gently washed with distilled water and acetone, dried, and analysed using Field Emission Scanning Electron Microscopy (FESEM, Carl Zeiss SUPRA 40VD, Germany) coupled with Energy Dispersive X-ray Spectroscopy (EDX). The atomic percent (in wt.%) of Ca and P, as determined by EDX analysis, was used to calculate the Ca/P ratios for the glass ceramic samples.

3. Results and Discussion

3.1 Characterization of Starting Materials

Table 1 shows the XRF analysis results for the chemical composition of limestone and silica sand. The limestone is primarily composed of calcium oxide (CaO) with a percentage of 54.56%, followed by trace amounts of MgO, SiO_2, Al_2O_3, Fe_2O_3, etc. While silica sand showed silicon oxide (SiO_2) as the predominant oxide with a content of 99.51%. Based on the XRF results, the limestone used in this study was defined as magnesian limestone as defined by Hamizah *et al.*, [16], while the silica sand was classified as a high-quality grade containing between 99.0 and 99.8% SiO_2 [17].

Table 1. Chemical composition of limestone and silica sand (%)

Raw material	Oxides (%)										Loss of Ignition (LOI) (%)
	SiO_2	Al_2O_3	CaO	MgO	Fe_2O_3	K_2O	Na_2O	TiO_2	MnO	P_2O_5	
Limestone	0.72	0.20	54.56	0.67	0.13	0.05	0.09	0.03	0.01	0.03	43.49
Silica sand	99.51	0.03	0.05	0.01	0.02	0.01	0.02	0.06	0.01	-	0.27

Figure 4 presents the XRD analysis for phase identification of limestone, which confirmed the existence of calcite ($CaCO_3$; PDF 01-085-1108) as the major phase mineral. While the predominant peak detected in the silica sand sample corresponded to the mineral quartz (SiO_2; PDF 00-046-1045).

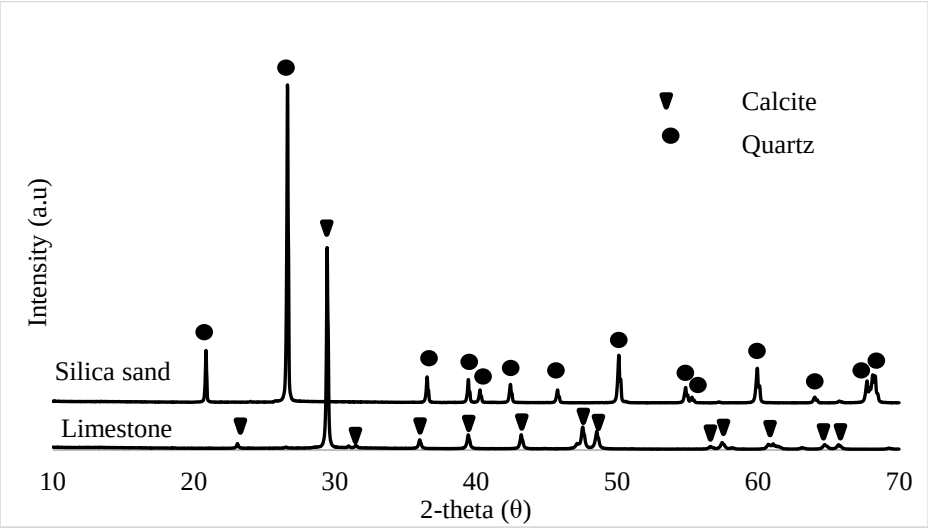


Fig. 4. XRD pattern of limestone and silica sand

In order to be used as biomaterials for implants and devices, the limestone and silica sand were also evaluated for heavy metal leaching in compliance with the standard ASTM F1538-03 Specification for Glass and Glass Ceramic Biomaterials for Implantation [18]. Table 2 revealed the results of the ICP analysis for the heavy metal elements of arsenic (As), cadmium (Cd), lead (Pb), and mercury (Hg) in limestone and silica sand, as well as the allowable limit of standard. The heavy metal content of limestone and silica sand was lower than the standard, demonstrating that both raw materials met the standard requirements for use as biomaterials. Therefore, the limestone and silica sand were proven to be safe for use as starting materials in the production of bioactive glass ceramics that are suitable for use in the human body.

Table 2. Heavy metal elements content of limestone and silica sand

Sample	Heavy element content (mg/L)			
	As	Cd	Pb	Hg
Standard ASTM F1538-03	3.000	5.000	30.000	5.000
Limestone	2.174	0.752	0.816	0
Silica sand	0.584	1.475	4.199	0

3.2 Characterization of Glass and Glass-ceramics

XRD analysis of glass and glass-ceramic profiles after heating at various temperatures (i.e., 850, 950, and 1000°C) is shown in Figure 5. It is obvious that the XRD pattern of the glass powder examined before heat treatment was fully amorphous. After heat treatment, the diffraction peaks matched with

the two types of crystalline phase, which corresponded to wollastonite ($CaSiO_3$; PDF 400-042-0547) and nepheline ($NaAlSiO_4$; PDF 00-05-0424). Based on the XRD pattern, an increase in temperature from 850 to 1000 °C did not cause significant changes for the wollastonite and nepheline crystalline phases. However, when the glass ceramic was heated to 950 °C, high crystalline of wollastonite showed an increased peak intensity at 2θ = 27, 28.5, and 30 °C, while nepheline showed a high peak at 2θ = 21° and 23°. When the glass ceramic was heated at 1000 °C, the nepheline crystal decreased in phase intensity at 2θ = 21° and disappeared at 2θ = 23°. The halo peak at 2θ = 12° was observed in all the glass ceramics samples, which indicates the presence of residual amorphous glassy phases. According to Teixera *et al.*, [19], the high content and complex structure of silica caused some silicate forms in the short range to not be detected by XRD, resulting in an amorphous phase.

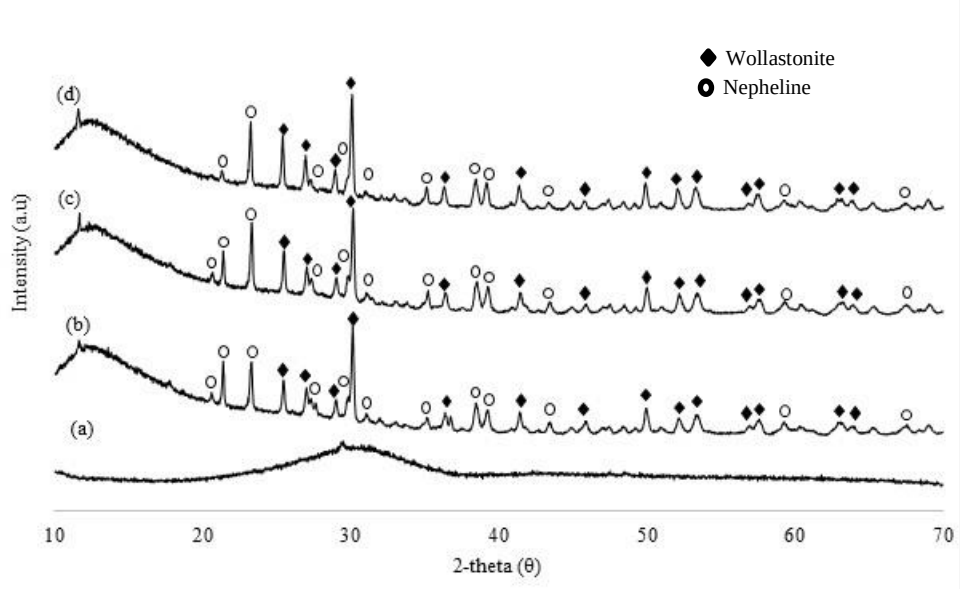


Fig. 5. XRD pattern of the (a) glass powder and glass ceramic sintered at (b) 850 °C, (c) 950 °C, and (d) 1000°C

Figure 6 represents the Vickers hardness and density measurements of the glass ceramic pellet sintered at 850, 950, and 1000 °C. From the graph, it was obvious that the Vickers hardness value increases as the heat treatment temperature increases from 850 to 950 °C with maximum values of 4.60 GPa. The same observation was observed for the density measurement of glass ceramic after heat treatment. The density value of glass ceramic after heat treatment increases with increasing sintering temperatures up to 950 °C. This increment is mightily attributed to the high crystallisation of wollastonite and nepheline phases revealed by the XRD result. In general, the sintering temperature is one of the significant factors influencing the densification of the glass ceramic. Based on the XRD result and Vickers hardness and density measurements, glass ceramic heat treated at 950 °C has been chosen for bioactivity testing due to its ability to develop highly crystalline phases and higher hardness and density.

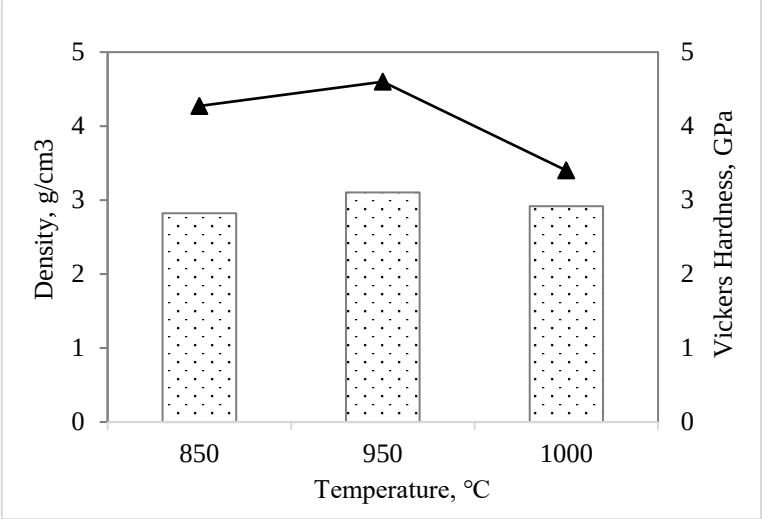
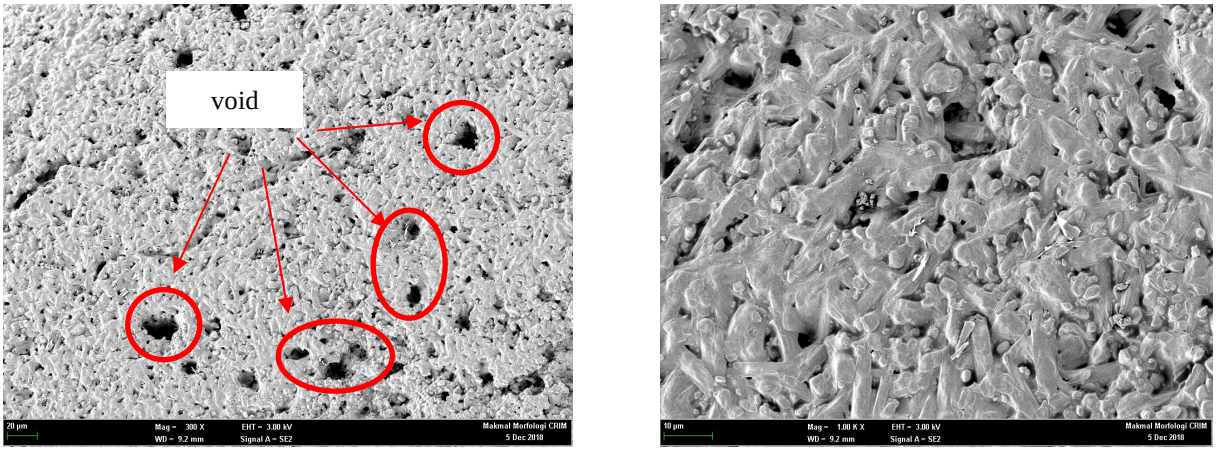


Fig. 6. Vickers hardness and density measurements for glass ceramic sintered at various temperatures

3.3 Bioactivity Evaluation

According to Kokubo and Takadama [15], the implanted biomaterial ability to form interfacial bonds with tissues and bone *in vivo* can be predicted by the apatite layer formation on the surface of the glass ceramics in SBF solution. Figure 7 shows the FESEM micrograph of the glass ceramic heat-treated at 950 °C before being immersed in the SBF solution. It was found that the glass ceramic pellet had a rough surface and a presence of voids. The surface structure of the glass ceramic (Figure 7(b)) shows a large size of whisker-like crystals, and this formation is typical of wollastonite-type glass ceramics formed at 1000 °C [20].



(a) (b)

Fig. 7. Morphology of bioactive glass ceramic sintered at 950 °C before immersion in SBF with the magnification of (a) 300x and (b) 1000x

FESEM images of the crystalline specimens after 1, 3, 7 and 14 days of immersion in SBF are presented in Figure 8. After immersion for 1 and 3 days in SBF, the glass ceramic demonstrated crystal development, as shown in Figure 8(a) and (b). On day 7 after immersion, it is evident that a new layer known as the apatite layer, which mimics coral reefs, was clearly visible on the surface of the glass

ceramic, as illustrated in Figure 8(c). Then, after 14 days of immersion, an apatite layer was well-growing in size and covered the whole surface of the glass ceramic.

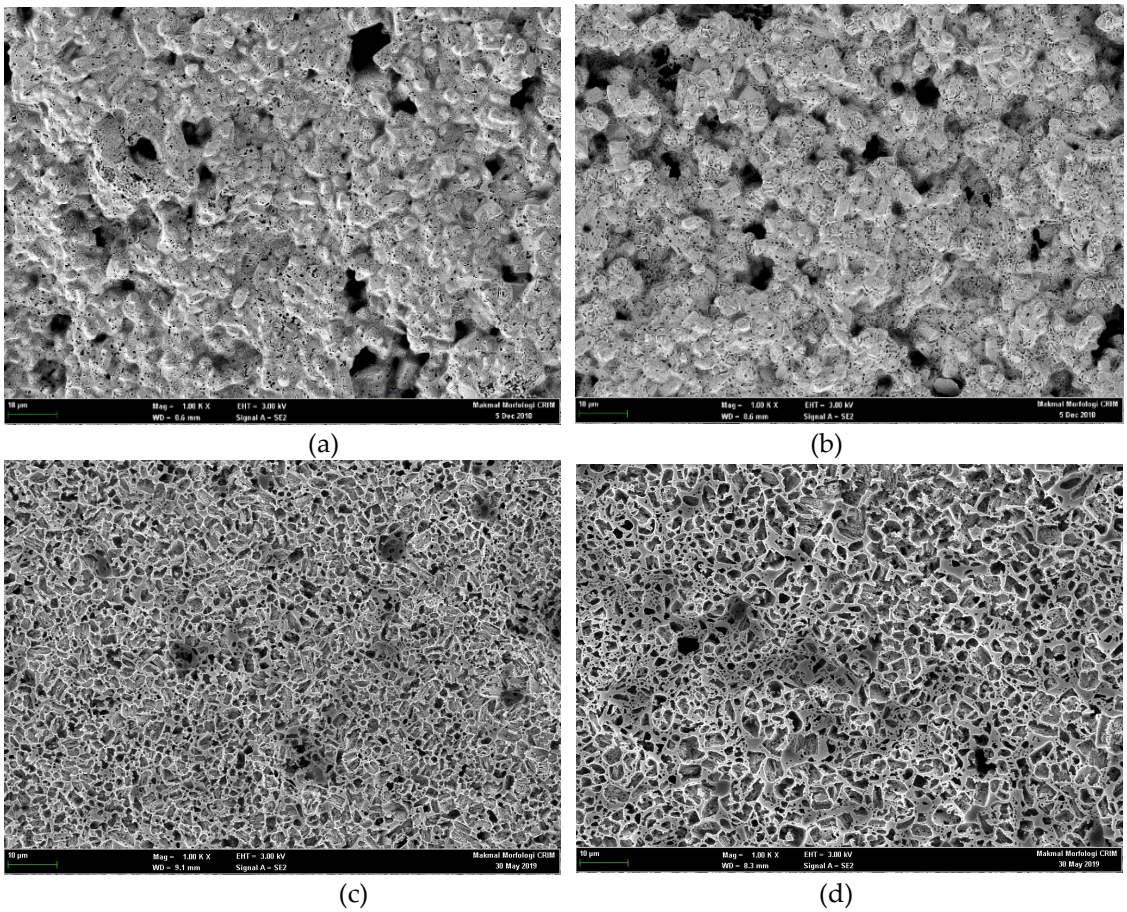
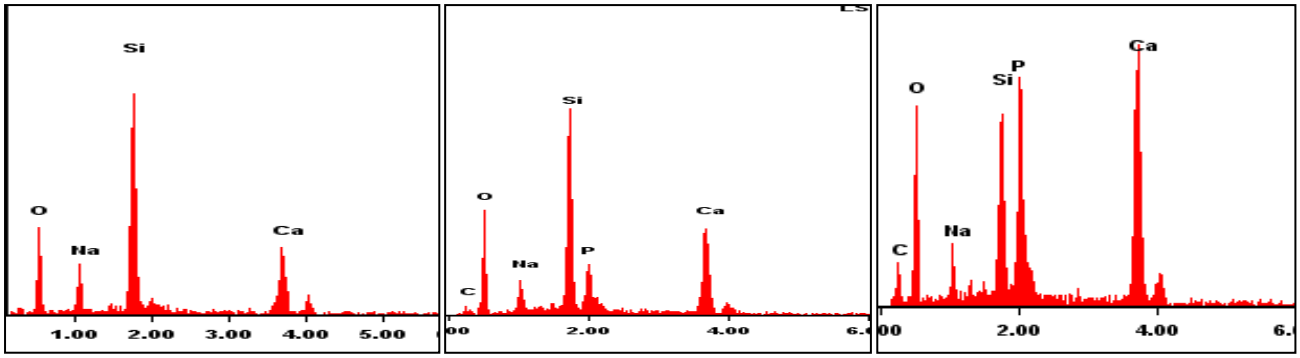


Fig. 8. Morphology of bioactive glass ceramic sintered at 950 °C after immersion in SBF for (a) 1 day and EDX spectrum (b) 3 days (c) 7 days (d) 14 days and EDX spectrum with the magnification of 1000x

The EDX spectra of bioactive glass ceramic before and after immersion of 1 and 14 days in SBF solutions are shown in Figure 9(a), (b), and (c), respectively. The EDX analysis of glass ceramic before immersion revealed the presence of high levels of silicon (Si), calcium (Ca), sodium (Na), and oxygen (O) that were attributed to the elemental composition of glass ceramic. After being immersed for 1 day (Figure 9(b)), the element phosphorus (P) was detected and its presence was in an appreciable amount, reflecting the bioactivity that occurred due to the immersion in the SBF solution. The EDX results showed that the Si content decreased while the concentrations of Ca and P increased significantly after 14 days of immersion (Figure 9(c)), which indicates that the layer formed on the sample surface is rich in calcium and phosphorus.



(a) (b) (c)

Fig. 9. EDX spectrum of bioactive glass ceramic sintered at 950 °C (a) before immersion (b) (b) after immersion in SBF for 1 day and (c) for 14 days

Table 3 represents the pH measurement, the elemental composition of Si, Ca, and P, as well as the Ca/P molar ratio before and after immersion in SBF. According to Liu *et al.,* [21], the bioactivity mechanism of glass and glass ceramics involves two processes: dissolution and precipitation. The release of ions such as Ca^{2+} and Si from the sample will occur during the immersion process due to the interaction of the sample with the SBF solution, which causes the development of a silica-rich layer phase containing silanol on the surface of the sample. Then, Ca^{2+} ions will dissolve into the solution and exchange with H^{+} ions. The dissolution of Ca^{2+} ions from the glass ceramic sample into the SBF solution caused the pH value of this study to rise from 7.37 to 7.98 after 1 day of immersion, which was confirmed by an increase in P concentration. These ions were then deposited on the surface of the sample, forming an apatite layer rich in calcium and phosphate. After 14 days of immersion, the bioactive glass ceramic sample's Ca/P ratio was determined to be between 1.54 and 1.83, suggesting the development of an apatite layer.

Table 3. pH value, elemental composition of Si, Ca, P and Ca/P ratio of bioactive glass ceramic before and after immersion in SBF solution

Immersion period (day)	ph value	Si (wt%)	Ca (wt%)	P (wt%)	Ca/P molar ratio
0	7.37	18.43	16.76	2.04	-
1	7.98	10.98	9.79	6.21	1.57
3	8.13	9.72	12.66	6.89	1.83
7	8.05	9.14	14.55	8.93	1.63
14	7.89	8.76	19.08	12.34	1.54

4. Conclusions

A bioactive glass ceramic based on SLS glass formation was successfully developed in this work by using mineral deposits of limestone and silica sand. Glass ceramic pellets sintered at 950 °C exhibited good physical and mechanical properties, as well as high crystalline phases of wollastonite and nepheline. The bioactivity evaluation by immersion in SBF solution revealed high bioactivity, which promotes the formation of an apatite layer on the surface of glass ceramic. Thus, Malaysian limestone and silica sand demonstrated a great potential for use as starting materials for the development of bioactive glass ceramics, which can be employed in medical and dental applications.

Funding

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