

# Effect of curing temperature and time on the mechanical properties of hydroxyapatite/calcined kaolin

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**ABSTRACT:** In the current study, improvement of HAP/Calcined kaolin (CK) strength as a function of its curing regime was studied. The influence of curing temperature and time on the compressive strength of hydroxyapatite combined with calcined kaolin (HAP/CK) samples was investigated using statistical analysis. Curing temperatures were ranged 40, 60, 80 °C and curing times were 2, 7, 14, 21, 28 days, respectively. Prolonged curing time and increased curing temperature improved the compressive strength of the samples. The curing time and temperature significantly affected the compressive strength of HAP/CK samples, while there was no interaction between curing time and curing temperature. The highest compressive strength, 37.8 MPa, was realized by curing the sample at 80 °C for 28 days. The optimal process was curing HAP/CK at 60 °C for 14 days to achieve a high compressive strength.

**KEYWORDS:** compressive strength, bone substitute materials, calcium phosphate bioceramics, geopolymer

## INTRODUCTION

Nowadays, a number of bone substitute materials including metals, polymers, and ceramics have been developed as alternatives for bone repair, augmentation or substitution<sup>1</sup>. The aim of development of bone substitutes is into create materials that have mechanical and chemical properties closest to those of human bone. Due to their bioactivity and biocompatibility,  $\text{Ca}_3(\text{PO}_4)_2$  bioceramics, such as hydroxyapatite (HAP) and  $\beta$ -tricalcium phosphate ( $\beta$ -TCP), have been widely employed for bone repairs, fixing defects or filling voids. These are available in various forms, such as powders, porous scaffolds, blocks, or beads<sup>2-4</sup>. Unfortunately, low strength, fracture toughness and brittleness of HAP and  $\beta$ -TCP have limited its application to just bone repairs or low weight bearing monolithic implants. Geopolymer is a three-dimensional aluminosilicate polymeric structure, which consists of Si-O-Al bonds

a chemical reaction between  $\text{SiO}_2$  and  $\text{Al}_2\text{O}_3$  under highly alkali conditions<sup>5</sup>. The materials that are currently attracting interest as bone substitutes or fillers are geopolymers and synthetic aluminosilicates<sup>6-9</sup>. Calcined kaolin (CK) is an aluminosilicate material that can be used as a starting material to obtain high strength geopolymers<sup>10-13</sup>. To produce biomaterials, clean source materials with minimum contamination are needed. The white coloured CK seems to be the best source material for this purpose.

On a review of literature, the property that is most often used to characterize the mechanical behaviour of bone substitute is their compressive strength. The compressive strength of porous human bone varies between 2 and 42 MPa for cancellous bone and between 100 and 230 MPa for cortical bone<sup>14,15</sup>. From previous research, mixtures of HAP with CK were investigated for their strengths and apatite formation<sup>16</sup>. The maximum compressive strength obtained from an HAP:CK mix at a ratio of

1:3 was 32.9 MPa when cured at 60 °C for 48 h. This HAp:CK material exhibited good bioactivity after immersion in simulated body fluids for 28 days. However, varying curing time and temperature may have substantial effects on the ultimate properties of the final product. This study investigated the improved strength of HAp mixed with CK in terms of the curing regime used. The effect of curing time and temperature on the compressive strength of the HAp:CK final product was also investigated. Statistical analysis was done to determine the level of influence of each factor. SPSS was used assuming a normal distribution. Two-way ANOVA and Duncan's multiple range tests were used in the statistical analysis.

## MATERIALS AND METHODS

### Preparation of starting materials

Hydroxyapatite (HAp) and calcined kaolin (CK) powders were used as the raw materials in this study. For the preparation of HAp powder, calcium carbonate ( $\text{CaCO}_3$ ) produced from golden apple snail shell (calcined at 600 °C for 3 h<sup>17</sup>) and dicalcium phosphate dihydrate ( $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$ , DCPD, Sigma Aldrich) were mechanochemically mixed at a theoretical stoichiometric Ca/P molar ratio (1.67) to produce HAp. This was done in a ball mill for 24 h. Then the material was heat-treated at a temperature of 1100 °C for 1 h. The crystalline phase of prepared HAp powder was identified using an X-ray diffraction equipment (XRD, Bruker D8). The XRD analysis was carried out with  $\text{CuK}\alpha$  radiation operating at a scanning rate of 2.4 °C 2 $\theta$ /min in 0.02 °C 2 $\theta$  increment.

Calcined kaolin (CK) was prepared by calcination of metakaolin at a temperature of 600 °C in an electric furnace. The metakaolin used as the starting material was obtained from the eastern region of Thailand. Chemical composition of CK was determined using an X-ray fluorescence (WDXRF, AXIOS mAX). NaOH (10 M) and sodium silicate (15%  $\text{Na}_2\text{O}$ , 33%  $\text{SiO}_2$  and 52%  $\text{H}_2\text{O}$ ) with a mass ratio of 1.0 were used as a liquid activated binder for the HAp and CK powders.

### Material preparation and evaluation

Hydroxyapatite (HAp) and calcined kaolin (CK) powders with an HAp:CK mass ratio of 1:3 (HAp/CK 25) was selected to study the influence of curing time and temperature on the compressive strength of the synthesized samples. This ratio was selected due to the high compressive strength and

good bioactivity of samples prepared at this ratio<sup>16</sup>. The HAp/CK 25 powders were mechanically mixed with an activated binder solution using a planetary mixer for 5 min. The resulting sample paste was then rapidly poured into a 25×25×25 mm<sup>3</sup> acyclic cube mould, and then mechanically vibrated for 10 s to remove entrapped air. Samples were carefully wrapped with a plastic film to prevent moisture loss. The cast samples were then separately cured in electric ovens.

In this study, two factors were varied, curing temperature and time, to investigate their effect on the compressive strength of HAp/CK 25. Three curing temperatures, 40, 60, and 80 °C, and five curing times, 2, 7, 14, 21, and 28 days, were evaluated. The samples cured for less than 28 days were removed from their moulds, wrapped in a plastic film and then stored at 23 °C until the 28 days. It has been reported that the temperature curing of 2 days produced geopolymer with a sufficiently high strength<sup>18–20</sup>. Hence the strength test at 2 days was performed in this experiment. Compressive strength tests were conducted on three cubed samples per each condition and the results were reported as the average values according to ASTM C109<sup>21</sup>. The statistical methods were employed to analyse the level of each factor using SPSS version 19.0. This analysis began with checking the adequacy of the model by testing the normality of the parameter value distributions for all treatments using Shapiro-Wilk's test ( $p$ -value > 0.05), since there were less than 50 data points. Then two-way ANOVA at a 95% confidence interval ( $\alpha = 0.05$ ) was employed to test the differences between the mean values. Duncan's multiple range test was used to compare the means of the five levels of curing times (2, 7, 14, 21, and 28 days) and the three levels of curing temperature (40, 60, and 80 °C).

## RESULTS AND DISCUSSION

### Characterization of starting materials

Fig. 1 shows the XRD pattern of the synthesized HAp powder. It was found from the figure that the major component of powder was HAp with a small amount of  $\beta$ -TCP in a crystalline phase. A similar behaviour was observed for HAp produced via a mechanochemical reaction<sup>22–24</sup>. Chemical composition of CK showed that it was composed mainly of 59%  $\text{SiO}_2$ , 36.71%  $\text{Al}_2\text{O}_3$ , 1.43%  $\text{TiO}_2$ , 1%  $\text{Fe}_2\text{O}_3$ , 0.25%  $\text{CaO}$ , 0.07%  $\text{MgO}$  and 0.03%  $\text{SO}_3$ .

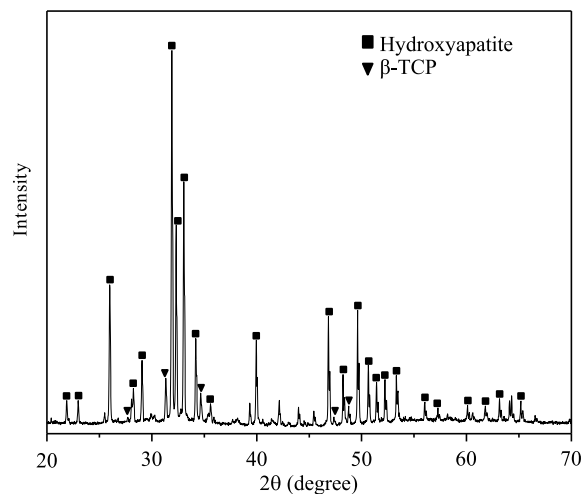


Fig. 1 XRD pattern of the hydroxyapatite synthesized from golden apple snail shell.

### Compressive strength

The average compressive strengths of samples cured at 40, 60, and 80 °C with various curing times are shown in Fig. 2. These three different curing temperatures were selected from observations of kaolin-based geopolymers, indicating that heat is beneficial for strength development and curing at temperatures below 100 °C and that it has a significant contribution to the geopolymeric reaction in kaolin<sup>25</sup>. The curing times of 2, 7, 14, 21, and 28 days were selected to allow sufficient time for the geopolymerization process. From Fig. 2, it can be seen that the strength of the samples increased with increasing curing time at all temperatures. The strength linearly increased with increasing curing time for the samples cured at 40 °C whereas the strength of samples cured at 60 and 80 °C increased slightly over 7 days of curing. The highest strength, 37.8 MPa, was observed from samples cured at 80 °C for 28 days, while the compressive strength of cancellous bone is 2–45 MPa<sup>14,15</sup>. From these results, it can be concluded that the appropriate curing conditions are a temperature of 80 °C for at least 7 days for strong development of HAp/CK 25. This range of strength is potential for cancellous bone graft substitute. Statistical analyses were used to elucidate the influence of curing time and temperature on the strength of HAp/CK 25 samples.

### Statistical analysis

The compressive strength of HAp/CK 25 was investigated using 15 treatments; five levels of curing

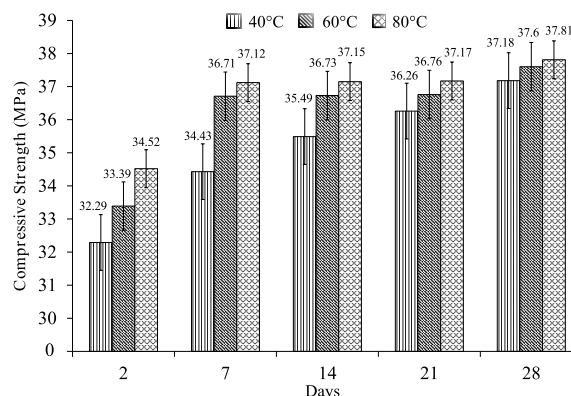


Fig. 2 Average compressive strengths of HAp/CK 25 samples after curing at 40, 60, and 80 °C for various curing periods

Table 1 Statistical data and Shapiro-Wilk’s test of compressive strength of HAp:CK 25 using 15 treatments to test for normality.

	Day_Temp	Shapiro-Wilk		
		Statistic	df	Sig.
Strength	2_40	0.952	3	0.578
	2_60	1.000	3	1.000
	2_80	0.996	3	0.873
	7_40	0.980	3	0.729
	7_60	0.864	3	0.278
	7_80	0.864	3	0.278
	14_40	0.797	3	0.108
	14_60	0.999	3	0.935
	14_80	0.970	3	0.670
	21_40	0.945	3	0.549
	21_60	0.967	3	0.654
	21_80	0.827	3	0.181
	28_40	0.947	3	0.558
	28_60	0.999	3	0.956
	28_80	0.935	3	0.506

time and three levels of curing temperature. Three replicates were made for each treatment and they were analysed statistically. The model adequacy was investigated using Shapiro-Wilk’s test to verify data normality. The results show that the compressive strength data of HAp/CK 25 was normally distributed ( $p$ -value > 0.05) (Table 1). The results indicated that these data were adequate for further statistical analysis, assuming a normal distribution.

A two-way ANOVA at a 95% confidence interval was employed to investigate the effects of curing temperature and time on the compressive strength of HAp/CK 25. The factors were considered sep-

**Table 2** Two-way ANOVA test of the effects of curing temperature and time on the compressive strength of HAp/CK 25. Tests factor: interaction effects; dependent variable: strength

Source	Type III SS	df	Mean Square	F	Sig.
C <sup>†</sup> Model	114.9 <sup>a</sup>	14	8.2	6.3	0.0
Intercept	58 459.5	1	58 459.5	45 124.2	0.0
Day	88.6	4	22.2	17.1	0.0
temp	20.6	2	10.3	8.0	0.0
Day Temp	5.7	8	0.7	0.6	0.8
Error	38.9	30	1.3		
Total	58 613.3	45			
C <sup>†</sup> Total	153.8	44			

<sup>a</sup> R<sup>2</sup> = 0.747 (Adjusted R<sup>2</sup> = 0.629). Type III SS = Type III sum of squares. C<sup>†</sup> = corrected.

arately and in aggregate. The results are shown in Table 2. The ANOVA results revealed that both curing time and curing temperature significantly affected ( $p$ -value < 0.05) the compressive strength of HAp/CK 25, while the interaction between curing time and curing temperature showed no significant effect ( $p$ -value > 0.05) on the compressive strength of HAp/CK 25, (Table 2).

Furthermore, the Pearson correlation coefficient (Table 3) showed that the effects of curing time and temperature on compressive strength are related in a positive linear sense. The correlation value between curing time and compressive strength was 0.660 and between curing temperature and compressive strength was 0.359. This means the compressive strength of HAp/CK 25 increased with increasing curing time. Also, at a higher curing temperature, the compressive strength was greater than at a lower curing temperature. Furthermore, it was found that the correlation between curing time and compressive strength was much stronger than that between curing temperature and compressive strength ( $0.660 > 0.359$ ) as shown in Table 3. Many studies on geopolymers conformed that curing temperature and curing time significantly influence the compressive strength<sup>19, 20, 26, 27</sup>.

Duncan's multiple range test was then used to compare the range of a subset of the compressive strengths of HAp/CK 25 based on curing time and another range of a subset of the compressive strengths of HAp/CK 25 based on curing temperature. The results are shown in Table 4.

The compressive strength of HAp/CK 25 increased significantly with increased curing time. However, there were no significant differences in

**Table 3** Pearson correlation of curing time and curing temperature with the compressive strength of HAp/CK 25.

		Day	Temp	Strength
Day	Pearson correlation	1	0.000	0.660
	Sig. (2-tailed)		1.000	0.000
	N	45	45	45
Temp	Pearson correlation	0.000	1	0.359
	Sig. (2-tailed)	1.000		0.016
	N	45	45	45
Strength	Pearson correlation	0.660	0.359	1
	Sig. (2-tailed)	0.000	0.016	
	N	45	45	45

**Table 4** Duncan's multiple range test of curing temperature and curing time on the compressive strength of HAp/CK 25.

C <sup>†</sup> time	N	Subset			C <sup>†</sup> temp.	N	Subset	
		1	2	3			1	2
2.00	9	33.4			40.0	15	35.1	
7.00	9		36.1		60.0	15		36.2
14.0	9		36.5	36.5	80.0	15		36.8
21.0	9		36.7	36.7	Sig.		1.0	0.2
28.0	9			37.5				
Sig.		1.0	0.3	0.1				

N = Sample size, Sig. = Significance. C<sup>†</sup> = cured.

the compressive strengths of HAp/CK 25 after 7, 14, and 21 days of curing at a 95% confidence interval. The highest compressive strength, 36.7 MPa, was observed after 21 days. Furthermore, after 14, 21, and 28 days of curing, there were no significant differences in the compressive strengths of HAp/CK 25 at a 95% confidence interval. The highest compressive strength, 37.5 MPa, was after 28 days.

The compressive strength of HAp/CK 25 increased significantly with increased curing temperature. There were significant differences in compressive strength of HAp/CK 25 with curing at 40 °C among all pairs of curing temperatures at a 95% confidence interval. However, there were no significant differences in compressive strength of HAp/CK 25 with curing at 60 °C and 80 °C with a 95% confidence interval. The highest compressive strength, 36.8 MPa, was observed after curing at 80 °C. From these results, it can be concluded that curing at 60 °C for 14 days represents the optimal curing conditions to achieve the maximal compressive strength of HAp/CK 25. Future research is required to evaluate the bioactivity and other mechanical and physical properties, such as bending

strength, porosity, surface morphology of HAp/CK 25 under optimal curing condition.

## CONCLUSIONS

The effects of curing time (2, 7, 14, 21, and 28 days) and temperature (40 °C, 60 °C and 80 °C) of hydroxyapatite combined with calcined kaolin (1:3 mass ratio) were investigated. Statistical analyses were used to determine the level of influence of each factor. The conclusions of the current study are as follows.

- (1) The compressive strength of the HAp/CK 25 increased significantly with increasing curing temperature and curing time.
- (2) The highest compressive strength of HAp/CK 25, 37.8 MPa, was achieved after curing the sample at 80 °C for 28 days.
- (3) Statistical analyses revealed that both curing temperature and curing time significantly affected compressive strength, while there were no interactions between these two factors.
- (4) The optimal curing conditions for HAp/CK 25 to achieve its highest compressive strength was 60 °C for 14 days.
- (5) The combinations between HAp/CK 25 provided suitably high compressive strength for the bone substitute material.

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