

Engineering of Carbonate Apatite Bone Substitute Based on Composition-Transformation of Gypsum and Calcium Hydroxide

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Abstract

Even though a lot of research has been carried out concerning the preparation of carbonate apatite (CHA), they were related to CHA in the form of powder. In the present study, macroporous CHA bone substitutes were prepared through composition-transformation of gypsum and Ca-hydroxide. Here, we investigated the effect of added Ca-hydroxide to gypsum, carbonation periods, and hydrothermal temperatures for phosphatization to understand the basic principle of composition-transformation of gypsum added Ca-hydroxide to fabricate CHA bone substitutes. The specimens were characterized in terms of chemical and physical properties, such as extent of transformation of macroporous gypsum added Ca-hydroxide into CHA body, type and content of carbonate, and crystal morphology. It was observed that the transformation was faster with higher hydrothermal temperature. However, higher hydrothermal temperature caused de-carbonation phenomena which resulted in the lack of carbonate ions of the product. Moreover, the higher the percentage of Ca-hydroxide added to gypsum, caused the complete transformation of gypsum into CHA to be slower. These findings have been applied to the standard fabrication procedure of carbonate apatite, which in turn will allow scaling up process, and will be provided for biomedical purposes for the Indonesian community.

Keywords: Carbonate Apatite (CHA), Bone Substitute, Composition-Transformation, Gypsum, Calcium Hydroxide

1. Introduction

Bone tissue is one of the most frequently used tissues for transplantation. Annually more than a million patients are treated worldwide for skeletal problems in the field of orthopedic surgery, plastic surgery, maxillofacial surgery, and neurosurgery. The use of autologous bone grafts is still considered to be the golden standard, but there are several major disadvantages in this technique, for example: 1) low availability of transplantable tissue, 2) postoperative morbidity, and 3) lack of functional shape of the transplant. Meanwhile, despite four decades of the researches to provide synthetic bone substituting materials, the engineered bone-substituting materials are still largely inferior to auto- or allografts as the golden standard. The clinical success of the current generation of bone-substituting materials is disappointingly limited

since they lack the high functionality of bone tissue in terms of biological and mechanical properties [1]. This lack of biofunctionality is related mainly to an engineering attitude towards design of novel biomaterials that lacks a biological mindset [2,3]. Therefore, the development of alternatives to autologous bone is a very relevant engineering issue in bone reconstructive surgery.

To provide the answer to the issue, the basic knowledge on the composition of human bone mineral must be understood. In view of this, it is known from the current studies that the mineral phase of human hard tissue consists of hydroxyapatite (HA: $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$) containing a variety of impurity ions such as carbonate, sodium, and magnesium, etc. [4-8]. Carbonate is one of the most abundant impurity ions and its content is about wt 4-8% [9-11]. In this sense, hard tissue is regarded as carbonate-substituted HA (CHA).

It has been reported that synthetic CHA revealed the biological activity better than synthetic HA because the incorporation of carbonate into HA caused an increase in solubility, a decrease in crystallinity, a change in crystal morphology, and an enhancement of chemical reactivity owing to the weak bonding [5,12,13]. In this context, CHA is actually more soluble in vivo than HA and increases the local concentration of calcium and phosphate ions that are necessary for new bone formation [14-16]. For these reasons, recent studies have focused on CHA in comparison with HA.

Even though a lot of research has been carried out concerning the preparation of CHA, they were related to CHA in the form of powder [10,12,17-19]. In the present study, macroporous CHA bone substitutes were prepared through composition-transformation of gypsum and Ca-hydroxide, the abundant minerals in Indonesia. The successful composition-transformation process will allow the modification of the macroporous CHA body to provide bone-substitutes with proper mechanical and biological properties, based on the needs of reconstructive surgeon. The specimens were characterized in terms of chemical and physical properties, such as extent of transformation of CaCO_3 body into CHA body, type and content of carbonate, and crystal morphology.

This research was also basically directed to find a low cost technology and environmental friendly method to prepare CHA bone substitutes. Its aim is to provide lower cost bone substitutes for the Indonesian market to help the people improve their quality of life. Many related cases showed low access to bone-substitution treatment due to the high cost of medical treatment in which it needs engineering approach to solve the problems.

2. Materials and Method

2.1. Composite Preparation

Calcium sulphate hemihydrate ($\text{CaSO}_4 \cdot 1/2\text{H}_2\text{O}$) of Wako Pure Chemical (Japan) was added by 20, 40, 50, and 100 weight% of Calcium hydroxide ($\text{Ca}(\text{OH})_2$) of Nacalai Tesque, Kyoto (Japan). The weight% of added Ca-hydroxide applied in this study was based on the preliminary studies done ranging from 0 to 100% Ca-hydroxide additions. The gypsum added Ca-hydroxide powders were used to prepare a monolith by mixing the powder with water in 1:2 W/P. The mixture was put in a stainless steel mold (6 mm in inner diameter by 3 mm thickness) and pressed uniaxially at 10 MPa to prepare $\text{CaSO}_4/\text{Ca}(\text{OH})_2$ composite. The composite was then put into 37°C incubator with 80% humidity for 24 hour to allow it to set completely.

2.2. Composite Carbonation

The composite was placed in carbon dioxide reaction vessel for 72 hours at room temperature. The reaction vessel (approximately 5L) was saturated with water vapor and carbon dioxide gas was supplied at a rate of 0.15 to 0.20L/min.

2.3. Hydrothermal Treatment

The composite monolith was then treated hydrothermally in 1M phosphate solutions at 60°C for various times, ranging from 3 to 14 days. The phosphate used was tri-sodium phosphate ($\text{Na}_3\text{PO}_4 \cdot 12\text{H}_2\text{O}$; Wako Chemicals, Japan). Each monolith after the hydrothermal treatment was washed with distilled water for 6h to eliminate soluble ions.

2.4. X-Ray Diffraction Analysis

The purity and crystallite size of the CHA formed were determined by XRD (RINT 2500V, Rigaku, Japan) analysis. The XRD pattern was obtained in the range of 2 θ ° of 10 to 60 in a continuous mode at a scanning rate of 2°/min.

2.5. Fourier Transformed Infrared (FT-IR) Analysis

Infrared spectra between 400 and 4,000 cm^{-1} were recorded on the FT-IR spectrometer (Spectrum 2000, Perkin-Elmer, USA) to examine structural changes by the hydrothermal treatment, in particular, CO_3^{2-} ions substituting for PO_4^{3-} and/or OH^- ions.

2.6. Scanning Electron Microscope (SEM) Analysis

The morphology of fracture surface and crystal shape were observed by SEM (JSM 5400LV, JEOL, Japan) at an acceleration voltage of 15kV after gold sputtering.

2.7. Chemical Analysis

Chemical analysis was also done to determine carbon and nitrogen contents using CHN elemental analyzer (CHN coder MT-6, Yanaco, Japan). Calcium and phosphorus content were determined by Ion-meter (TOA-DKK Model IM-55G, TOA, Japan) and a spectrometer (UV-Spectrometer, JASCO, Japan) respectively.

2.8. Mechanical Analysis

Diametral tensile strength of the composite monolith was evaluated at a constant crosshead speed of 1mm/min on a

universal testing machine (SV-301, IMADA, Japan). For each experimental condition ten specimens were tested.

3. Results and Discussion

3.1. X-Ray Diffraction Patterns

Figure 1 shows XRD patterns of gypsum added Ca-hydroxide composite after 72 hours carbonation. It was detected that CaCO_3 (calcite) peaks were developed by carbonation regardless the percentage of Ca-hydroxide addition, shown by the peak development at around 2θ of 28.5° . However, the complete transformation into CaCO_3 was faster in the monolith containing 100% Ca-hydroxide. Other compositions (20, 40, and 50% addition) transformed into biphasic DCPD/ CaCO_3 which was shown by peaks (121) at around 2θ of 21° , (141), (112) and (121) at around 2θ of 29 to 31° , and at around 2θ of 34° for (022) and (150).

It was also shown in the **Figure 1** that the CaCO_3 resulted in the carbonation reaction has broader peaks than the commercially available one, indicating that the crystallite size of CaCO_3 resulted by carbonation was much smaller. Ca-hydroxide was completely transformed into CaCO_3 after 72 hours carbonation, in a single phase or in the form of biphasic DCPD/ CaCO_3 . Up to this reaction stage, it is expected that the carbonation products will show good tissue response to be applied for biomedical purposes. This is due to the composition transformation into low crystalline CaCO_3 that has similar biological properties with aragonite, which is the constituent of marine coral [20]. In case of biphasic DCPD/ CaCO_3 , it was also reported that DCPD is considered to be HA precursor [21].

Figure 2 shows XRD patterns of gypsum added Ca-hydroxide after phosphatization by $1\text{M Na}_3\text{PO}_4 \cdot 12\text{H}_2\text{O}$ for 7 days (a) and 14 days (b). Hydroxyapatite (HA) phase was detected as a main reaction product regardless of the percentage of Ca-hydroxide added into gypsum. The composition transformations into HA phase were marked by peaks development at around 2θ of 25.5° and 32.5° . Meanwhile peaks at around 2θ of 28.5° decreased in their intensity. Transformation of CaCO_3 or DCPD/ CaCO_3 into HA is seemed to proceed faster by lower concentration of added $\text{Ca}(\text{OH})_2$. In higher Ca-hydroxide concentrations (50 and 100%), the CaCO_3 peaks at around 2θ of 28.5° still remained high even after 14 days hydrothermal treatment in phosphate solution. In the lower concentrations of Ca-hydroxide (20 and 40%), the CaCO_3 peaks also remained although the intensity was lower than those of with higher Ca-hydroxide concentrations. This is probably due to high pH in $\text{Na}_3\text{PO}_4 \cdot 12\text{H}_2\text{O}$ solution, which resulted in the stabilization of CaCO_3 compared with carbonate apatite [22,23]. Moreover in the composition where CaCO_3 remained, the peaks

around 2θ of 32° were merged, showing different feature with the composition without remaining CaCO_3 (gypsum without added Ca-hydroxide) where it was found clear separation between (211) and (112) diffraction peaks. Regarding the peak separation, LeGeros [24] observed that the lattice parameter of a-axis in a hexagonal apatite crystal decreased with the increasing CO_3 content. In this study, it was theoretically predicted that the more the percentage of Ca-hydroxide added into gypsum, the faster the composition transformation of the composite into CaCO_3 monolith after carbonation.

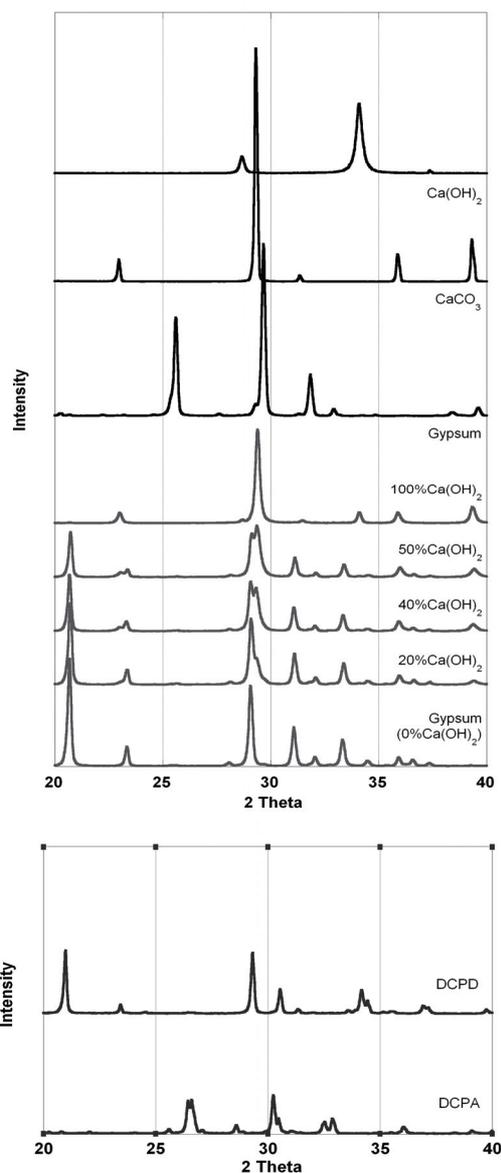


Figure 1. X-Ray diffraction patterns of gypsum added Ca-hydroxide after 72 hours carbonation. Peak development at around 2θ of 28.5° was observed regardless the amount of Ca-hydroxide additions. Another pattern shows diffraction of DCPD and DCPA for comparison.

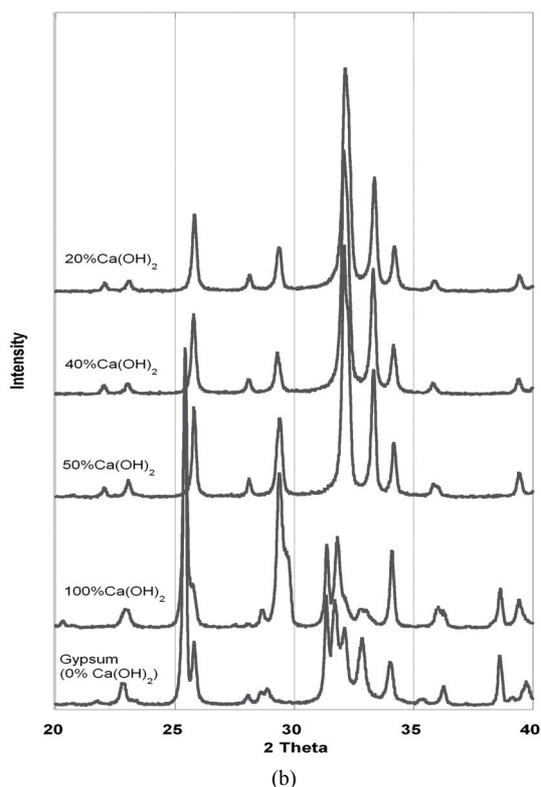
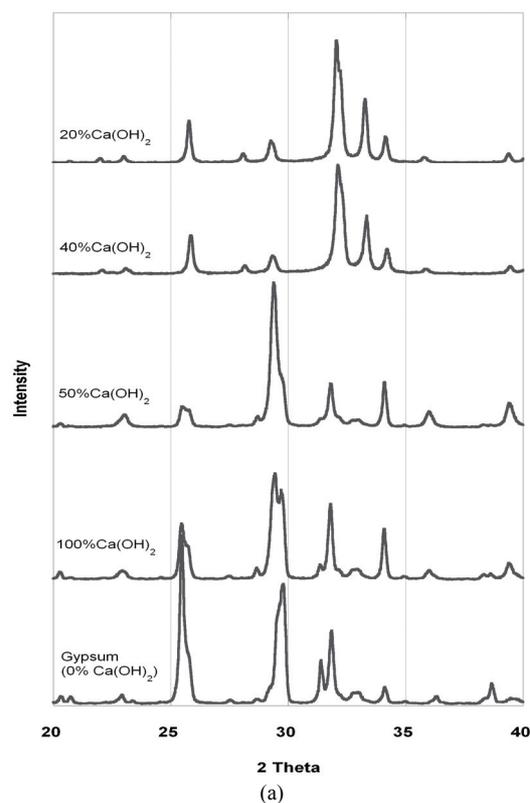


Figure 2. X-Ray diffraction patterns of gypsum added Ca-hydroxide after 7 days (a) and 14 days (b) phosphatization in 1M $\text{Na}_3\text{PO}_4 \cdot 12\text{H}_2\text{O}$.

Table 1 summarizes the qualitative analysis of XRD showing the composition transformation after carbonation and phosphatization.

3.2. FT-IR Spectra

Figure 3 shows the FT-IR spectra of the specimens after carbonation and 7 days phosphatization. Although typical CO_3^{2-} bands were detected between 1000 and 900 cm^{-1} and 1600 to 1400 cm^{-1} but the bands between 1600 - 1400 cm^{-1} were broad indicating that the transformation into CHA was not completed yet by 7 days process, irrespective of the percentage of Ca-hydroxide added into gypsum. After carbonation and 7 days phosphatization, bands around 1100 cm^{-1} were developed in all specimens, regardless the amount of Ca-hydroxide added into gypsum. These bands were correlated with PO_4^{3-} of the typical B-type CHA [15,25,26] but also A-type CHA indicated by CO_3^{2-} bands development at around 810 - 800 cm^{-1} . The higher the amount of $\text{Ca}(\text{OH})_2$ added, the more the absorbance indicating CO_3^{2-} bands at around 980 - 960 cm^{-1} was observed. The complete composition transformation into B-type CHA was detected by prolonging phosphatization to 14 days as shown in the typical composition transformation process (**Figure 4**).

Figures 4 and **5** show the FT-IR spectra of the process after carbonation, continued by 7 days phosphatization, and 14 days phosphatization. After 14 days phosphatization, the typical CO_3^{2-} bands were developed at 1455 - 1410 cm^{-1} and 880 - 860 cm^{-1} in which the absorbance was higher by longer phosphatization period. On the same figure, the typical PO_4^{3-} bands were observed at 600 - 560 , and 1100 - 960 cm^{-1} indicating the development of B-type CHA. The PO_4^{3-} band at around 600 - 560 was well separated. The existence of carbonate ion bands in the spectra shows the formation of CO_3^{2-} substituted HA (CHA). Elliot [23] in his explanation of the structure and chemistry of apatites mentioned that B-type CHA shows CO_3^{2-} band at 1410 and 1455 cm^{-1} while A-type at about 1545 and 1455 cm^{-1} . In the final reaction products, the CO_3^{2-} band appeared at around 1410 and 1455 cm^{-1} .

Table 1. Qualitative analysis of XRD showing the composition transformation of gypsum added Ca-hydroxide after carbonation and phosphatization in continuous treatment.

Content of Ca-hydroxide (wt%)	Composition After 72 hours Carbonation	Composition After 7 days Phosphatization	Composition After 14 days Phosphatization
Gypsum (0% $\text{Ca}(\text{OH})_2$)	DCPD, Gypsum	Gypsum, CHA	CHA
20% $\text{Ca}(\text{OH})_2$	DCPD, CaCO_3	CHA, CaCO_3	CHA
40% $\text{Ca}(\text{OH})_2$	DCPD, CaCO_3	CHA, CaCO_3	CHA
50% $\text{Ca}(\text{OH})_2$	DCPD, CaCO_3	CaCO_3	CHA, CaCO_3
100% $\text{Ca}(\text{OH})_2$	CaCO_3	CaCO_3	CHA, CaCO_3

This observation showed that the composition transformation reaction by carbonation and phosphatization resulted in B-type CHA. **Figure 5** show extended bands found in the study. Landi *et al.* [18] reported that the increasing of CO_3^{2-} band at about 1545 and 1455 cm^{-1} caused by the migration of CO_3^{2-} band at 1410 and 1455 cm^{-1} to OH^- lattice site with heat treatment caused a decrease in crystallinity. The decrease in crystallinity attributed to the ability of hydroxyl site to accept more vacancies and the increasing degree of freedom of CO_3^{2-} ion. Based on that phenomenon, in this study, lower hydrothermal treatment (60°C) was applied after experimenting different temperatures ranging from 100 to 60°C to allow formation of low crystalline B-type CHA. It was observed that the longer the phosphatization period, the lower the crystallinity obtained by composition transformation of gypsum added Ca-hydroxide ended with B-type CHA.

Table 2 summarizes the composition of CHA prepared by composition transformation of gypsum added Ca-hydroxide. The Ca/P molar ratio ranged between 1.53 to 2.01 molar ratio. Without Ca-hydroxide addition into

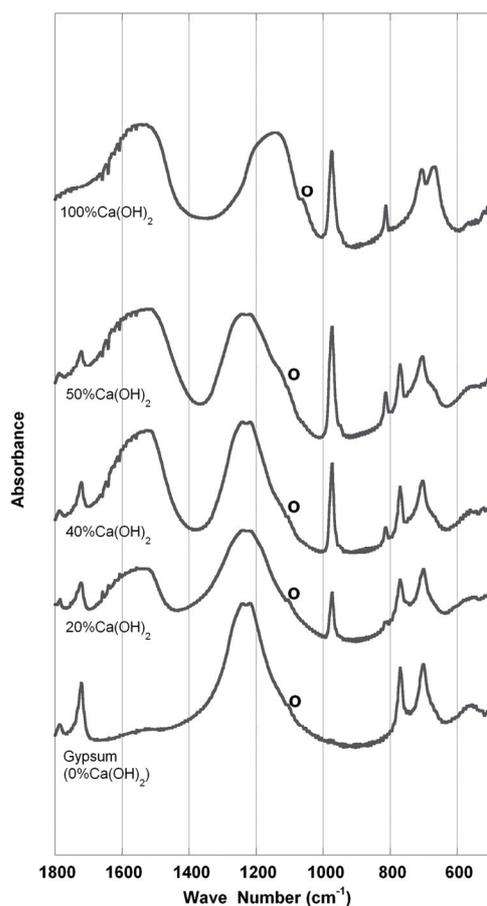


Figure 3. FT-IR spectra of the monoliths after 72 hours carbonation and 7 days phosphatization, showing CHA peaks development which was not completed.

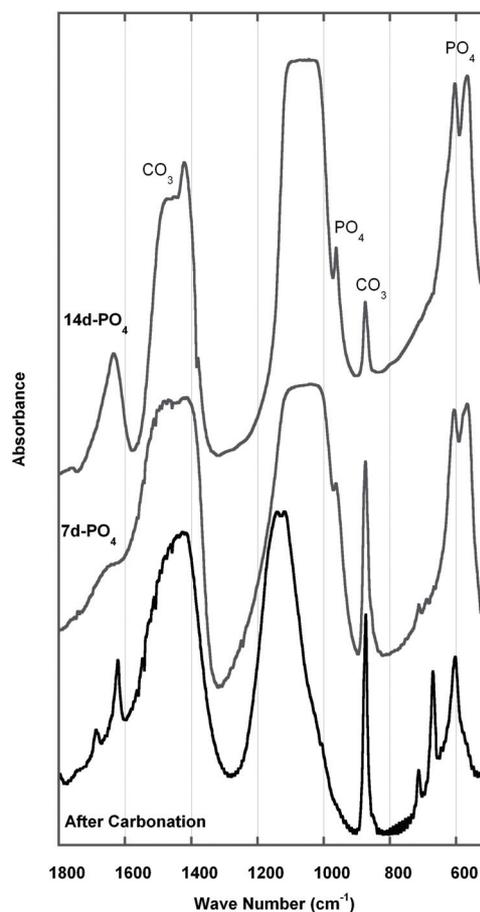


Figure 4. FT-IR spectra of gypsum added 40 weight% Ca-hydroxide after carbonation, continued by 7 days phosphatization, and prolonged up to 14 days phosphatization.

Table 2. Chemical composition of CHA prepared by composition transformation of gypsum added Ca-hydroxide.

Content of Ca-hydroxide (wt%)	Ca/P Molar ratio	CO_3^{2-} (wt%)	OH (wt%)
Gypsum (0% $\text{Ca}(\text{OH})_2$)	1.53 ± 0.04	0.58	0.55
20% $\text{Ca}(\text{OH})_2$	1.71 ± 0.02	3.36	0.47
40% $\text{Ca}(\text{OH})_2$	1.83 ± 0.02	6.12	0.52
50% $\text{Ca}(\text{OH})_2$	1.88 ± 0.01	3.10	0.53
100% $\text{Ca}(\text{OH})_2$	2.01 ± 0.02	3.27	0.58

Table 3. Qualitative analysis on setting reaction on the gypsum added Ca-hydroxide.

Content of Ca-hydroxide (wt%)	Setting Reaction
Gypsum (0% $\text{Ca}(\text{OH})_2$)	Good
20% $\text{Ca}(\text{OH})_2$	Good
40% $\text{Ca}(\text{OH})_2$	Good
50% $\text{Ca}(\text{OH})_2$	Poor (Difficult to handle)
100% $\text{Ca}(\text{OH})_2$	Very Poor (Very difficult to handle)

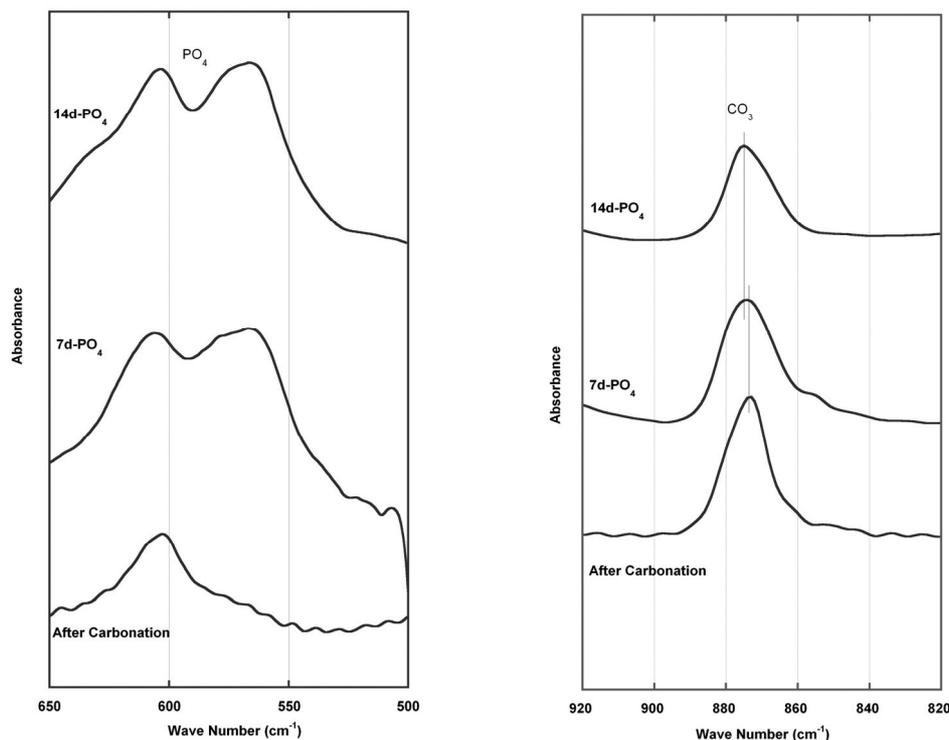


Figure 5. Extended CO_3^{2-} and PO_4^{3-} bands of FT-IR spectra found by composition transformation reaction.

gypsum, the Ca/P ratio was slightly lower than that of stoichiometric HA, that is 1.67, indicating that calcium deficient apatite was formed. By addition of Ca-hydroxide into gypsum followed by carbonation and phosphatization treatment using $\text{Na}_3\text{PO}_4 \cdot 12\text{H}_2\text{O}$, the Ca/P ratio are 1.71, 1.83, 1.88, and 2.01 molar ratio for 20, 40, 50, and 100% additions respectively. These results showed that by Ca-hydroxide additions, PO_4^{3-} was replaced by CO_3^{2-} which led to the formation of B-type CHA.

The CO_3^{2-} content of the specimens ranging from 0.58 (for gypsum without Ca-hydroxide addition), 3.10, 3.27, and 3.36 for 50, 100, and 20 weight% Ca-hydroxide additions respectively, to 6.12 weight% (for the specimen with 40 weight% Ca-hydroxide addition). Only by addition of 40 weight% Ca-hydroxide the carbonate content resulted by composition transformation is on the range of bone mineral, that is 4-8 weight% [9,10].

Figure 6 shows SEM photographs of CHA obtained in various addition of Ca-hydroxide. Without addition of Ca-hydroxide into gypsum, the CHA crystal showed a needle like apatite morphology after carbonation and phosphatization reaction (Figure 6(b)). By 20 weight% addition of Ca-hydroxide into gypsum, the CHA resulted both a needle like morphology and fine globular crystal (Figure 6(d)). Increasing the amount of Ca-hydroxide (to 40 and 50 weight%) added into gypsum resulted in fine globule-like CHA crystals (Figure 6(f) and 6(h)). Inter connective porosity is observed on the microstruc-

ture of the monoliths.

Figure 7 shows the changes in DTS (diametral tensile strength) of the specimens. The mechanical strength increased after carbonation and phosphatization reactions, and the increased strength after carbonation and phosphatization was found 50% in pure set Ca-hydroxide, 25% in gypsum added by 20 or 40 weight% of Ca-hydroxide, 20% in gypsum added by 50 weight% of Ca-hydroxide, and remained the same in the gypsum without Ca-hydroxide addition. The difference in DTS is probably due to the crystallite size and shape of the specimens.

In this study, we investigated the effect of added Ca-hydroxide to gypsum, carbonation and phosphatization temperatures and periods to understand the basic principle of composition-transformation of gypsum added Ca-hydroxide to fabricate carbonate apatite bone substitutes. It was observed that faster transformation was achieved with higher hydrothermal temperature. However, higher hydrothermal temperature caused de-carbonation phenomena which resulted in the lack of carbonate ions in the product. Decreasing phosphatization temperatures required longer reaction periods. Moreover, the higher the percentage of Ca-hydroxide added to gypsum resulted in slower complete transformation of gypsum into CHA, indicated by remaining CaCO_3 phase inside the composition. These findings have been applied to the standard fabrication procedure of carbonate apatite that is provided for biomedical purposes in Indonesia. Cell studies and in vivo animal studies are being carried out

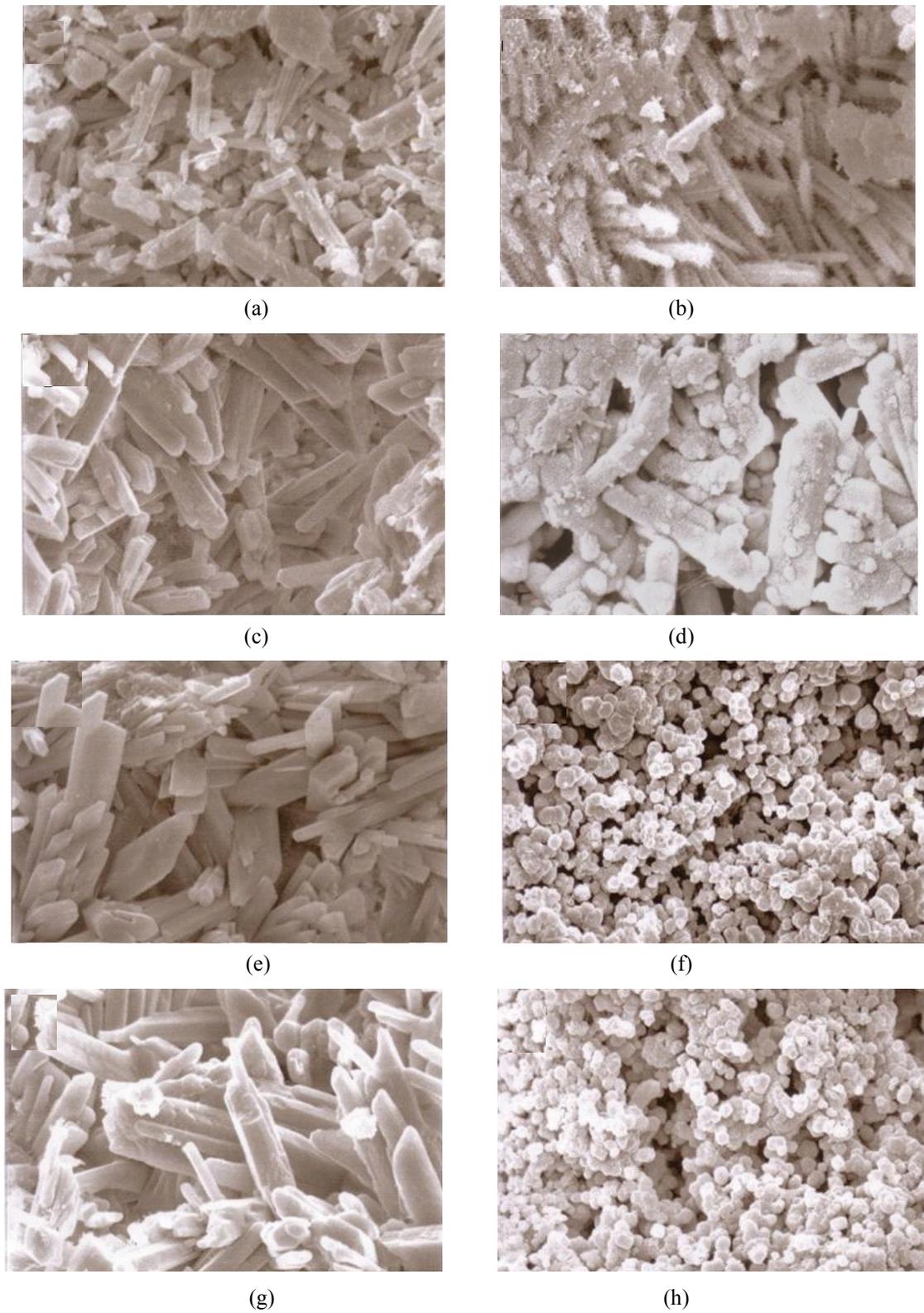


Figure 6. Scanning electron microscopic observation of set gypsum, gypsum added Ca-hydroxide by 20, 40, and 50 weight% before treatment (a, c, e, g) and after carbonation and phosphatization (b, d, f, h).

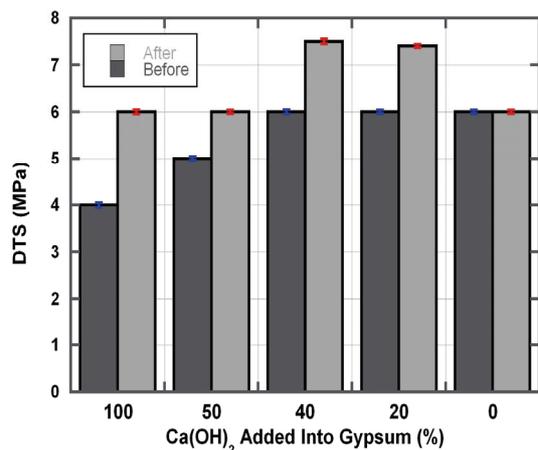


Figure 7. Changes in DTS (MPa) before and after carbonation and phosphatization reactions.

in our laboratory. For the continuation of these studies, it is suggested to start preliminary clinical trials, so as to have an overview study to scale up fabrication, and to precede valorization and actual bone substitute production for the Indonesian market.

4. Acknowledgements

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