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'IN-VITRO' BIOACTIVITY STUDY OF SYNTHETIC WOLLASTONITE PRODUCED FROM MALAYSIAN LIMESTONE AND SILICA SAND

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ABSTRACT

Wollastonite ($CaO.SiO_2$ or $CaSiO_3$) is an industrial mineral comprising of calcium, silicon and oxygen. Synthetic wollastonite was synthesised via solid state reaction using Malaysian limestone and silica sand. The in-vitro bioactivity was investigated by soaking the synthetic wollastonite pellet in simulated body fluid (SBF) solution for 1, 3, 5, 7 and 14 days at 36.5^oC. XRD revealed the presence of hydroxyapatite phase as the soaking time of synthetic wollastonite pellet in SBF was increased. SEM coupled with EDX showed the formation of granules of agglomerated apatite particles on the surface of the soaked synthetic wollastonite pellet. During the formation of apatite, phosphate ions from SBF solution were consumed and this was confirmed by ICP where the concentration of the ions decreased after the soaking process. FTIR showed that the peaks for phosphate and carbonate ions increased when the apatite layer formed on the surface of synthetic wollastonite pellet. This study indicated that synthetic wollastonite produced from Malaysian limestone and silica sand was bioactive and may be used for preparation of implantable biomaterial.

Keywords: synthetic wollastonite, in vitro bioactivity, limestone, silica sand

1. INTRODUCTION

Malaysian has abundant limestone ($CaCO_3$) and silica sand (SiO_2) resources. According to Malaysian Minerals Year Book 2009, the production of limestone and silica sand in 2009 is 35,808,507 tonnes and 630,394 tonnes respectively. Malaysian limestone is used mainly in construction and agriculture industry while Malaysian silica sand is widely used in glass industry. Therefore, it is essential to diversify the usage of Malaysian limestone and silica sand into advance technology such as in biomaterial applications.

Bioactive material can elicit a specific biological response at the interface of the material, which results in the formation of a bond between the tissues and the material (Hench and Wilson, 1999). Recent studies (Liu and Ding, 2004) have shown that calcium silicate material has the potential to be used as biomaterials due to their excellent bioactivity. Some calcium silicate material such as wollastonite (β-$CaSiO_3$), pseudowollastonite (α-$CaSiO_3$) and larnite (Ca_2SiO_4) can bond to bone when in contact with simulated body fluid (SBF) (Gou and Chang, 2004).

Wollastonite or monocalcium silicate is an industrial mineral that appears in three forms of mineral. Two forms which occur naturally at low temperature are wollastonite and parawollastonite (β $CaO.SiO_2$ or β $CaSiO_3$). At high temperature, pseudowollastonite ($CaO.SiO_2$ or α $CaSiO_3$) can be obtained from the mixture of lime and silica and also when either of low temperature mineral are synthesised to above temperature of 1125^oC (Lea, 1970). Pseudowollastonite is very rare in natural wollastonite. The binary phase diagram for system CaO-SiO_2 system is shown in Figure 1 (Lea, 1970). Currently, wollastonite mineral is widely used in ceramics, paint, friction products, joint compounds, refractories, rubber, wallboard and metallurgical.

In this study, a solid state reaction has been used to synthesis synthetic wollastonite from Malaysian limestone and silica sand. The in-vitro bioactivity of the synthetic wollastonite was evaluated by soaking them in simulated body fluid (SBF) solution. The objective of this study is to examine the formation behaviour of hydroxyapatite on the surface of synthetic wollastonite powder.

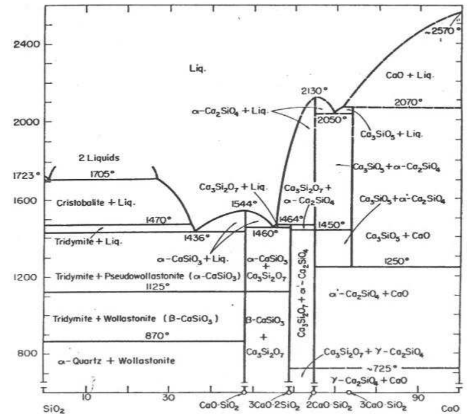

Figure 1 System $CaO\text{-}SiO_2$

2. METHODOLOGY

2.1 Preparation of Synthetic Wollastonite Powder

Theoretically, synthetic wollastonite powder can be synthesised by solid state reaction according to the equation:

$$CaCO_3 + SiO_2 \longrightarrow CaSiO_3 + CO_2 \qquad (1)$$

Malaysian limestone from Simpang Pulai, Perak and silica sand from Kota Tinggi, Johor were ground to obtain finer particle size 100% passing through -75 μm. The batch mixture of Malaysian limestone and silica sand with molar ratio of 1: 1 was synthesised at 1450^oC for 4 hours at a heating rate of 5^oC/min (Modutemp). The wollastonite powder was characterised by X-ray diffraction (XRD) [Bruker D8 Advanced XRD] [Bruker] and Scanning electron microscopy (SEM) coupled with Energy-dispersive X-ray spectroscopy (EDX) [Supra 40VP].

2.2 In-vitro Bioactivity Evaluation of Synthetic Wollastonite Powder

An amount of 1 g of synthetic wollastonite powder was pressed into a disc of 13 mm in diameter and 7 mm in thickness. The SBF solution was prepared by dissolving reagent grade sodium chloride (NaCl), sodium hydrogen carbonate ($NaHCO_3$), potassium chloride (KCl), di-potassium hydrogen phosphate trihydrate ($K_2HPO_4.3H_2O$), magnesium chloride hexahydrate ($MgCl_2.6H_2O$), calcium chloride ($CaCl_2$) and sodium sulphate (Na_2SO_4) in distilled water at 36.5^oC. The details of the composition of the SBF solution are given in Table 1 (Kokubo and Takadama, 2006). The ion

Table 1 Reagent for preparing the simulated body fluid solution

Order	Reagent	Amount	Purity (%)
1	NaCl	8.035 g	99.5
2	$NaHCO_3$	0.355 g	99.5
3	KCl	0.225 g	99.5
4	$K_2HPO_4.3H_2O$	0.231 g	99.0
5	$MgCl_2.6H_2O$	0.311 g	98.0
6	1.0 M HCl	39 ml	-
7	$CaCl_2$	0.292 g	95.0
8	Na_2SO_4	0.072 g	99.0
9	Tris	6.118 g	99.0
10	1.0 M HCl	0-5 ml	-

concentrations of SBF were adjusted to those in human blood plasma (Table 2) with hydrochloric acid (HCl) and tris-hydroxymethyl aminomethane (($(HOCH_2)3CNH_2$) (Tris)) at pH value of 7.30 ± 0.05 (Kokubo and Takadama, 2006). The pellets of synthetic wollastonite powder were soaked in SBF for 1, 3, 5, 7 and 14 days at 36.5^oC. The soaked and dried synthetic wollastonite pellets were characterised by X-ray diffraction spectrometer (XRD) [Bruker D8 Advanced XRD], Scanning electron microscopy (SEM) [Supra 40VP] and Fourier transform infrared spectrometer (FTIR) [Bruker Optics]. The concentration of Ca,Si and P in the SBF solution was determined by Inductive coupled plasma spectrometer (ICP) [Perkin Elmer].

Table 2 Ion concentrations of SBF in comparison with human blood plasma

	Concentration (mmol/l)	
	SBF	Human Blood Plasma
Na^+	142.0	142.0
K^+	5.0	5.0
Ca^{2+}	2.5	2.5
Mg^{2+}	1.5	1.5
Cl^-	147.8	103.8
HPO_4^{2-}	1.0	1.0
HCO^{3-}	4.2	27.0
SO_4^{2-}	0.5	0.5

3. RESULT AND DISCUSSION

3.1 Characteristics of Limestone, Silica Sand and Synthetic Wollastonite Powder

Figure 2 shows the XRD patterns of limestone, silica sand and wollastonite powder. The peak of crystalline calcite and quartz was respectively obtained from Malaysian limestone and silica sand. Pseudowollastonite was found as a major phase in the XRD pattern of synthetic wollastonite.

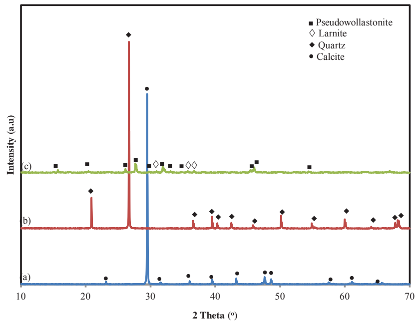

Figure 2 XRD patterns of (a) limestone (b) silica sand and (c) synthetic wollastonite

Figure 3 illustrates SEM micrograph and EDX spectrum of limestone, silica sand and synthetic wollastonite. It indicates the irregular shape of Malaysian limestone and silica sand grains whereas synthetic wollastonite exhibits a needle-like structure. The EDX spectrum confirmed the chemical composition for limestone, silica sand and synthetic wollastonite.

3.2 Characteristics of soaked synthetic wollastonite

Figure 4 shows the XRD patterns of synthetic wollastonite pellet after soaking in the SBF solution for various periods. The peaks for hydroxyapatite started to appear in the XRD pattern after the pellet is left 1 day soaked in the

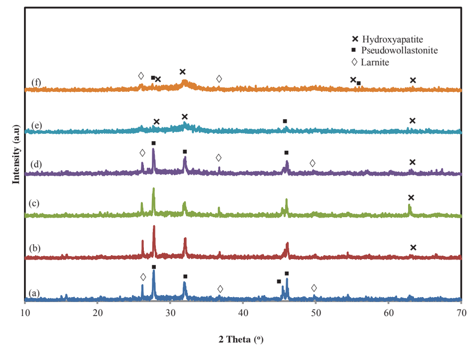

Figure 4 XRD patterns of (a) 0 day, (b) 1 day, (c) 3 days, (d) 5 days, (e) 7 days and (f) 14 days of soaked synthetic wollastonite in the SBF solution.

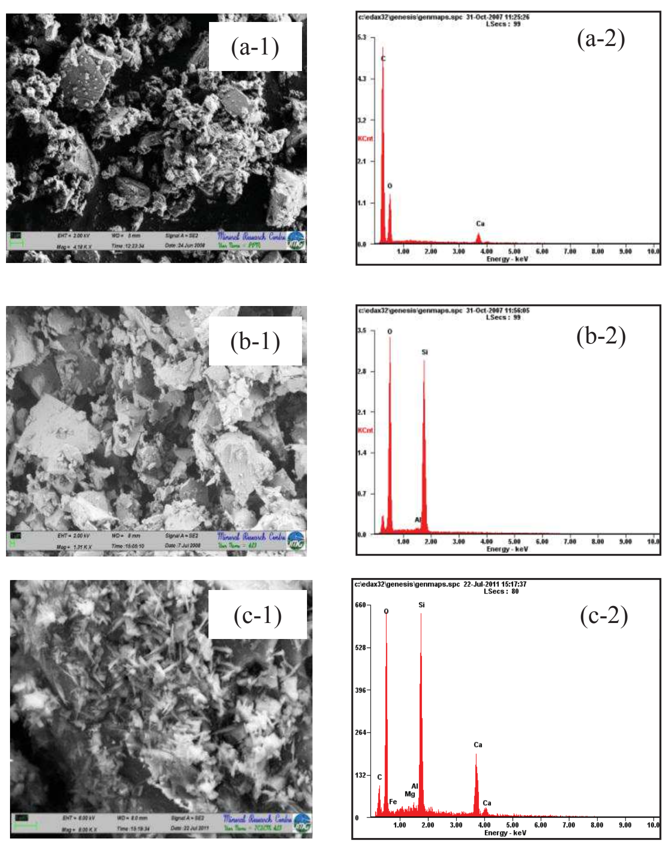

Figure 3 SEM micrographs and EDX spectrum of (a) limestone (b) silica sand and (c) synthetic wollastonite

SBF solution. With prolonged soaking time from 1 day until 14 days, the intensities of hydroxyapatite increased which indicate the formation of hydroxyapatite on the surface of wollastonite powders.

Figure 5 shows the SEM micrographs and EDX spectrum of wollastonite pellets after soaking in the SBF solution for various soaking time. As a comparison with the structure of synthetic wollastonite before soaking in the SBF solution, it shows formation of granules agglomerated apatite particles on the surface of synthetic wollastonite. These tiny granules of agglomerated particles of apatite started to form after 1 day it was soaked. Moreover, it was also found that by increasing the soaking time, larger granules of agglomerated particles of

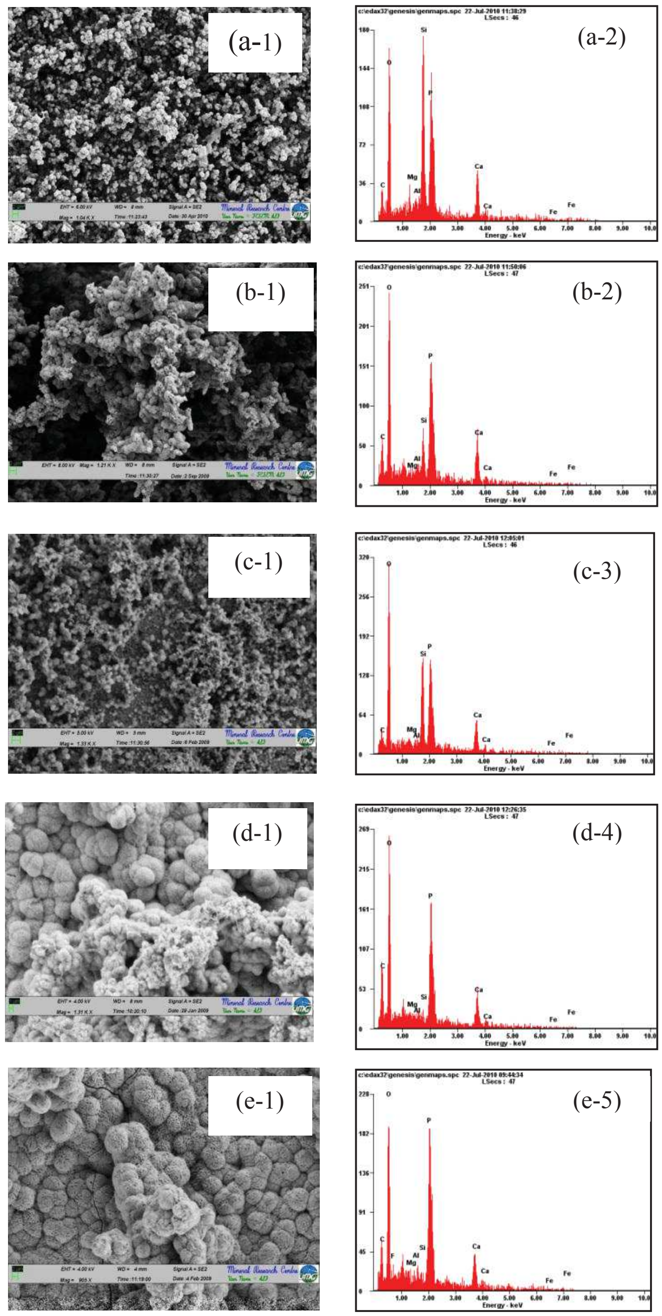

Figure 5 SEM micrographs of synthetic wollastonite after soaking in the SBF solution for (a) 1, (b) 3, (c) 5, (d) 7 and (e) 14 days.

apatite appeared on the surface of synthetic wollastonite. It involves the precipitation process during the deposition of apatite layer on the surface of synthetic wollastonite. Observation of the SEM micrographs is confirmed by the EDX spectrum. It shows the changes in chemical composition of elements during the formation of apatite layer on the surface of synthetic wollastonite powder. The increase of intensity of P peak and the ratio of Ca/P obtained (Table 3) show that the formation process of apatite has occurred for every soaking time.

Table 3 The Ca/P ratio of synthetic wollastonite powder after soaking in the SBF solution for various periods

Soaking time (day)	Ca/P ratio
1	1.22
3	1.10
5	1.12
7	0.84
14	0.91

The FTIR spectrums (Figure 6) show the change of absorption bands of silica and carbonate to apatite. When the soaking time increased, the intensity of silica rich layer started to decrease. It indicates that the silica rich layer has provided a favorable site for apatite nucleation (Hench and Wilson, 1999). The C-O stretching carbonate is increased as the soaking time increased. It promotes the formation process of carbonate-containing apatite layer on the surface of synthetic wollastonite. The P-O stretching of phosphate started to reduce after 1 day. It shows that during the soaking time, phosphate ions in the

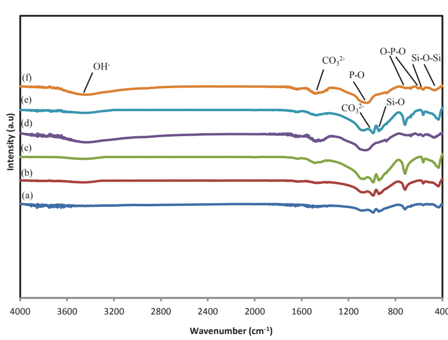

Figure 6 Soaked spectrum of synthetic wollastonite in SBF solution for (a) 0, (b) 1 day, (c) 3 days, (d) 5 days, (e) 7 days and (f) 14 days.

SBF solution are consumed when the apatite layer is formed (Gou and Chang, 2004).

Figure 7 shows the change in concentration of Ca, Si and P in the SBF solution measured by ICP after synthetic wollastonite is soaked for various periods. Calcium ions and silicate ions are released from the synthetic wollastonite powder while phosphate ions is used to form the Ca-P layer. The Ca, Si and P concentrations became almost constant after prolonged soaking period. It shows that an equilibrium state is obtained between the dissolution of synthetic wollastonite powder and the formation of apatite layer.

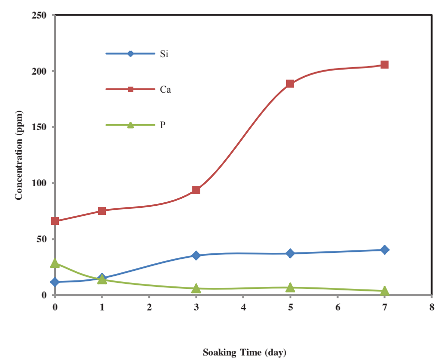

Figure 7 Changes of Ca, Si and P concentrations in the SBF solution.

4. CONCLUSION

This study led to the following conclusions:

- A solid state route can be successfully applied for the preparation of synthetic wollastonite from Malaysian limestone and silica sand.
- Apatite layers formed on the surface of synthetic wollastonite suggest that synthetic wollastonite possess good bioactivity.
- Malaysian limestone and silica sand may be potentially used as implantable biomaterial.

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