

Synthesis of Tricalcium Phosphate at Different pH Condition

M. F. Md Nor^a, M. R. Abdul Hanan^b, Z. N. Zamal^c, N. M. Daud^d, Z. Tapsir^e and S. Saidin^{*}

Faculty of Biosciences & Medical Engineering, Universiti Teknologi Malaysia, 81310 UTM
Johor Bahru, Johor, Malaysia.

^aam.ferdaus@utm.my, ^bredzuanhanan@gmail.com, ^czarithnadira@gmail.com, ^dnurizzatimohddaud@gmail.com, ^ezafirahtapsir1@gmail.com, ^{*}syafiqahsaidin@biomedical.utm.my

Abstract – Calcium element is crucial for bone health and bone remodeling process. Its compound is often used as a material for bone scaffold. One of the common calcium compounds utilized in the development of bone scaffold is tricalcium phosphate (TCP) due to its bioactive and high degradation property. In this study, TCP is synthesized from the precursors of calcium oxide (CaO) and diammonium hydrogen phosphate, (NH₄)₂HPO₄. The synthesization was set at three different pH condition – pH 4, pH 7 and pH 10. Both precursors were mixed at a stoichiometric Ca/P ratio of 1.5, stirred under heat, left to cool, filtered and dried in an oven to solidify. It was then crushed to form powders and subjected to SEM-EDX and wettability analyses. The morphology images showed that the TCP4 was formed as thin-plate-flakes whereby, the TCP7 and the TCP10 were formed as feathery-thick-blocks materials. As the pH increased, the Ca/P ratio and wettability were increased. These results indicated that the synthesization in an alkaline environment favored to the formation of TCP compared to the acidic environment. **Copyright © 2015 Penerbit Akademia Baru - All rights reserved.**

Keywords: Tricalcium Phosphate, pH, Bone Scaffold

1.0 INTRODUCTION

Tricalcium phosphate (TCP) or Ca₃(PO₄)₂, has been extensively used as a scaffold material for bone tissue engineering due to its main elements, calcium and phosphorus [1]. Wide studies of resorption rate show that TCP is beneficial for bone formation [2]. The scaffold materials ought to be ideally osteoinductive, osteoconductive and osseointegrative [3]. Osteoinduction is the ability to stimulate and recruit progenitor cells that will differentiate into pre-osteoblast cells, while osteoconduction is the ability of osteoblast cells to grow on the implant surface. Osseointegration, on the other hand, is the integration and direct anchor between bone and implant [3,4]. Tricalcium phosphate is being used to treat illnesses such as joint arthritis, tumors or injuries which may require operations to supplant or restore lost and defective bone [5].

Tricalcium phosphate is a resorbable material that can be arranged in different conditions [6]. It can be divided into several forms such as β-TCP, α-TCP and α'-TCP [1]. β-TCP has been perceived as a suitable bioactive material, implying that it will bolster bone ingrowth and osseointegration when utilized in the orthopedic, dental and maxillofacial applications [6,7]. Presently, the β-TCP is prepared by mechanical methods and chemical methods. Since, the products obtained by mechanical methods had deficiencies in bigger crystal size and more

impurities, numerous chemical methods are used to overcome those disadvantages [7]. The common chemical methods been used are electrospinning, gel casting, slip casting, fiber compaction, freeze casting, gas foaming and phase separation [3]. All these techniques require the preparation of liquid/gel solvent by a wet chemical method, which is usually involve double decomposition reactions.

There are several parameters need to be considered during the preparation of liquid/gel solvent such as solvent composition, temperature, pH, ageing time and drying time. Therefore, this study was intended to investigate the effect of different pH (acidic, neutral and alkaline) conditions on the formation of TCP powders for later use as a bone scaffold. The chemical composition, microstructure and wettability properties of the synthesized TCP powders were then investigated.

2.0 METHODOLOGY

2.1 Sample Preparation

Tricalcium phosphate (TCP) solvent was synthesized by titrating 0.3 M calcium oxide, (CaO) into 0.2 M diammonium phosphate, ((NH₄)₂HPO₄) at 40°C under stirring condition. The concentration of both solution were determined based on the stoichiometric of TCP to produce Ca/P ratio of 1.5. The pH was then adjusted to 4, 7 and 10, separately, by adding either ammonium (NH₄) or nitric acid (HNO₃). The TCP solvents were further stirred at 40°C for 4 hours. The homogenized TCP solvents were leaved at room temperature for 16 hours to allow complete reaction and precipitation. After that, the TCP precipitates were filtered using Smith filter paper and dried in an oven at 110°C for 5 hours. The dried TCP was crushed using mortar and pestle to form fine TCP powders and labeled as TCP4, TCP7 and TCP10 for the synthesization at pH 4, 7 and 10, respectively.

2.2 Morphology and Chemical Composition Analyses

Morphology and chemical composition of the TCP powders were examined by scanning electron microscopy (SEM, TM3000, HITACHI, Japan) equipped with energy dispersive X-ray spectroscopy (EDX, HITACHI swiftED300), respectively. The image of TCP powders was observed under magnification of 1,000× and 2,000×. The chemical composition of the TCP powders was analyzed as the average of three consecutive spots on each sample.

2.3 Wettability Analysis

The TCP powders were compressed with a pellet compressor to form a pellet. The pellets with a diameter of 1.4 cm and a thickness of 0.3 cm were subjected to wettability analysis using a video contact angle instrument (VCA, Optima, AST products, Inc., US). The angle of dropped 0.25 mL de-ionised (DI) water was captured within 10 seconds. The average data were determined by measuring the water drop angle at three difference spots.

3.0 RESULTS & DISCUSSION

The morphology and chemical composition of the TCP powders, synthesized at different pH were obtained by SEM-EDX instrument as shown Figure 1. The TCP4 formed like thin-plate-flakes with a more distinctive structure compared to the TCP7 and TCP10. Increasing the pH contributed to feathery-thick-blocks materials. The powders were highly scattered with a size,

approximately in between 3 and 35 μm . Previous study reported that the formation of thin-plate flakes imitated the octacalcium phosphate (OCP), another variation of calcium phosphate [8]. While the thick blocks represented various phases of calcium phosphate such as TCP, tetracalcium phosphate (TTCP), hydroxyapatite (HA), etc [9,10].

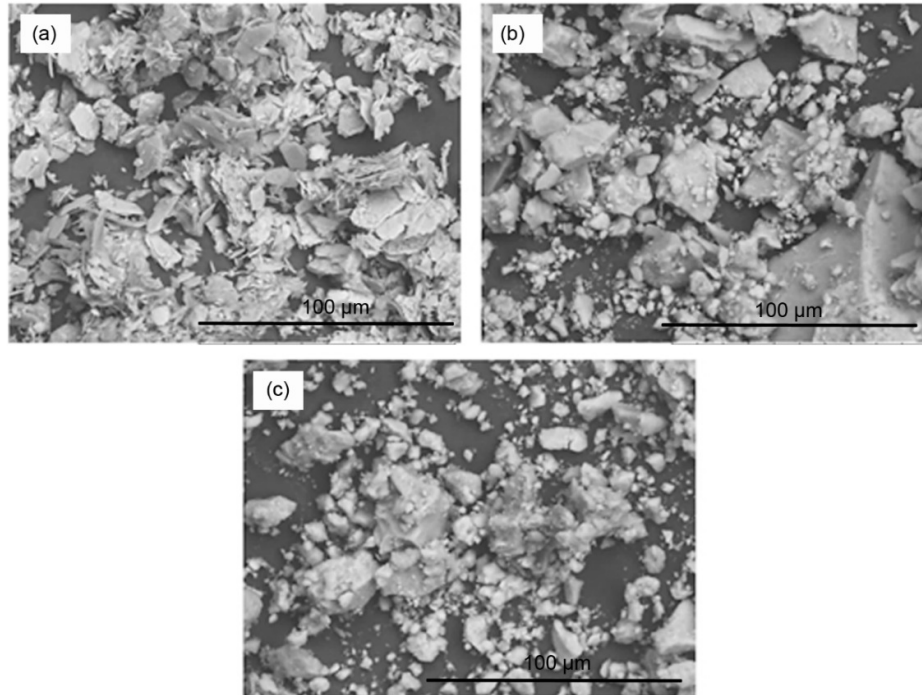
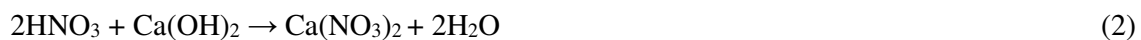


Figure 1: SEM images of synthesized (a) TCP4, (b) TCP7 and (c) TCP10

The Ca/P ratio in Table 1 further confirmed the phases of calcium phosphate. The results demonstrated that the initial Ca/P ratio, 1.50, set during the synthesis process has dropped significantly to 1.16 at pH 4. Interestingly, the ratio increased to 1.81 at pH 7 and subsequently decreased to 1.72 at pH 10.

Table 1: Ca/P ratio of TCP4, TCP7 and TCP10

Sample	Ca/P ratio (%)
TCP4	1.16±0.04
TCP7	1.81±0.25
TCP10	1.72±0.24



The reaction between HNO_3 and CaO at pH 4 has interrupted the synthesis of TCP due to the formation of calcium nitrate ($\text{Ca(NO}_3)_2$) based on equation (1) and (2). This reaction caused the reduction in calcium ions, thus producing low Ca/P ratio. The Ca/P ratio of 1.16, suggesting

the formation of OCP ($\text{Ca/P}_{\text{theoretical}} = 1.33$), confirming the morphology image in Figure 1(a). The high Ca/P ratio of TCP7 and TCP10 were obtained as there was no addition of HNO_3 during the synthesis process. Therefore, most calcium and phosphorus elements were reacted to each other to produce a variation of calcium phosphate. However, further analysis by X-ray diffractometer (XRD) should be conducted to identify each specific phase.

The contact angle measurement was used to investigate the surface wettability of TCP pellets. By referring to Figure 2, the contact angle on TCP4 states a degree of approximately $34.27 \pm 1.65^\circ$, higher than the average contact angle of TCP7 and TCP10 with $18.73 \pm 0.74^\circ$ and $13.70 \pm 0.99^\circ$, respectively. This testifies that the TCP10 was more hydrophilic compared to the TCP7 and the TCP4. The increment in pH condition led to greater wettability property which are substantial for assisting bone absorption and scaffold degradation after surgical restoration [11].

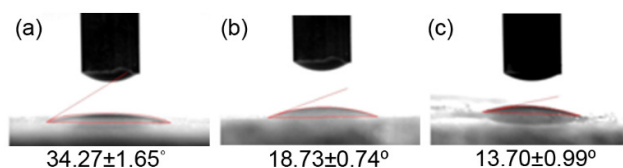


Figure 2: Water contact angle analyses on synthesized (a) TCP4, (b) TCP7 and (c) TCP10

4.0 CONCLUSION

The TCP powders synthesized in the alkaline environment were in a form of feathery-thick-blocks materials. It has closest Ca/P ratio with the pure TCP and possessed high wettability property. The utilization of TCP is essential in the development of bone scaffold as the compound is bioactive and has high degradation property for bone ingrowth.

REFERENCES

- [1] B. Susmita, M. Roy, A. Bandyopadhyay, Recent advances in bone tissue engineering scaffolds, *Trends in Biotechnology* 30(10) (2012) 546-554.
- [2] R. Filmon, N. Retailleau-Gaborit, G. Brossard, F. Grizon-Pascaretti, M.F. Baslé, D. Chappard, MicroCT and preparation of β -TCP granular material by polyurethane foam method, *Image Analysis & Stereology* 28(2) (2009) 32-33.
- [3] G. Gergely, F. Wéber, I. Lukács, A.L. Tóth, Z.E. Horváth, J. Mihály, C. Balázs, Preparation and characterization of hydroxyapatite from eggshell, *Ceramics International* 36(2) (2010) 803-806.
- [4] J. Mitra, G. Tripathi, A. Sharma, B. Basu, Scaffolds for bone tissue engineering: role of surface patterning on osteoblast response, *RSC Advances* 3(28) (2013) 11073-11094.
- [5] T. Albrektsson, C. Johansson, Osteoinduction, osteoconduction and osseointegration, *European Spine Journal* 10 (2001) S96-S101.

- [6] A.S. Brydone, D. Meek, S Maclaine, Bone grafting, orthopaedic biomaterials, and the clinical need for bone engineering, Proceedings of the Institution of Mechanical Engineers, Part H: Journal of Engineering in Medicine 224(12) (2010) 1329-1343.
- [7] Y. Zhang, G. Yin, S. Zhu, D. Zhou, Y. Wang, Y. Li, L. Luo, Preparation of β - $\text{Ca}_3(\text{PO}_4)_2$ bioceramic powder from calcium carbonate and phosphoric acid, Current Applied Physics 5(5) (2005) 531-534.
- [8] Z. Gou, X. Yang, X. Gao, X. Zhang, K. Ting, B.M. Wuc, C. Gao, Octacalcium phosphate microscopic superstructure self-assembly and evolution by dual-mediating combination, Royal Society of Chemistry 11(8) (2009) 1585-1590.
- [9] C. Rey, M. Freche, M. Heughebaert, J.C. Heughebaert, J.L. Lacout, A. Lebugle, J. Szilagy and M. Vignoles, Apetite chemistry in biomaterial preparation, shaping and biological behaviour, in: W. Bonfield, G.W. Hastings, K.E. Tanner. Bioceramics, Butterworth-Heinemann Ltd., London, 1991, pp. 57-64.
- [10] R.Z. LeGeros, J.P. LeGeros. Dense hydroxyapatite, in: L.L. Hench, J. Wilson. An Introduction to Bioceramics, World Scientific Publishing Co. Pte. Ltd., Singapore, 1993, pp. 139 – 181.
- [11] F. Barrère, C.A. van Blitterswijk, K. de Groot, Bone regeneration: molecular and cellular interactions with calcium phosphate ceramics, International Journal of Nanomedicine, 1(3) (2006) 317-332.