Evaluation of the Physical Properties and Biological response of HAP/β-TCP

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Abstract

Hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$) is used in many biomedical applications including bone graft and joint replacements. Due to its structural and chemical similarities to human bone mineral and teeth, Hydroxyapatite (HAP) promotes growth of bone tissue directly on its surface. In this research, hydroxyapatite was prepared by precipitation method. The phase formations of these samples were confirmed by using X-ray Diffraction (XRD) technique. Surface morphology and particle size of HAP and HAP/β-TCP samples were studied by Scanning Electron Microscope (SEM). Finally, the bone bonding ability was evaluated by examining the formation of the new apatite layer on the surface after immersing in Simulated Body Fluid (SBF).

Keywords: calcium sulphate, HAP, XRD, SEM, SBF

Introduction

Among all biomaterials, hydroxyapatite: the mineral component of hard tissues in vertebrates is the most biocompatible material. Hydroxyapatite shows excellent biocompatibility not only with hard tissue but also with soft tissue. This material is capable of integrating biologically when directly implanted into a bone defect. Furthermore, it is not toxic and produces no harmful effect on the immune system with excellent osteoconductive behavior [1]. Tricalcium phosphate is also one of the most important biomaterials based on phosphates, currently recognized as ceramic material that significantly simulates the mineralogical structure of bone. Theoretically, the resorbable β-TCP is an ideal implant material [2].

The bone-bonding ability of a material is often evaluated by examining the ability of apatite to form on its surface in a Simulated Body Fluid (SBF) with ion concentrations nearly equal to those of human blood plasma. Haemo-A (Aeonmed Co., Ltd) was mixed with Haemo-B (Aeonmed Co., Ltd) in 1:1.2 ratio to form Simulated Body Fluid (SBF). This solution was mixed with DI water in 1:32.8 ratio. Each of this solution (5 ml) was put into 25 ml of three glass beakers. HAP, HAP/β-TCP and β-TCP pellets were put into the SBF solution in each beaker. The beakers were put into an incubator and set the temperature at 37 ℃ for 14 days. Then, the pellets were dried in desiccator for 2 days. These dried pellets were analyzed by SEM. SEM micrographs obtained from pellets before SBF test and after SBF test were compared.

Materials and Method

Experimental Procedure

Laboratory grade gypsum ($CaSO_4.2H_2O$) was used for starting material to synthesize hydroxyapatite (HAP) in the first stage. HAP synthesized from gypsum powder was achieved by precipitation method at 90°C. The flow chart in Figure 1 shows the details for conversion

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of gypsum powder to HAP. This reaction conversion has been achieved according to the reaction below.

$$10CaSO_4.2H_2O + 6(NH_4)_2HPO_4 \rightarrow Ca_{10}(PO_4)_6(OH)_2 + 6(NH_4)_2SO_4 + 4H_2SO_4 + 18H_2O$$

1.6 M of gypsum solution was prepared with 11.5 g of gypsum and 40 ml of deionized water. Then, the gypsum solution has been mixed with 40 ml of 1 M - $(NH_4)_2HPO_4$ solution in conical flask with water bath at the reaction temperature of 90° with magnetic stirring. The reaction time lasted for 4 hours. At the end of the reaction period, the solid products were washed with DI-water for 5 times and filtered to eliminate any water soluble remains. After washing, the solid residue was put in drying oven operating at 75°C for 24 hours. Again, the dry powder had been heat-treated at 500 ℃, 800 ℃, 1000℃, 1100 ℃ and 1150 ℃ for 2 hours each in a furnace. The HAP phase was formed at 500 ℃ and 800℃ for 2 hours. However, HAP converts into β-tricalcium phosphate (β-TCP) at 1000℃ and beyond. Therefore, this research focuses on biphasic HAP/β-TCP. The process of sample preparation of HAP is presented.

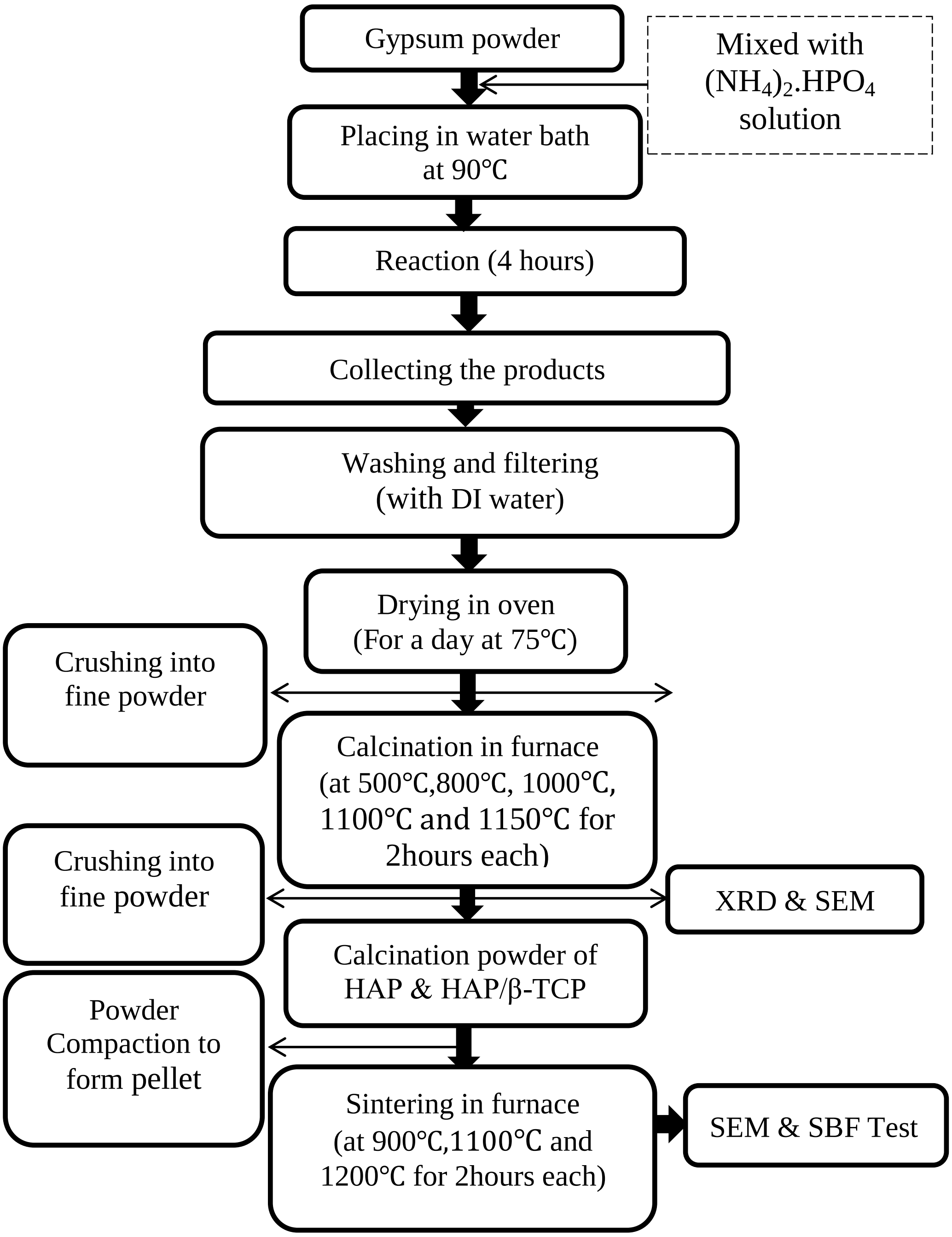

Figure 1. Flowchart of HAP, HAP/β-TCP and β-TCP

Results and Discussion

Phase Formation by XRD analysis

HAP powder was synthesized from gypsum powder by precipitation method at 90°C. The phase formation of HAP was found in the powder after calcination at 500°C and 800°C and in pellets sintering at 900°C. HAP/β-TCP was found in powder after calcination at 1000°C and 1100°C and also that of pellet after sintering at 1100°C. β-TCP was formed in the powder after calcination at 1150°C and in pellet after sintering at 1200°C. The pattern reveals that the phase precipitated out in the sample was hexagonal structure. There was no trace of starting materials. Therefore, it was identified that a single phase structure of HAP from gypsum has been formed via the precipitation method.

After calcination at 1000°C, some of the HAP converted into β-TCP. The lattice parameters ‘a’ and ‘c’ were calculated by using‘d’ value of the diffraction peaks. These lattice constants well agree with the typical values for HAP and β-TCP structures. The crystallite size was estimated from FWHM values and it was found that the HAP and β-TCP crystallites were formed in the nanometer scale. For HAP, the typical standard value of lattice parameter a, b is 9.432 Å and that of lattice parameter c is 6.8814 Å. For β-TCP, the typical standard value of lattice parameter a, b is 10.439 Å and that of lattice parameter c is 37.375 Å. The average lattice values and crystallite sizes of HAP and β-TCP are shown in Table 1 and Table 2. From all XRD diffractograms, the lattice parameters value well agrees with the typical values. The XRD diffractograms are illustrated in Figure 2.

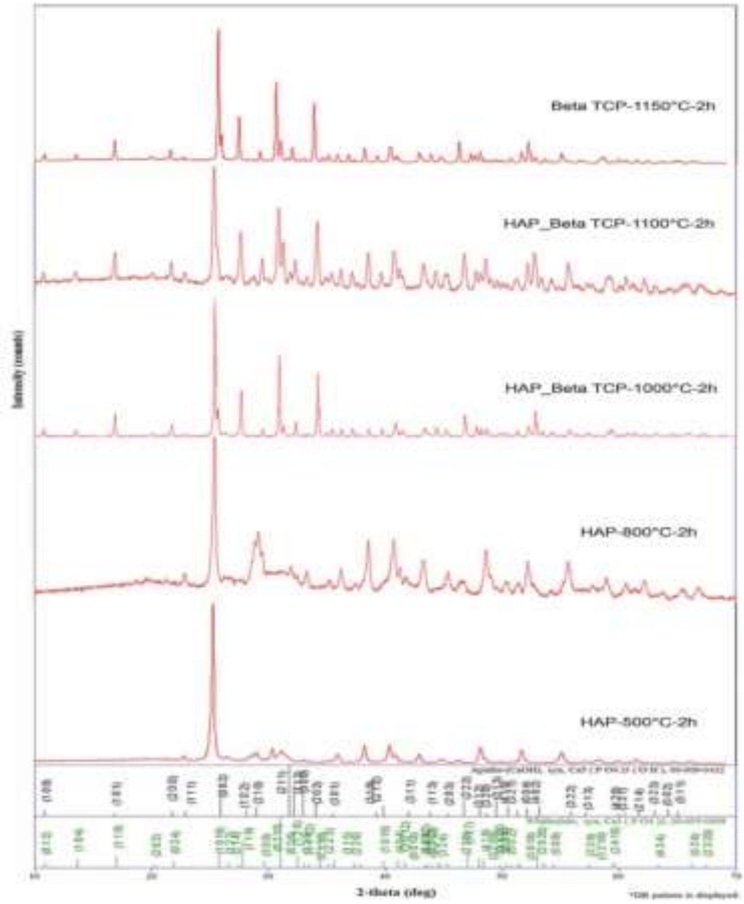

Figure 2 XRD diffractograms of HAP and β-TCP powder for different temperatures

Table 1. The lattice parameters (a, b and c) and crystallite size (D) of HAP Power

Sr. No.	Sample	Heat-treatment temperature (°C)	a (Å)	b (Å)	c (Å)	D (nm)
1	HAP	500 (Calcination)	9.409	9.409	7.023	31.48
2	HAP	800 (Calcination)	9.251	9.251	6.707	26.56

Table 2. The lattice parameters (a, b and c) and crystallite size (D) of HAP/β-TCP and β-TCP powder

Sr. No.	Sample	Heat-treatment temperature (°C)		a (Å)	b (Å)	c (Å)	D (nm)
1	HAP/ β-TCP	1000 (Calcination)	HAP	9.404	9.404	6.831	66.10
			β -TCP	10.469	10.469	37.455	69.64
2	HAP/ β-TCP	1100 (Calcination)	HAP	9.317	9.317	7.026	41.47
			β -TCP	10.486	10.486	37.561	46.98
3	β-TCP	1150 (Calcination)	β -TCP	10.471	10.471	37.469	66.95

Morphological Analysis

The SEM micrographs of powder and pellets samples at different temperature are shown in Figures 3 and 4.

According to the SEM micrograph, the grain sizes of HAP after calcination at 500°C were found to be in the range of 0.50 μm to 1.00 μm. It was observed that the small grains were being agglomerated with the small crystallites which were in nanometer range. The homogeneity of the topography exhibited the formation of pure HAP powder which agreed well with the XRD analysis.

From the SEM micrograph of HAP after calcination at 800°C for 2 hours in a furnace, the grain sizes were estimated by using Line Intercept Method and found to be 1.22 μm. According to the microstructure of the sample calcination at 1000°C and 1100°C, it was found that some parts of the HAP had transformed into β-TCP. Therefore, the smaller grains of HAP were more numerical than the larger grains of β-TCP. However, after calcination at 1150°C, almost all of the HAP had transformed into β-TCP. From the SEM micrograph of HAP after sintering at 900°C, the average grain size was about 1.45 μm and that of β-TCP pellet after sintering at 1200°C, the average grain size of β-TCP was about 6.55 μm. It was the largest grain size of all temperatures. It was worth to note that the grain sizes in biphasic pellets were about two times larger than those in biphasic powder. The comparison on grain sizes of HAP and β-TCP powder and pellet and that of HAP/β-TCP powder and pellets are shown in Tables 3 and 4.

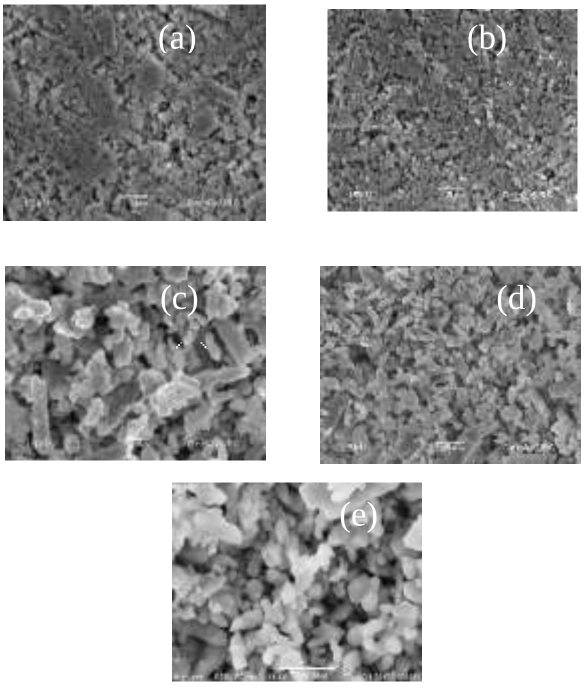

Figure 3 The SEM micrographs of the HAP powder at (a) 500℃, (b) 800℃, (c) HAP/β-TCP powder at 1000℃, (d) at 1100℃ and (e) β-TCP powder at 1150℃ for 2 hours

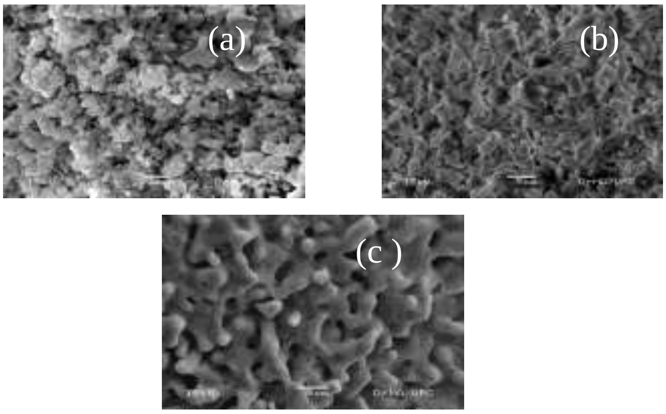

Figure 4 The SEM micrograph of the HAP pellet at (a) 900℃ (b) HAP/β-TCP pellet at 1100℃ and (c) β-TCP pellet at 1200℃ for 2 hours

Table 3. The average grain sizes of heat-treatment HAP powder and pellet

Sr. No.	Sample	Heat-treatment Temperature (℃)	Grain Size (µm)
1	HAP	500 (Calcination)	0.50-1.00
2	HAP	800 (Calcination)	1.22
3	HAP	900 (Sintering)	1.45

Table 4. The average grain sizes of heat-treatment HAP /β-TCP and β-TCP powder and pellet

Sr. No.	Sample	Heat-treatment Temperature (℃)	Grain Size (µm)	
			HAP	β-TCP
1	HAP/β -TCP	1000 (Calcination)	0.67	1.45
2	HAP/β -TCP	1100 (Calcination)	0.84	2.51
3	β -TCP	1150 (Calcination)	ND	1.75
4	HAP/β -TCP	1100 (Sintering)	1.23	2.42
5	β-TCP	1200 (Sintering)	ND	6.55

ND= Non Detected

Apatite Formation in Simulated Body Fluid (SBF)

The SEM images for the pellets before and after immersing in SBF are presented in Figures 6 to 8. It is worth to note that the morphology of the pellets immersed in SBF was changed distinctly. As seen in the SEM images the formation of apatite layers on each surface of HAP, HAP/β-TCP and β-TCP pellets was confirmed by the difference in the surface texture of the sample. Importantly, the β-TCP exhibited the faster rate of formation of apatite layer compared to the pure HAP and biphasic HAP/β-TCP. Based on the SEM analysis, β-TCP seemed to have better bioactivity than HAP in this work. The apatite layer formed on the surface of the HAP, HAP/β-TCP and β-TCP can bond to living bone. Therefore, the HAP, HAP/β-TCP and β-TCP prepared in this research have potential biomaterials which can be applied for bone replacement.

Figure 5 The pellets in SBF solution

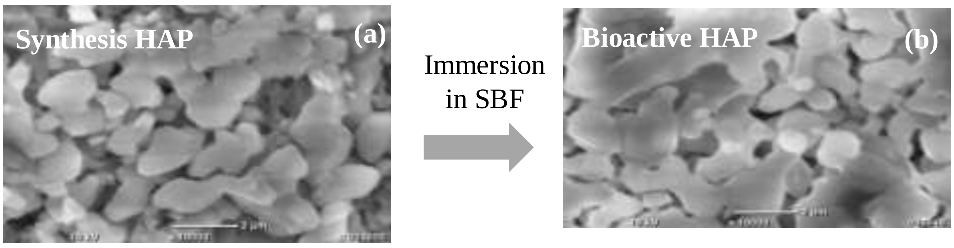

Figure 6 SEM micrographs of HAP pellets at the calcination temperature of 900 ℃ in 10K × magnification (a) before and (b) after immersion in SBF

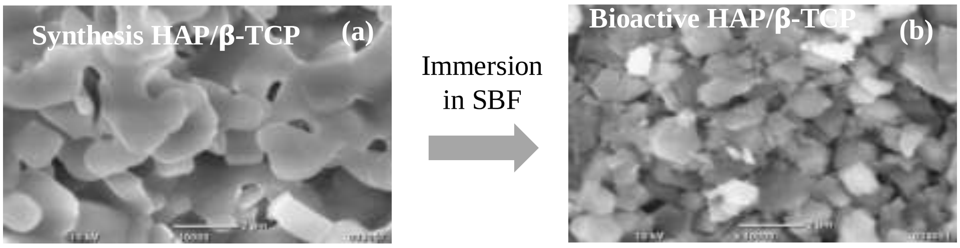

Figure 7 SEM micrographs of HAP/β-TCP pellets at the calcination temperature of 1100 ℃ in 10 K × magnification (a) before and (b) after immersion in SBF

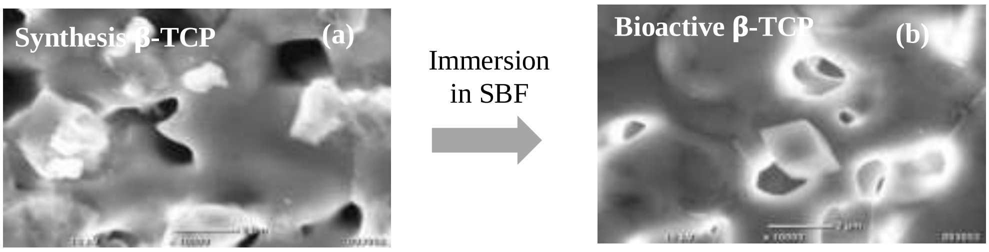

Figure 8 SEM micrographs of β-TCP pellets at the calcination temperature of 1200 ℃ in 10 K × magnification (a) before and (b) after immersion in SB

Conclusion

Hydroxyapatite, $Ca_{10}(PO_4)_6(OH)_2$ was prepared from gypsum by precipitation method. The raw materials were well characterized prior to the preparation of HAP. A single phase structure of HAP from gypsum was successfully formed via the precipitation method after heat-treated at 500℃ and 800℃ for 2 hours. It is worth to note that some parts of HAP converted to β-TCP phases after heat-treated at 1000℃ for 2 hours. It is interesting to note that transformation to β-TCP started by calcination at 1000℃ and the transformation rate increased with increase in temperature. The average lattice constants well agree with the typical values for HAP and β-TCP structure. From the SEM micrographs of different calcination and sintering temperatures, the small grains were being agglomerated with the small crystallites which were in nanometer range at calcination temperature 500℃. The grain sizes could be calculated from the SEM micrographs of other remaining temperatures. The grain sizes of the highest sintering temperature at 1200℃ used in this research was about 6.55 μm. It was the largest grain size of all temperatures. It is worth to note that the grain sizes in biphasic pellets were about two times larger than those in biphasic powder. The SBF soaking results revealed that all the studied materials are biologically active which confirmed its application as bone in living things.

Acknowledgements

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References

[1] Angelescu, N., Ungureanu, D.N. and Anghelina F.V. (2011). Synthesis and Characteristic of Hydroxyapatite Obtained in Different Experimental Conditions, Valahia University of Targoviste, Romania.

[2] Bahman Mirhadi, Bahman Mehdikhani and Nayereh Askari, (2012). Sintering effects on the hardness of β-TCP, Journal of Ceramic Processing Research, Vol. 13, No.4.

[3] Hitachi High-Tech Science Corporation, (1985). Thermal Analysis of Gypsum, TA No.22, Tokyo.

[4] Kim, S.R., Lee, J.H., Riu, D.H., Jung, S.J., Lee, Y.J., Chung, S.C., Kim, Y.H., (2003). Synthesis of Si, Mg substituted hydroxyapatite and their sintering behaviors. Biomaterials, 24, 1389-1398.

[5] Onur Rauf Bingöl and Caner Durucan (2011). Hydrothermal Synthesis of Hydroxyapatite from Calcium Sulfate Hemihydrate, Department of Metallurgical and Materials Engineering, Middle East Technical University, Ankara, Turkey.

[6] Orlovskii, V.P.et al., (2002). Hydroxyapatite and Hydroxyapatite-Based Ceramics, Inorganic Materials, Vol.38

[7] Living with Gypsum: From Raw Material to Finished Products. (n.d.). Retrieved July 7, 2017 from http://www.eurogypsum.org.