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Contributed Paper

## Comparison Among Alpha-tricalcium Phosphate Synthesized by Solid State Reaction and Wet Chemical Reaction for Calcium Phosphate Cements

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### ABSTRACT

Recently, calcium phosphate cement has been used in bone tissue engineering and regenerative medicine due to its bioactivity and biocompatibility. Alpha-tricalcium phosphate has been known previously to be an essential raw material of self-setting calcium phosphate cement. The most established method for alpha-tricalcium phosphate synthesis is the solid state reaction of Dicalcium phosphate anhydrous (DCPA) and Calcium carbonate ( $\text{CaCO}_3$ ) at high temperatures, follow by quenching immediately to room temperature. It has been reported that alpha-tricalcium phosphate synthesized by wet chemical reaction from calcium nitrate ( $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ ) and phosphoric acid ( $\text{H}_3\text{PO}_4$ ) at high temperature, being unnecessary quenching for obtaining high purity alpha-tricalcium phosphate. The aim of the present work was to study the effect of comparative alpha-tricalcium phosphate powder obtained by solid state reaction and wet chemical reaction on the properties of calcium phosphate cements. The obtained powder was characterized by particle size analysis and X-ray diffraction. Cement powders consisting of the different synthesis of alpha-tricalcium phosphate powders were mixed with the liquid solution to obtain cement paste. After setting cement samples were determined by setting time, compressive strength, phase analysis in vitro test (simulated body fluid). Although the calcium phosphate cement was prepared using alpha-tricalcium phosphate by solid state reaction showed a slightly better performance, it was likely that the resulting cement from both syntheses was not significantly different.

**Keywords:** calcium phosphate cement, bone cement, alpha-tricalcium phosphate, solid state synthesis, wet chemical synthesis

## 1. INTRODUCTION

Alpha-tricalcium phosphate ( $\alpha$ -TCP) has been used as the one component for bone cements, reacting with water lead to calcium-deficient hydroxyapatite (CDHA) which has a similar chemical and composition of the mineral phase of bones. Hydroxyapatite induced the new bone formation, leading to the proliferation of bone cells [1]. It was found that alpha-tricalcium phosphate is difficult to synthesize because of its instability at ambient temperature. This alpha-tricalcium phosphate is stable in the temperature range of 1140-1470 °C. Although it is quite difficult to obtain the pure alpha-tricalcium phosphate, it has been investigated in other methods and applications [2]. There are many methods of synthesis which have been published. The synthesis of  $\alpha$ -TCP is most basically described as the thermal transformation of a precursor with a molar ratio Ca/P  $\approx$  1.5 or by solid state reaction of a mixture of solid component at high temperatures. The solid state reaction, mixing solid precursors is quite popular method in the publications which have been reported so far [3-6].

The solid state reaction is synthesized by the mixture of solid reagents, followed by wet milling for the homogenous phase. After mixing, the mixture must be immediately heated above the transformation temperature between 1250 and 1500 °C. After long holding calcination time, it must be quenched suddenly to room temperature to avoid the conversion of  $\alpha$ -TCP phase which could transform to  $\alpha$ -TCP at  $\sim$  1125 °C. The obtained powder often displays some of other phases, mostly  $\alpha$ -TCP or hydroxyapatite [7]. Camire et al. [8] produced the purity of  $\alpha$ -TCP from a mixture of dicalcium phosphate and calcium carbonate at 2 : 1 molar ratio, followed by heating at 1350 °C for 4 h. Duncan et al.[9] obtained

the pure phase of  $\alpha$ -TCP by solid state reaction, mixing monetite (DCPA, dicalcium phosphate) and calcium carbonate in acetone which monetite was prepared by aqueous precipitation method using  $\text{Ca}(\text{OH})_2$  and  $\text{H}_3\text{PO}_4$  as the reagents. After mixing, the mixture was dried at 80 °C and then directly heated at 1300 °C for 16 h, reground and heated again at 1300 °C for another 8-12 h, followed by quenching to room temperature.

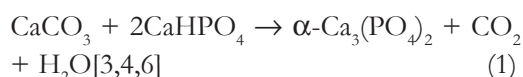
The wet chemical reaction has been known as the new method for the synthesis of  $\alpha$ -TCP, as this method was able to synthesize the  $\alpha$ -TCP with no use of quenching. Some researchers have evaluated and identified the appropriated factors to provide the  $\alpha$ -TCP since 2012 [10]. This method has been improved to produce the high purity of  $\alpha$ -TCP. However, it is critically important to obtain  $\alpha$ -TCP by the new method of synthesis in order to improve material properties and/or reduce cost. The purpose of this work is to study the influence of  $\alpha$ -TCP obtained by different routes of synthesis on the properties of bone cements.

## 2. MATERIALS AND METHODS

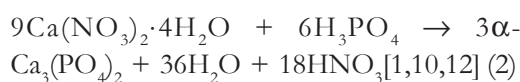
### 2.1 Synthesis of $\alpha$ -TCP powder

Two different routes of  $\alpha$ -TCP synthesis were prepared by (a) solid state reaction method and (b) by wet chemical reaction method as followed the Eq.(1-2) respectively, representing the  $\alpha$ -solid and  $\alpha$ -wet to be used as one of the precursor for cement preparation.

Solid state reaction



Wet chemical reaction



The  $\alpha$ -solid was obtained by solid state reaction as originally described by Srakaew et al [11]. Briefly, dicalcium phosphate anhydrous ( $\text{CaHPO}_4$ ) was mixed with calcium carbonate ( $\text{CaCO}_3$ ) at a 2:1 molar ratio. The mixture was heated to 1350 °C, left to dwell for 4 h and then rapidly quenched to room temperature. The  $\alpha$ -wet from wet chemical reaction was done as followed Thirmer et al.[12], 0.5 M calcium nitrate ( $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ ) was mixed with 0.5 M phosphoric acid ( $\text{H}_3\text{PO}_4$ ). After mixing, the obtained solution was aged at 90 °C for 24 h and dried at 120 °C. The obtained powder was calcined at 1400 °C for 1 h without quenching in air. The obtained powder from both methods was milled and sieved through 325 mesh to achieve a fine powder.

## 2.2 Phase Characterization

The phase compositions of  $\alpha$ -TCP powder and cements after immersion in SBF solution for the period of time were determined by X-ray diffraction (XRD Bruker/D2-phaser) in the range 20 of 10 to 40 (5-powder), and in step size of 0.02. The quantitative phase compositions of cements were measured by means of Rietveld refinement analysis using the TOPAS software (Bruker AXS, Karlsruhe, Germany). The chemical functional groups were characterized by using Fourier transform infrared spectroscopy (ATR-FTIR Bruker/Tensor27-Hyperion) with the range of 4000 to 400  $\text{cm}^{-1}$ .

## 2.3 Particle Size Analysis

Particle size distribution of both  $\alpha$ -solid and  $\alpha$ -wet was detected by using laser scattering particle size distribution analyzer (Horiba/LA-950V2). 100 mg of powder was dispersed in 200 ml isopropanol by applying ultrasound for 15 min [13].

## 2.4 Setting Time Measurement

The initial and final setting time ( $t_i$  and  $t_f$ ) of the cement pastes were measured by using the standard Gillmore needles with a light-thick needle (2.13 mm, 113.4 g) and a heavy-thin needle (1.06 mm diameter, 453.6 g), according to the standard ASTM C266-99 [14]. The setting time of all specimens was recorded when there was no visible mark on the surface of cements.

## 2.5 Compressive Strength

$\alpha$ -solid and  $\alpha$ -wet were mixed with other components, as referred to Srakaew et al.[11] providing the homogenous powder phases, denoted as CPC-solid and CPC-wet. The liquid phases were the mixture of 1 M disodium hydrogen phosphate ( $\text{Na}_2\text{HPO}_4$ ) and 1 M sodium dihydrogen phosphate ( $\text{NaH}_2\text{PO}_4$ ). For this procedure, the cement paste was prepared by mixing in the range of 0.35 and 0.4 L/P liquid to powder ratio. The homogenous paste was directly poured into the Teflon mold size (6 mm diameter and 12 mm height) according to the standard ASTM F451-95[15]. The specimens were removed and maintained in 100 % humidity at 37 °C for 24 h, then immersed in SBF solutions for 7 and 14 days, respectively. The compressive strength of all specimens was tested using Universal testing machine (UTM capacity 100 kN/MUL-125 TTR : THAI, according to ISO/IEC 17025) with a 10 kN load cell at a cross speed of 1  $\text{mm}/\text{min}^{-1}$ . Eight specimens were tested for each condition.

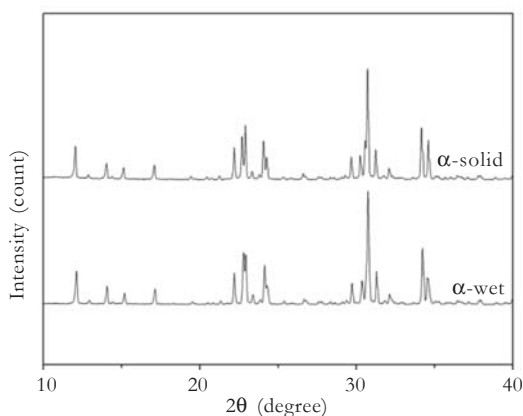
## 2.6 Microstructure Analysis

The scanning electron microscopy was shown on the morphologies of  $\alpha$ -TCP powder used with an energy dispersive X-ray (EDX OXFORD INSTRUMENTS/X-max20) analyzer, and that of the fracture surfaces after compression test. The bioactivity

was investigated by detecting whether the precipitated hydroxyapatite occurred on the surface of samples or not after immersion in SFB solution for 7 and 14 days, using (SEM JEOL/JSM-6010LV). All specimens received a gold coating before observation.

### 3. RESULTS AND DISCUSSION

As shown in Figure 1, It can be seen that there is no significant difference between the XRD pattern of both  $\alpha$ -TCP obtained by solid state reaction and wet chemical reaction. The high purity phase of  $\alpha$ -TCP was detected, with corresponding to the standard file ( $\alpha$ -TCP: JCPDS 09-0348 and 29-0359).



**Figure 1.** The XRD pattern of  $\alpha$ -TCP obtained by solid state reaction ( $\alpha$ -solid), and wet chemical reaction ( $\alpha$ -wet).

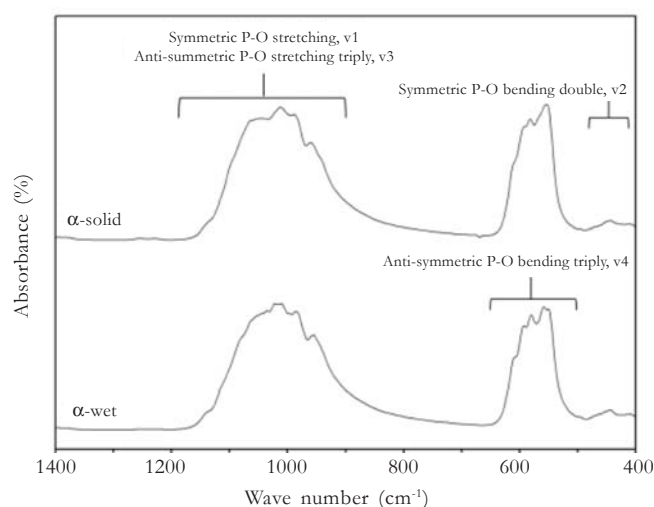
The  $\alpha$ -TCP obtained by wet chemical reaction was calcined at varying temperatures from 1300-1500 °C, has been published in Thörmer et al.[12] providing the higher pure phase of  $\alpha$ -TCP with the increasing of temperature calcination to 1500 °C. However, this study showed the high purity phase of  $\alpha$ -TCP at 1400 °C which is unnecessary for the higher calcination

(1500 °C) to obtain the higher pure phase of  $\alpha$ -TCP.

The synthesis of  $\alpha$ -TCP with high purity phase has been studied with the use of long dwell time at high temperature (greater than 1200 °C) not less than 4 h, was known as the standard method. Moreover, the quenching to room temperature was the main point to inhibit the transformation of  $\alpha$ -TCP in  $\alpha$ -TCP after cooling period. Duncan et al.[9] studied the effect of cooling rate on the quantitative transformation of  $\alpha$ -TCP, resulting the higher  $\alpha$ -TCP with the increasing of cooling rate.

The spectra of  $\alpha$ -solid and  $\alpha$ -wet were also investigated in transmission mode using the ATR technique of FTIR, as shown in Figure 2. The spectra of  $\alpha$ -TCP powder from literature reviews [2, 7] compared with  $\alpha$ -solid and  $\alpha$ -wet, bands were identified in accordance with the vibrations of phosphate groups that consisted of  $\nu_1$  bands determined to symmetric P-O stretching and  $\nu_3$  bands determined anti-symmetric P-O stretching triply degenerate (1200-900  $\text{cm}^{-1}$ ),  $\nu_2$  bands determined symmetric P-O bending double degenerate (410-470  $\text{cm}^{-1}$ ), and  $\nu_4$  bands determined anti-symmetric P-O bending triply degenerate (650-500  $\text{cm}^{-1}$ ), illustrated in Table 1.

Figure 2 shows the spectrum of the  $\alpha$ -solid powder that is quite similar to that of the  $\alpha$ -wet spectrum, but some bands in the region of  $\nu_2$ ,  $\nu_3$ , and  $\nu_4$  vibrations were not identified comparing to the bands of  $\alpha$ -wet and  $\alpha$ -TCP from literature reviews, as shown in Table 1. Some noticeable bands are poorly resolved showing very low intensity. This may be due to the low intensive bands overlap with other higher intensive bands [2]



**Figure 2.** The FTIR spectra of  $\alpha$ -TCP obtained by two methods ( $\alpha$ -solid,  $\alpha$ -wet).

**Table 1.** Main bands and characteristic wave number of  $\alpha$ -TCP ( $\alpha$ -solid and  $\alpha$ -wet) compared with  $\alpha$ -TCP from literature review.

Normal mode	Free $\text{PO}_4^{3-}$ ( $\text{cm}^{-1}$ )	$\alpha$ -solid ( $\text{cm}^{-1}$ )	$\alpha$ -wet ( $\text{cm}^{-1}$ )	$\alpha$ -literature ( $\text{cm}^{-1}$ ) [2, 7]
Symmetric P-O stretching, v1	938	960	955	954
Symmetric P-O bending double degenerate, v2	420	412 443	412 444 463 470	415 430 454 463 471
Anti-symmetric P-O stretching triply degenerate, v3	1017	988 1012 1038 1047	984 1012 1022 1036 1045	984 997 1013 1025 1039 1055
Anti-symmetric P-O bending triply degenerate, v4	567	553 582	550 559 580 609	551 563 585 597 613

Particle size distributions of both  $\alpha$ -TCP powders ( $\alpha$ -solid and  $\alpha$ -wet) were narrow distribution (data not shown).  $\alpha$ -solid and  $\alpha$ -wet had mean particle size of 13.739  $\mu\text{m}$ , and 16.9  $\mu\text{m}$ , respectively which were in the same range of size (13.33  $\mu\text{m}$  and 16.80  $\mu\text{m}$  by milling time at 30 and 60 min ) reported by Thurmer et al. [12] determined by light

scattering. The average particle size of  $\alpha$ -wet was in the range, corresponded to the literature [12]. Although the mean size of  $\alpha$ -solid is smaller than that of  $\alpha$ -wet, it was not a significant difference, as displayed in Table 2. Many publications have reported the influence of the particle size distribution on the properties of bone cement.

**Table 2.** Particle size distribution of powders.

Samples	particle size ( $\mu\text{m}$ )			SD
	mean	median	mode	
$\alpha$ -solid	13.73781	12.48384	12.4326	6.9112
$\alpha$ -wet	16.90078	14.2971	14.1644	10.6565

The setting time of cements was measured at room temperature with two constant values of 0.35 and 0.4  $\text{ml g}^{-1}$  liquid to powder ratio in order to observe which ratio was the most suitable for moldability of cements after mixing powder and liquid phase. The results showed that the setting time of CPC-solid raised up from 13.5 min to 25 min and that of CPC-wet raised up from 15 min to 26.5 min with the increasing of liquid to powder ration at 0.35  $\text{ml g}^{-1}$  and 0.4  $\text{ml g}^{-1}$  respectively. As expected, the CPC-solid specimen contained a smaller mean particle size of  $\alpha$ -solid powder, which performed a slight shorter setting time than that of CPC-wet. The particle size of reactants and liquid to powder ratio were reported as the factor that involved the setting time performance. These factors became a strategy to reduce the setting time of cements [16]. Furthermore, the amount of hydroxyapatite phase after immersion cements in SBF solution for 7 and 14 days analyzed by Reitveld refinement of the XRD diagrams, resulting with no significant differences between both cements as well as

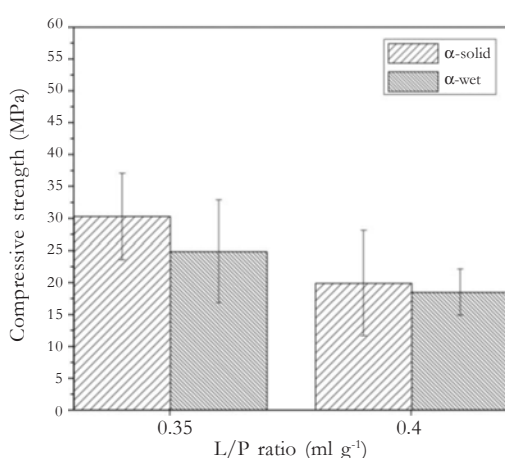
the resulting of setting time, as shown in Table 3.

It has been proved that the particle size of  $\alpha$ -TCP and liquid to powder ratio affect the properties of cements [17]. It can be seen that a slightly bigger size of particle, the range of size corresponds to [12], and increasing of liquid to powder ratio reduced the compressive strength and the formation of hydroxyapatite after immersion in SBF solution, while setting time increase, as illustrated in Table 3. The results of this study are in accordance with the publication [13, 18].

The compressive strength of all samples was presented by mean  $\pm$  standard deviation using one way analysis of variance (ANOVA). A comparison between two means was applied by Tukey post hoc test (P0.05) as reported in Table 3. In spite of the compressive strength histogram of CPC-wet is lower than that of CPC-solid, the mean of one way analysis statically performed with no significant difference between both cements at the same liquid to powder ratio, as displayed in Figure 3.

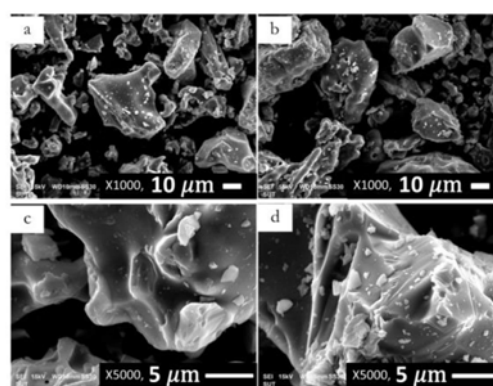
**Table 3.** Percentage of hydroxyapatite after immersion of cement in SBF solution for 7 and 14 days, setting time measurement, and compressive strength of samples.

Samples	% HA formation (Rietveld refinement)				Setting time (min)				Compressive strength (MPa)	
	L/P = 0.35		L/P = 0.4		L/P = 0.35		L/P = 0.4		L/P = 0.35	L/P = 0.4
	ml g <sup>-1</sup>		ml g <sup>-1</sup>		ml g <sup>-1</sup>		ml g <sup>-1</sup>		ml g <sup>-1</sup>	ml g <sup>-1</sup>
	7 days	14 days	7 days	14 days	t <sub>i</sub>	t <sub>f</sub>	t <sub>i</sub>	t <sub>f</sub>		
CPC-solid	31.67	37.94	33.96	41.67	13.5	55	25	80	30.32±6.75	19.92±8.21
CPC-wet	31.65	36.58	34.03	39.17	15	58	26.5	86	24.85±8.02	18.51±3.63

**Figure 3.** Histogram showing the mean compressive strength of samples with L/P ratio 0.35 and 0.4 ml g<sup>-1</sup>.

The scanning electron microscope (SEM) was used for morphological determination of  $\alpha$ -TCP, both powders ( $\alpha$ -solid and  $\alpha$ -wet) presented irregular shape crystals without agglomeration. The size range of  $\alpha$ -solid and  $\alpha$ -wet, representing as Figure 4a-d, both powders were able to confirm the results of particle size distribution analysis.

The Ca/P molar ration of powders was examined by energy dispersive X-ray (EDX) to ensure that Ca/P molar ratio of both synthesized  $\alpha$ -TCP was about 1.5 as calculated from the ratio of starting reagents, resulting in Table 4.

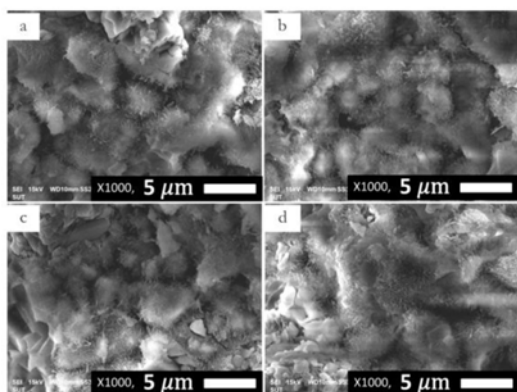
**Figure 4.** SEM micrographs of different  $\alpha$ -TCP powders,  $\alpha$ -solid  $\times 1000$ - $\times 5000$  (a-b),  $\alpha$ -wet  $\times 1000$ - $\times 5000$  (c-d).**Table 4.** The quantitative results of two different powder of  $\alpha$ -TCP, using EDX spectrum analyzer.

Sample	P (Atomic%)	Ca (Atomic%)	Ca/P ratio
$\alpha$ -solid	40.03	59.97	1.498
$\alpha$ -wet	39.93	60.07	1.504

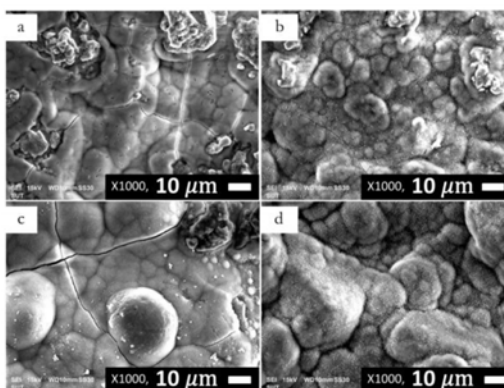
Figure 5 shows the SEM images of the fracture surface micrograph of cements (CPC-solid, and CPC-wet) at 0.35 and 0.4 ml g<sup>-1</sup> liquid to powder ration after immersion in SBF solution for 7 days. As the results, hydroxyapatite needles' formation was observed surrounding some

of the incompletely solubilized starting powder phases. The hydroxyapatite structure formed by the dissolution of 5-TCP and other powders after mixing with liquid phase, released Ca and P ions followed by the reprecipitation process of the network of entangled crystals on the starting powder, which the hydroxyapatite became a thicker layer as the reaction time continued [16]. It can be seen that both cements consisting of different 5-TCP powder as one of the starting powders transforms to hydroxyapatite phase as described above.

After the immersion of cement samples in SBF solution for 7 days, the cements surface was covered by the visibly dense hydroxyapatite. Some of starting powder phase still remained that was in accordance with the fracture surface micrograph of cement in Figure 5. The growth of hydroxyapatite on the surface of cements occurred from the precipitation of Ca and P ions supersaturated SBF. The formation of precipitated hydroxyapatite on the cement surface presented an excellent bioactivity of material, promoting the new bone formation (Figure 6).



**Figure 5.** Fracture surface micrograph of different cements, (a-b) CPC-solid at 0.35 and 0.4 ml g<sup>-1</sup>, (c-d) CPC-wet at 0.35 and 0.4 ml g<sup>-1</sup>, soaked for 7 days in SBF solution.



**Figure 6.** SEM micrograph of the surface of different cements, (a-b) CPC-solid at 0.35 and 0.4 ml g<sup>-1</sup>, (c-d) CPC-wet at 0.35 and 0.4 ml g<sup>-1</sup>, soaked for 7 days in SBF solution.

#### 4. CONCLUSION

Although  $\alpha$ -solid displayed slightly better performances of cements, setting time, compressive strength, and percentage of hydroxyapatite formation as the final product, it can be proved that the high purity of  $\alpha$ -TCP from different synthesis (solid state reaction and wet chemical reaction) presented the properties of calcium phosphate cement with no significant difference. This is possibly another choice for the use of  $\alpha$ -TCP from wet chemical reaction instead of the solid state reaction method due to time efficiency and the reduced cost of the process.

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## REFERENCES

- [1] Thurmer M.B., Diehl C.E., Brum F.J.B. and Santos L.A., *Artif. Organs*, 2013; **37(11)**: 992-997. DOI 10.1111/aor.12236.
- [2] Kolmasa J., Kaflika A., Zimab A. and Slosarczykb A., *Ceram. Int.*, 2015; **41**: 5727-5733. DOI10.1016/j.ceramint.2014.12.159.
- [3] Camire C.L., Jegou Saint-Jean S., Hansen S., McCarthy I. and Lidgren L., *J. Appl. Biomater. Biomech.*, 2005; **3(2)**: 106-111.
- [4] Durucan C. and Brown P.W., *J. Mater. Sci.*, 2002; **37(5)**: 963-969.
- [5] Camire C.L., Nevsten P., Lidgren L. and McCarthy I., *J. Biomed. Mater. Res. B: Appl. Biomater.*, 2006; **79B**: 159-65. DOI 10.1002/jbm.b.30526.
- [6] Famery R., Richard N. and Boch P., *Ceram. Int.*, 1994; **20**: 327-336.
- [7] Carrodeguas R.G. and De Aza S., *Acta Biomater.*, 2011; **7**: 3536-3546. DOI 10.1016/j.actbio.2011.06.019.
- [8] Camire C.L., Gbureck U., Hirsiger W. and Bohner M., *Biomaterials*, 2005; **26**: 2787-2794.
- [9] Duncan J., MacDonald F.J., Hanna J.V., Shirotsaki Y., Hayakawa S., Osaka A., Skakle J.M.S. and Gibson I.R., *Mater. Sci. Eng. C*, 2014; **34**: 123-129. DOI 10.1016/j.msec.2013.08.038.
- [10] Thurmer M.B., Vieira R.S., Fernandes J.M., Coelho W.T.G. and Santos L.A., *Mater. Sci. Forum*, 2012; **727-728**: 1164-1169. DOI 10.4028/www.scientific.net/MSF.727-728.1164.
- [11] Srakaew N.L. and Rattanachan S.T., *J. Biomim. Biomater. Biomed. Eng.*, 2014; **21**: 3-16. DOI 10.4028/www.scientific.net/JBBB.21.3.
- [12] Thurmer M.B., Diehl C.E. and Santos L.A.L., *Ceram. Int.*, 2016; **42**: 18094-18099. DOI 10.1016/j.ceramint.2016.08.115.
- [13] Gbureck U., Barralet J.E., Radu L., Klinger H.G. and Thull R., *J. Am. Ceram. Soc.*, 2004; **87(6)**: 1126-1132.
- [14] Standard Test Method for Time of Setting of Hydraulic Cement Paste by Gilmore Needles," ASTM Designation C266-99. ASTM International, West Conshohocken, PA, 2002.
- [15] American Society for Testing and Materials, ASTM F451-95: Standard specification for acrylic bone cement.
- [16] Zhang J., Liu W., Schnitzler V., Tancret F. and Bouler J.M., *Acta Biomater.*, 2014; **10**: 1035-1049. DOI 10.1016/j.actbio.2013.11.001.
- [17] Montufar E.B., Maazouz Y. and Ginebra M.P., *Acta Biomater.*, 2013; **9**: 6188-6198.
- [18] Schumacher M., Henb A., Rohnke M. and Gelinsky M., *Acta Biomater.*, 2013; **9**: 7536-7544. DOI 10.1016/j.actbio.2013.03.014.
- [19] Ghosh R. and Sarkar R., *J. Mater. Sci. Eng. C*, 2016; **67**: 345-352. DOI 10.1016/j.msec.2016.05.029.