

IN-VITRO BEHAVIOUR OF BIPHASIC CALCIUM PHOSPHATE WITH DIFFERENT RATIO OF SILICA CONTENT IN SIMULATED BODY FLUID

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RINGKASAN

Hidroksiapatit (HA) dan trikalsium fosfat (TCP) adalah seramik bioaktif kalsium fosfat (CaP) berfungsi sebagai bahan yang memberangsangkan bagi menggantikan tulang kerana komposisinya hampir menyamai dengan komponen-komponen organik semulajadi tulang. Oleh itu, gabungan di antara kalsium fosfat yang bersifat lebih stabil dan lebih larut itu sesuai untuk di formulasikan menjadi kalsium fosfat dwifasa (BCP). Beberapa kajian melaporkan gabungan gelas bioaktif yang berasaskan silika ke atas CaP telah meningkatkan struktur bahan implan dalam tubuh manusia untuk membaiki dan menggantikan tulang yang berpenyakit atau rosak. Tujuan utama kajian ini adalah untuk menentukan kadar penguraian/kemerosotan BCP dalam nisbah yang berbeza daripada silika (Si) selepas di rendam dalam cecair simulasi badan (SBF). Peratusan nilai kehilangan dan nilai pH bagi kedua-dua nilai daripada 0-30 hari akan di tentukan. Kajian mendedahkan nilai pH meningkat daripada kandungan silika 0 wt.% kepada silika 5 wt.% serta ianya selari dengan peningkatan kadar masa rendaman di mana semua nilai kekal dalam keadaan neutral (7.0-7.9). Kehilangan berat sampel juga meningkat dengan peningkatan masa rendaman. Keputusan menunjukkan bahawa salah satu sampel silinder Si-BCP masing-masing akan merosot 625 hari untuk Si 0 wt.%, 735 hari untuk Si 1 wt.%, 518 hari untuk Si 3 wt.% dan 705 hari untuk Si 5 wt.%.

ABSTRACT

Hydroxyapatite (HA) and tricalcium phosphate (TCP) are bioactive calcium phosphate (CaP) ceramics which function as a promising material for bone substitute due to their almost similar composition to the inorganic components of natural bone (Zhang *et al.*, 2006). Thus, with the combination of both CaP's features of more stable and more soluble, it is possible to formulate a biphasic calcium phosphate (BCP). Several studies reported that the combination of silica-based bioactive glasses on the CaP enhanced the structure for implant materials in the human body to repair and replace diseased or damaged bone (Ning *et al.*, 2005). The main purpose of this study is to determine the degradation rate of BCP in different ratios of silica (Si) after immersion in simulated body fluid (SBF). The percentage of weight loss and pH value for both types of samples from 0-30 days were carried out. The study revealed that pH value increased from 0 wt.%Si to 5 wt.%Si, followed with increasing immersion time and both values remained at neutral condition (7.0-7.9). The weight loss also increased with increasing immersion time. Through the gradient of the graph, the results indicated that one cylinder disc of Si-BCP will degrade in 625 days for 0 wt.% Si, 735 days for 1 wt.% Si, 518 days for 3 wt.% Si and 705 days for 5 wt.% Si, respectively.

Keywords: Hydroxyapatite, Tetracalcium phosphate, Silica, Simulated body fluid.

INTRODUCTION

Promising as a biocompatible and biodegradable biomaterial, calcium phosphate (CaP) is the main component of bone and widely used as a bone substitute for bone reconstructive surgery (Rush, 2005). Among the CaP group, hydroxyapatite (HA) and tricalcium phosphate (TCP) have been used due to their similarity composition and crystalline structures to the mineral phase of bone. HA is known to be more stable in body environment while TCP is known to be more soluble. Thus, the combination of a balanced condition between a more stable and more soluble phase, is appropriate to formulate a biphasic CaP with a controlled dissolution rate and different mechanical properties.

However, several literature reported that another bioceramic material, called silica based bioactive glasses, have a superb bioactivity than simply CaP bioceramics (Ning *et al.*, 2005). The biocompatibility character of these materials led them to be investigated for use as implant in the human body to repair and replace damaged bone. Due to these advantages, silica is used to incorporate with BCP in order to increase their biological activity (Song, 2012).

The bioactivity (ability to directly bond to bone tissue) of these materials could be evaluated by examining the ability of a layer to form on their surface in a Simulated Body Fluid (SBF) with ion concentrations and pH values similar to those of human blood plasma (Kokubo *et al.*, 2006). In recent years, several studies reported the mechanism of bone-like apatite formation and dissolution behaviour of the bioactive materials through immersion in SBF for various durations (Ding, 2008). Kokubo *et al.*, reported the formation of bone-like apatite on artificial materials induced by functional groups such as Si-OH and Ti-OH, where it forms a negative charge and induce apatite formation through formation of amorphous CaP that ultimately transform into bone-like apatite. This unique bioactivity is highly desirable and hence CaP compound are widely studied and used as bone replacements or as coatings on implants (Otsuka *et al.*, 1994). However, the major limitation of CaP use as load-bearing biomaterials is its mechanical properties. Typical of ceramics, CaP biomaterials are brittle with poor fatigue resistance. It is for these reasons that the materials are used primarily as fillers and coatings.

The objective of this study is to determine and compare degradation rate of BCP in different ratio of silica (Si) after immersion in Simulated Body Fluid (SBF). The comparison of degradation was based on the conditions in neutral phase and how fast the samples degrade in SBF. Characterization of the Si-BCP was carried out using Scanning Electron Microscope (SEM) to observe homogeneity of silica in Si-BCP, ratio of BCP, and surface morphology of the composites after immersion. X-ray Diffractometer (XRD) was used to observe crystallinity of the composite, ratio of BCP, presence of impurities and other reaction between Si-BCP after immersion process.

MATERIALS AND METHODS

Synthesis of Apatite slurry

Apatite slurry is obtained using raw calcium hydroxide and phosphoric acid via wet precipitation technique at 1.60 Ca/P ratio. Phosphoric acid (85 % concentration) is slowly added to calcium hydroxide solution at a controlled rate. pH (maintained below 9) and temperature (below 80 °C) are controlled during reaction to obtain a stable suspension. Gelatinous apatite slurry was obtained at the end of the process and ready for spray drying.

Incorporation of Silica into apatite powder

The gelatinous apatite slurry was collected from material synthesis (i.e. after supernatant is poured out leaving behind concentrated gelatinous apatite mixture). Silica (1-5 wt.%) was then added into this mixture and spray dried using lab scale spray dryer.

Fabrication of cylinder discs for degradation and characterisation purposes

Apatite powder (~2 g) from spray drying process is fabricated into cylindrical discs (~20 mm diameter) using an automated handpress (Brand Carver, 1 tonne pressure for 1 minute) before sintering.

Sintering to obtain Si- BCP composite

Silica-apatite cylinder discs was sintered in box furnace at 900 °C (CMTS) to obtain Si- BCP composite (Figure 1). The sintering temperature that affects the composition and microstructure of Si- BCP at different ratios of BCP was studied in terms of grain size and melting phase for both Silica and BCP that could promote bioactivity of the composite.

Sintering process:

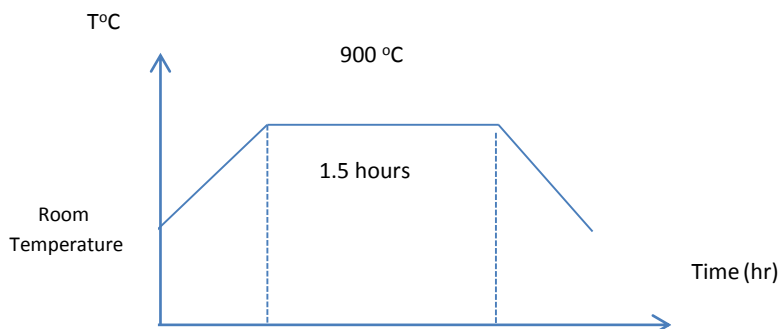


Figure 1. Sintering profile at 900 °C of Si-BCP

In-vitro chemical evaluation

For in-vitro chemical evaluation, each Si- BCP composites was sterilized in an autoclave (HVE-50, Hirayama) for 20 minutes at 121 °C. Samples were then immersed in vials containing 30 ml of Simulated Body Fluid (SBF) [pH 7.4 ± 0.1] maintained at 37.0 ± 0.5 °C in oven for various durations (3, 7, 14, 21 and 30 days). The preparation of the samples was conducted in biohazard safety cabinet (TELSTAR BIO-II-A). After immersion, corresponding to the identified durations, Si- BCP samples were taken out from the vials containing SBF, and subsequently dried with laboratory tissue for 24 hours at ambient temperature. Prior to sending the dried samples to SEM and XRD for characterisation, the dried samples were weighed using analytical balance to determine the rate of mass loss. After immersion, the SBF for each duration were stored in chiller, before being taken out and maintained at 37.0 ± 0.5 °C in water bath for pH measurement. Mass loss was calculated as Equation 1.

$$\text{Mass loss} = \frac{\text{Dry weight} - \text{Weight before immersion}}{\text{Initial weight}} \times 100 \quad (1)$$

Physical characterisation of Si-HA/TCP

X-Ray Diffractometer (XRD) Bruker D8 Advanced and Scanning Electron Microscope (SEM) LEO 1500 series was used to observe the difference between samples before and after immersion process.

RESULTS AND DISCUSSION

Mass loss

Table 1 and Figure 2 show the degradation rate of the Si- BCP samples after immersion in SBF. It was observed that weight loss of 0 wt. % Si was from 13.02 % at day 3 to 17.02 % at day 30. This trend of weight loss was followed by 16.28 % to 18.76 % for 1 wt. % Si and 16.72 % to 18.85 % for 3 wt. % Si, respectively. At high content of Silica at 5 wt. %, samples continue to degrade from 16.72 % at day 3 to 18.93 % at day 30 of immersion time. These results revealed that an increase in immersion time increased the weight loss. The Si- BCP samples will degrade in total of 625 days for 0 wt% Si, 735 days for 1 wt% Si, 518 days for 3 wt% Si and 705 days for 5 wt% Si based on the gradient of the graph.

Table 1: Weight loss of Si-HA/TCP after immersion in SBF

Immersion	Weight loss of Si-BCP							
	0wt% Si (g)	%	1wt% Si (g)	%	3wt% Si (g)	%	5wt% Si (g)	%
Day 0	2.1655	-	2.1655	-	2.1655	-	2.1655	-
Day 3	1.8835	13.02	1.8130	16.28	1.8034	16.72	1.8035	16.72
Day 7	1.8823	13.08	1.8102	16.41	1.8506	14.54	1.8820	13.09
Day 14	1.8437	14.86	1.7806	17.77	1.7574	18.84	1.7697	18.27
Day 21	1.8095	16.44	1.7680	18.36	1.7645	18.52	1.7880	17.43
Day 30	1.7970	17.02	1.7633	18.76	1.7570	18.85	1.7556	18.93

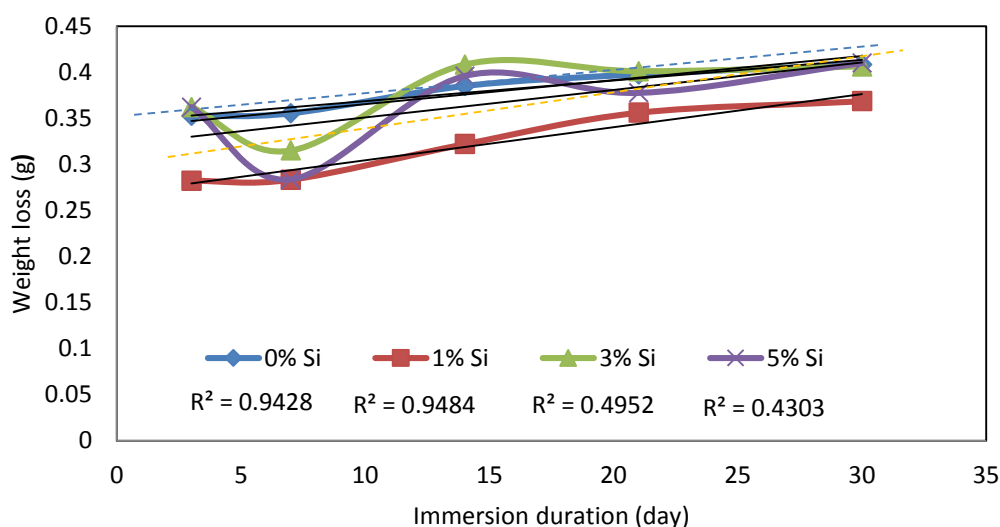


Figure 2. Degradation trends of Si-BCP after immersion in SBF

pH study

pH is the measurement of the acidity or alkalinity of a solution. Figure 3 shows the pH of Si-BCP after immersion in SBF solution. It can be observed that pH value for both types of samples remained in neutral condition (7.0-7.9) even though at high content of silica. An increasing of silica content from 0 wt.% to 5 wt.% will cause increase of pH value. These results were illustrated in Figure 3 where pH value of 5 wt.% silica is higher than others, respectively. However, it was observed that an increasing of immersion time from day 0 to day 30 did not affect the pH value, where it was still maintained in neutral condition, even though until 30 days of immersion.

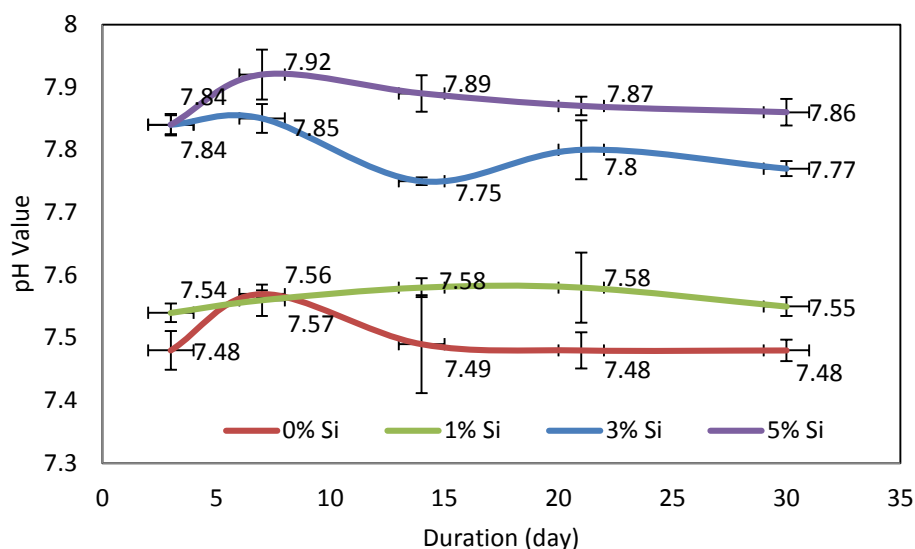


Figure 3. pH value against immersion of Si-BCP after immersion in SBF

Effect of Silica content on the degradation behaviour

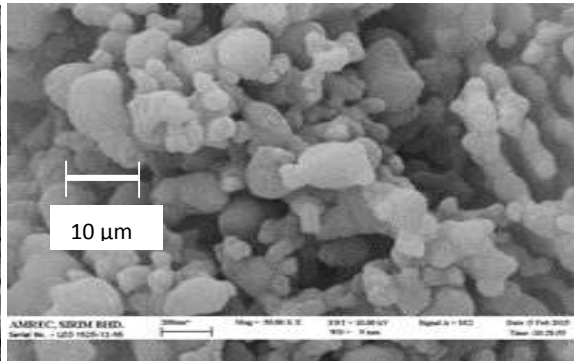
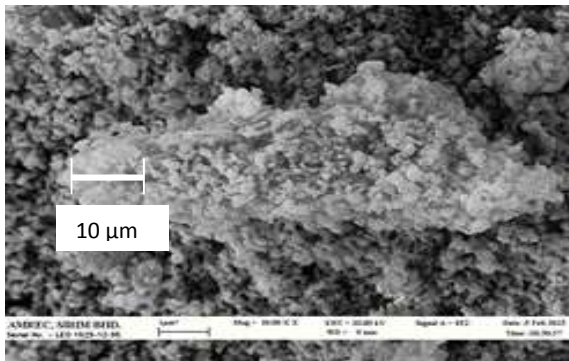
Scanning Electron Microscope (SEM)

Figures 4-7 show SEM image of Si-BCP after immersion in SBF solution for 0 day, 14 days and 30 days with different ratio of silica content, respectively. As can be observed, there are no changes for the structure with low silica content at 0 wt% and 1 wt%, even after 30 days immersion time. The structure is still maintained with tiny and smooth particles in multi-type irregular form of granules at 50,000x magnification. At high silica content of 3 wt% and 5 wt. %, the differences can be seen where silica component appeared with white splats at the images on the Si-BCP, especially at 50,000x magnification. The size of structure at high silica content also clearly increased even though at the same magnification, showing that silica content penetrated and expanded the structure, and maybe substituted one of the BCP components. It is believed that silica may be substituted by tricalcium phosphate (TCP) in BCP component due to it being resorbable material compared to HA which is non-resorbable material. Several literature also reviewed that silica substituted TCP at high content of 3 wt. % silica. Chang Weon Song *et al.* reported at high silica content of 3 wt.%, BCP powders were degraded and precipitation started to be formed. Characterisation using XRD also revealed silica may be substituted by TCP at 3 wt.% silica due to peaks corresponding to TCP phase that have disappeared.

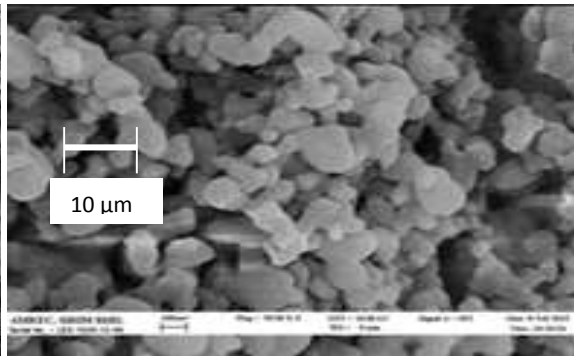
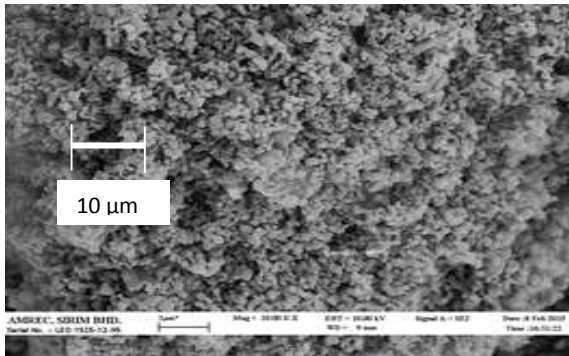
Magnification: 10,000x

Magnification: 50,000x

Day 3



Day 14



Day 30

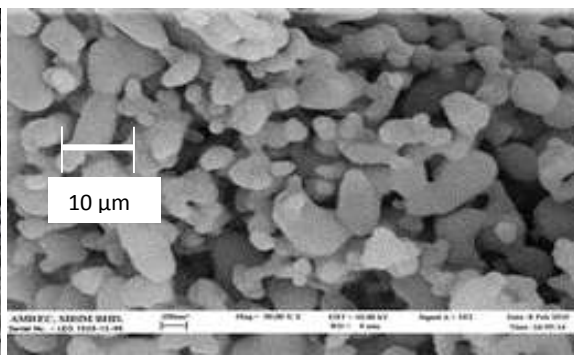
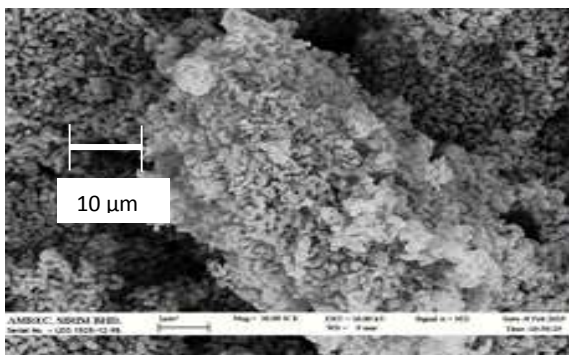
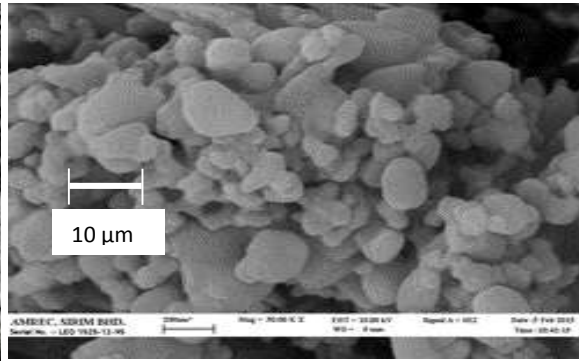
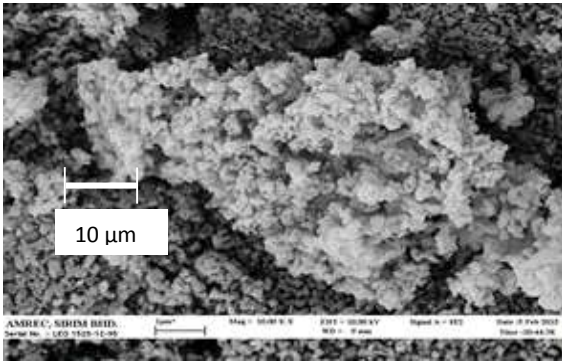


Figure 4. SEM image for 0 wt. % of Silica in Si-BCP after immersion in SBF

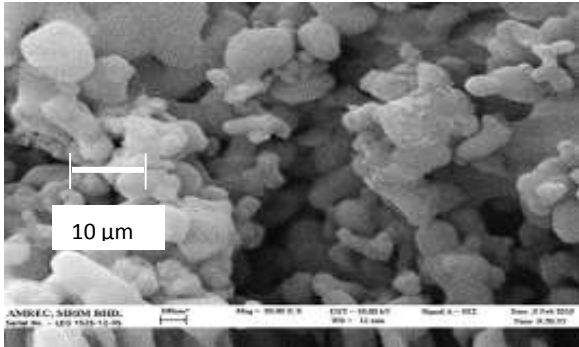
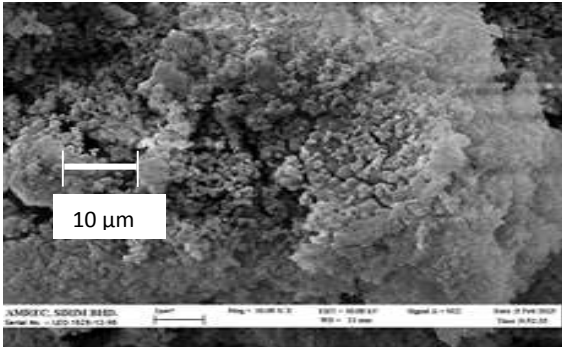
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Day 3



Day 14



Day 30

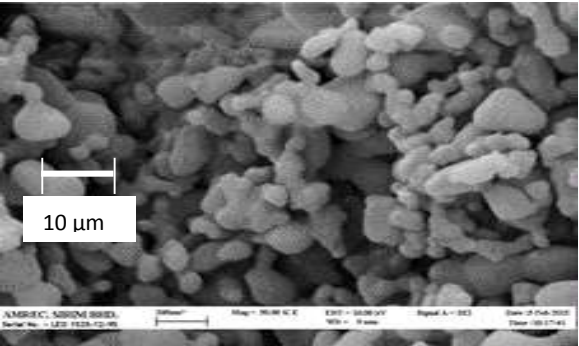
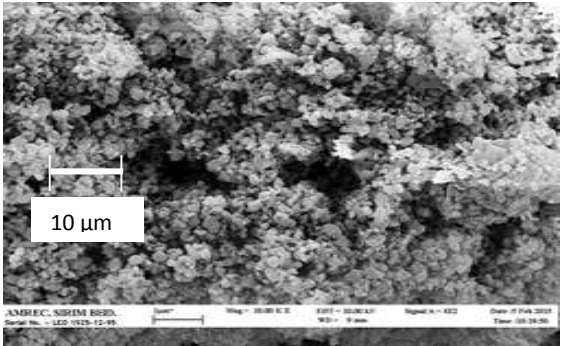
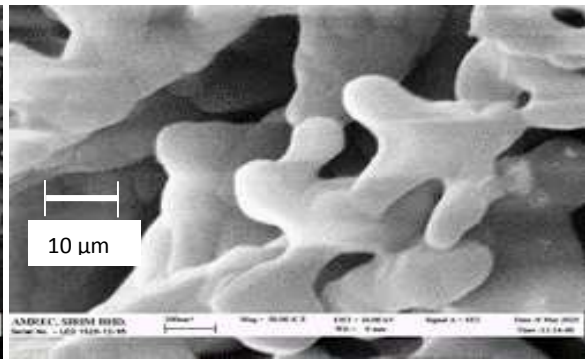
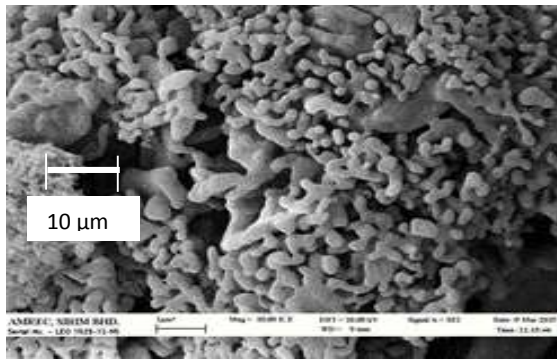


Figure 5. SEM image for 1 wt. % of Silica in Si-BCP after immersion in SBF

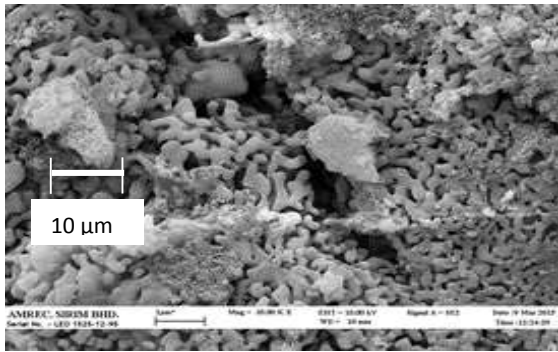
Magnification: 10,000x

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Day 3



Day 14



Day 30

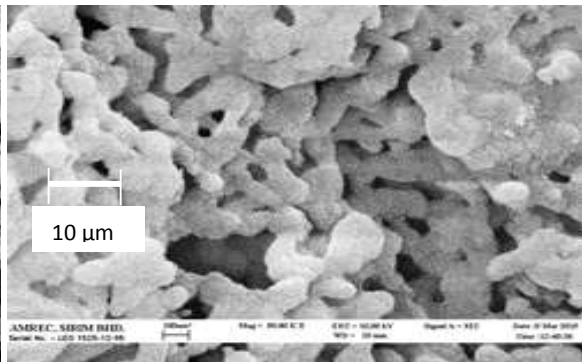
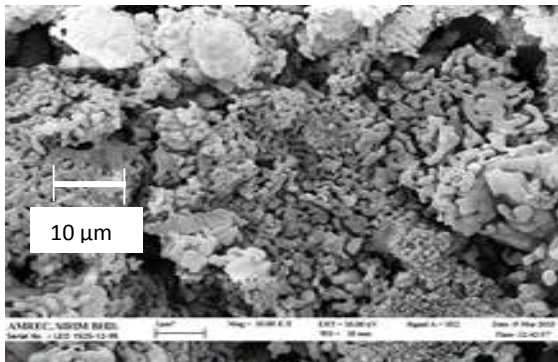


Figure 6. SEM image for 3 wt. % of Silica in Si-BCP after immersion in SBF

Magnification: 10,000x

Magnification: 50,000x

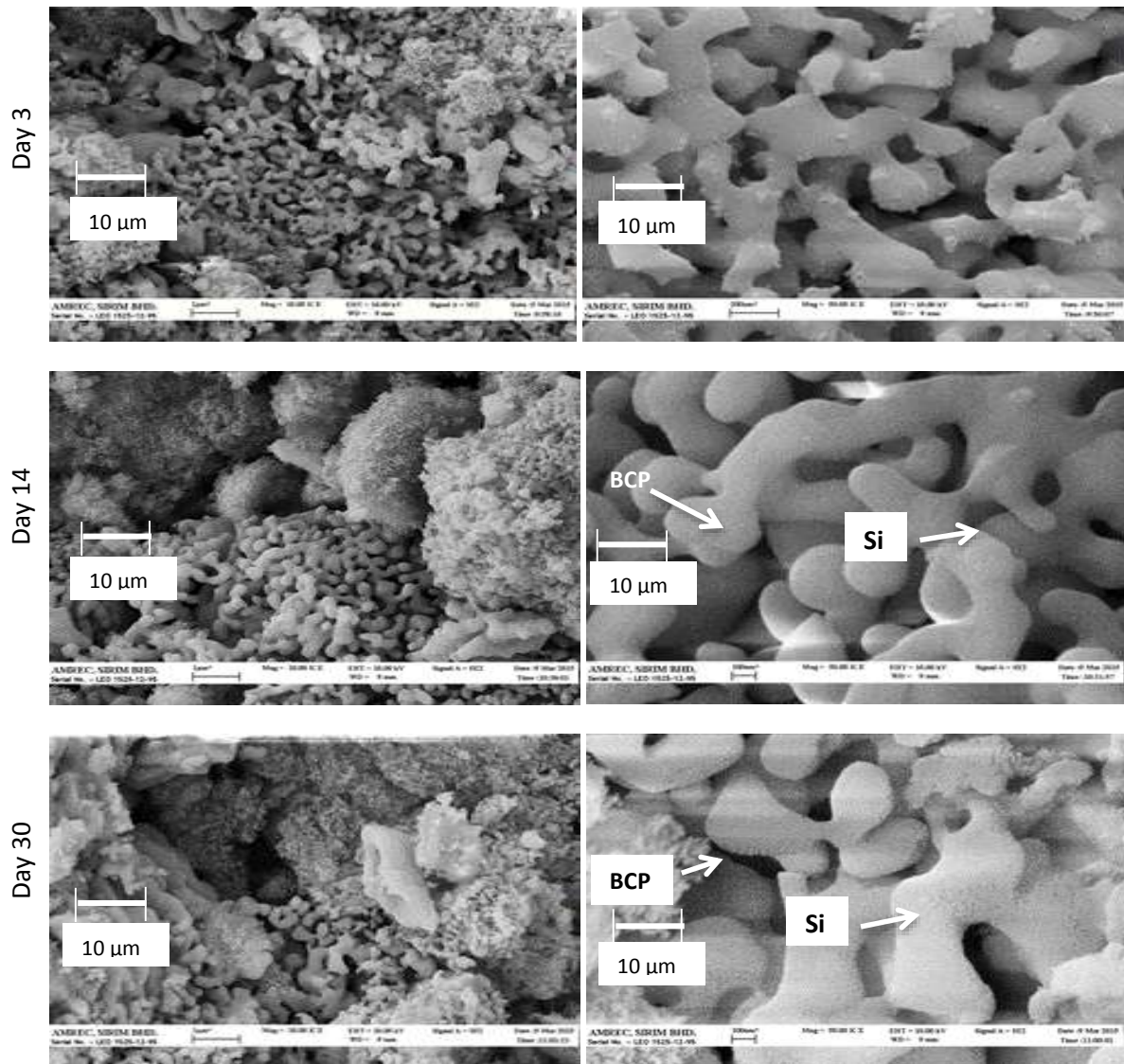


Figure 7. SEM image for 5 wt. % of Silica in Si-BCP after immersion in SBF

X-Ray Diffraction (XRD)

To further confirm whether silica may be substituted in TCP phase and in line with degradation behaviour observed by SEM, characterisation using XRD was performed (Figures 8-11). Figure 8 shows the XRD patterns of 0 wt. % of silica. HA and TCP peaks are seen with high crystalline and absolute intensity. The sharp peaks still remained even though after immersion for 30 days in SBF. Figure 9 shows 1 wt. % of silica content, with little difference compared to 0 wt. % of silica, which showed decrease in crystalline phase and intensity. It seems that the substitution of silica did not appear to interfere much in the diffraction pattern of the BCP phase when compared to 0 wt. % of silica content. These results tend to agree with SEM images as reported, where low silica content of 0 wt. % and 1 wt. % did not affect the BCP structure. On the other hand, XRD pattern for 3 wt. % and 5 wt. % of silica revealed that the peak intensity corresponding to the TCP phase decreased with increase in silica content and immersion time, while the HA peaks increases. This degradation phase could be attributed to the partial dissolution of the more soluble phase, as TCP in silica substituted BCP structure (Alexis *et al.*,

2005). However HA peaks in XRD pattern still remained in highly crystalline phase, showing their non-resorbable material features.

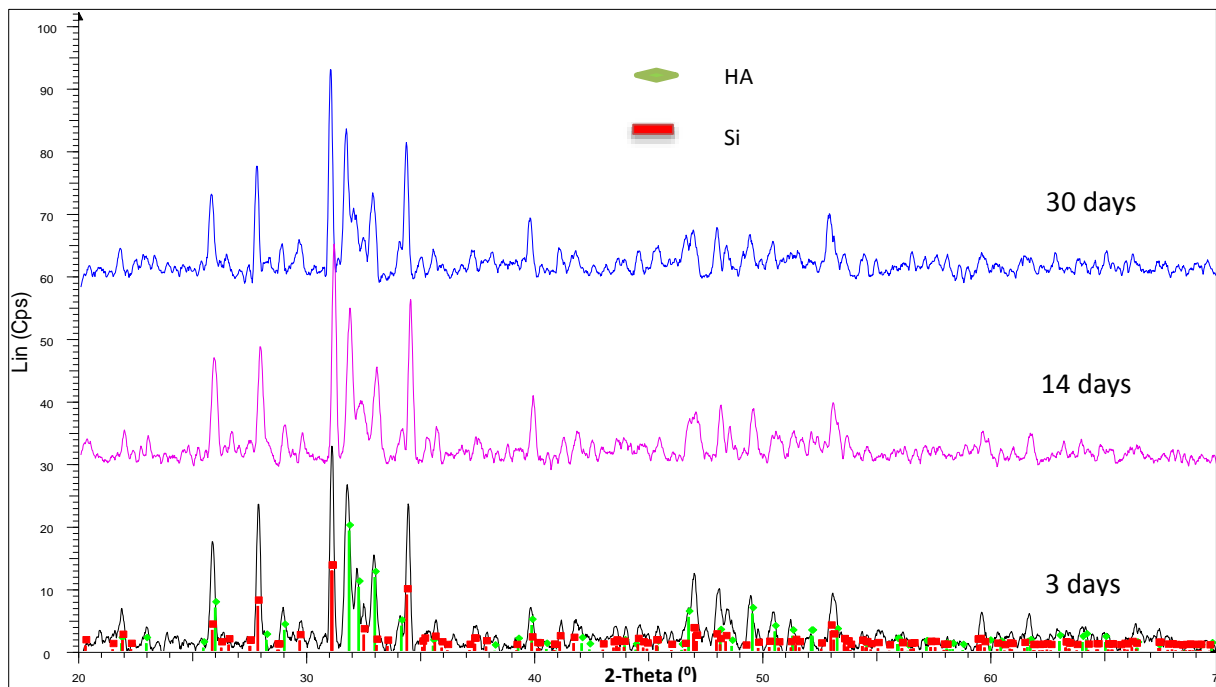


Figure 8. XRD results of 0 wt. % silica in Si-BCP for 3, 14 and 30 days after immersion in SBF

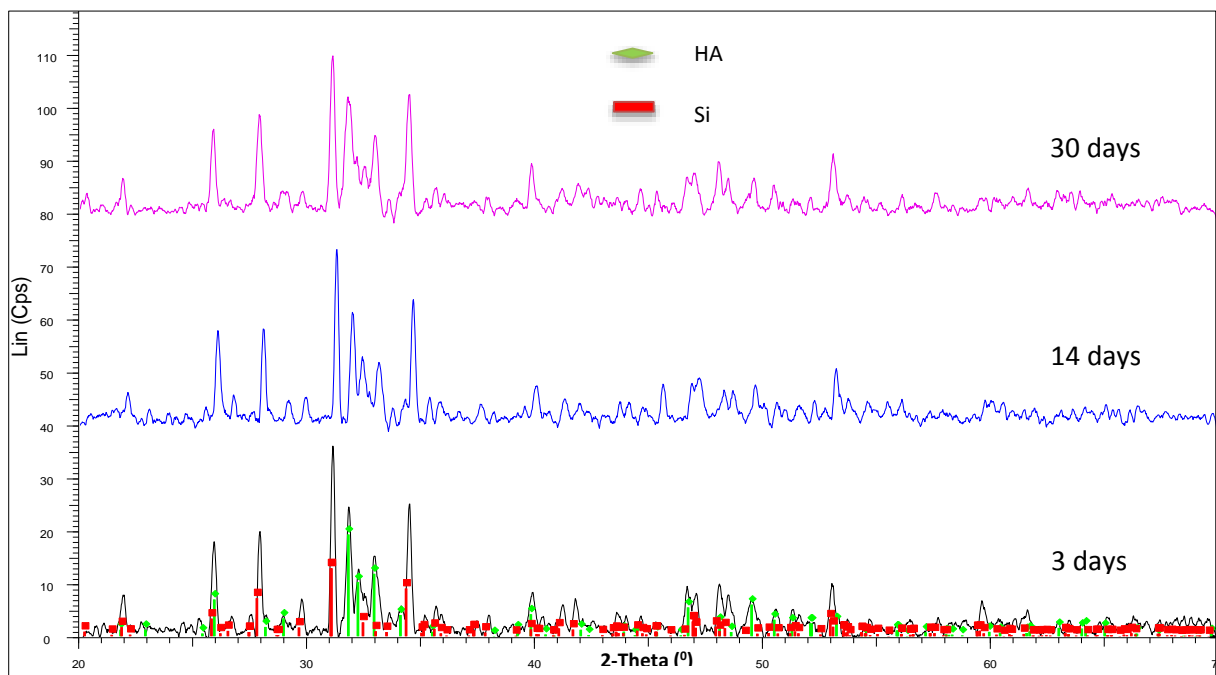


Figure 9. XRD results of 1 wt. % silica in Si-BCP for 3, 14 and 30 days after immersion in SBF

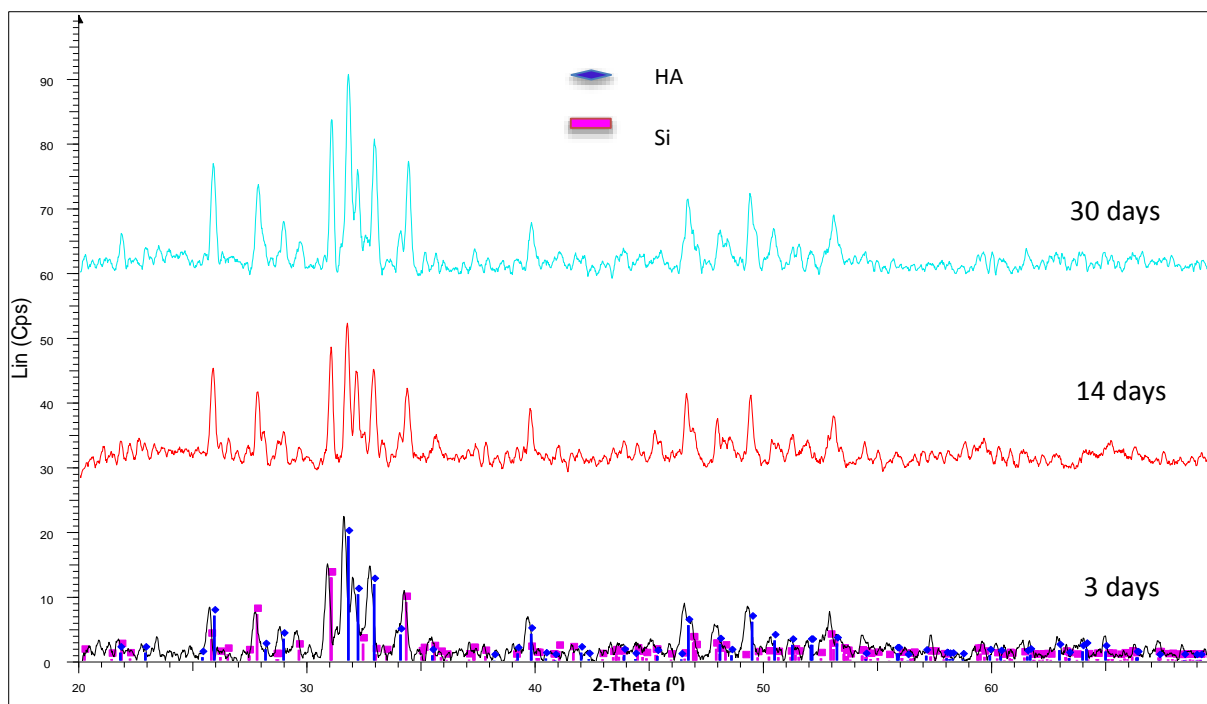


Figure 10. XRD results of 3 wt. % silica in Si-BCP for 3, 14 and 30 days after immersion in SBF

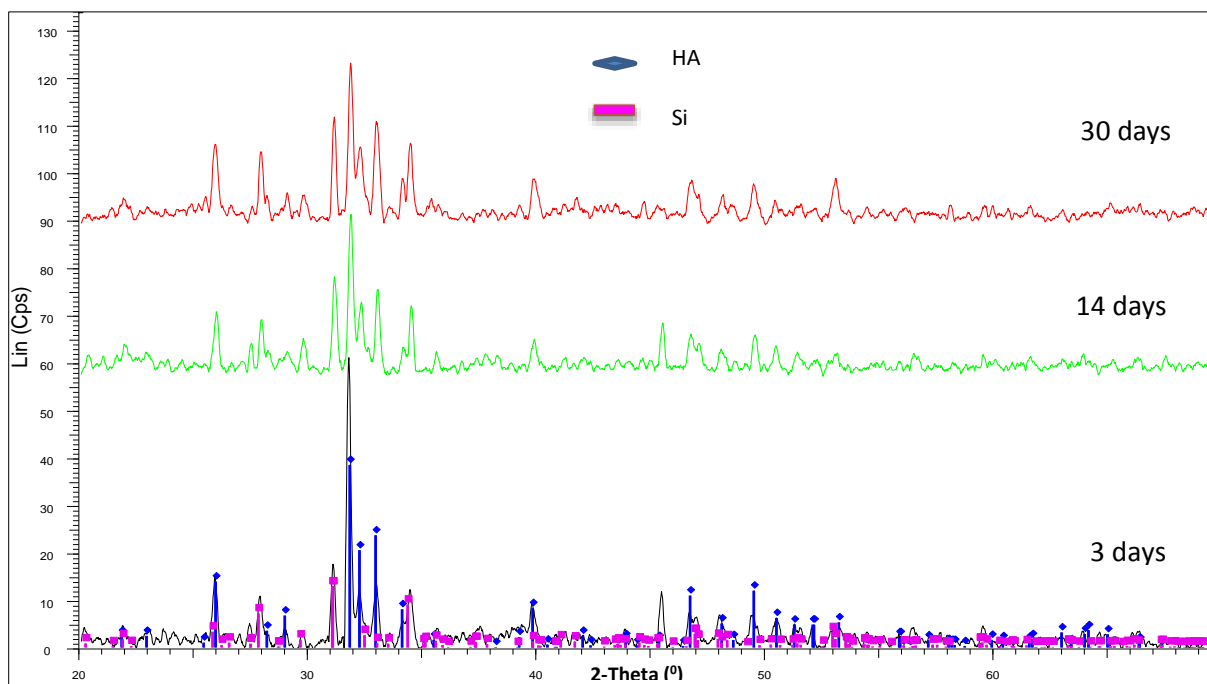


Figure 11. XRD results of 5 wt. % silica in Si-BCP for 3, 14 and 30 days after immersion in SBF

CONCLUSION

Silica content affected the degradation phase in Si-BCP structure after immersion in SBF, where at 0 wt.% of silica, the structure will degrade in total of 625 days, 735 days for 1 wt.% silica, followed by 518 days for 3 wt.% silica and 705 days for 5 wt.% silica content. This results indicated an increasing of silica content will increase their degradation rate. On the basis of these results, silica could be applied to merge with BCP in order to be functioned as replacement for quick response healing compared to BCP alone which has more retention time to produce the apatite phase in implantation operation, and concurrently increased bioactivity of BCP. Firing at 900 °C was used to incorporate silica to the BCP and due to the balance rate of HA and TCP observed, and both phases appeared in XRD characterization thus confirming the formation of biphasic mixtures.

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