

RESEARCH ARTICLE

Characterization of β -tricalcium phosphate derived from green mussel shells (Molarity variation)

Hillary Aurene Santoso*, Eddy**✉, Manuel Romario Kesnatri*, Tansza Setiana Putri**, Jackson Dipankara***, Astri Rinanti Nugroho****, Bang Le Thi*****

*Faculty of Dentistry, Universitas Trisakti, Jakarta, Indonesia

**Department of Dental Materials, Faculty of Dentistry, Universitas Trisakti, Jakarta, Indonesia

***Department of Oral Maxillofacial Surgery, Faculty of Dentistry, Universitas Trisakti, Jakarta, Indonesia

****Department of Environmental Engineering, Faculty of Landscape Architecture and Environmental Technology, Universitas Trisakti, Jakarta, Indonesia

*****School of materials science and engineering, Hanoi University of Science and Technology, Hanoi, Vietnam

*Jl Kyai Tapa No 1, West Jakarta, Indonesia; ✉ correspondence: eddydrg@trisakti.ac.id

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ABSTRACT

β -Tricalcium phosphate (β -TCP) is a widely used bioceramic material. In dentistry, it is commonly used as bone graft material. β -TCP is osteoconductive, bioresorbable, bioactive, and has biocompatibility properties. This study aims to evaluate the optimum molarity of CaO and H₃PO₄ to synthesize β -TCP from a natural source (*Perma viridis linn*). This is laboratory experimental research conducted by reacting calcium compounds from green mussel shells and phosphoric acid using the dissolution precipitation method with variations in molarity ratio. X-ray diffraction (XRD), scanning electron microscope (SEM), and fourier transform infrared (FTIR) were used to identify the characteristics of β -TCP synthesized from green mussel shells. The XRD chart pattern showed the formation of peaks identical to the β -TCP (Sigma-Aldrich). However, formation of whitlockite phase was also seen in the results. FTIR results showed that phosphate, hydroxyl, and carbonyl groups were shown on the graph and could be identified as β -TCP. SEM characterization showed that the sample consisted of small particles irregularly shaped to form like aggregates. β -TCP synthesized using molarity ratio of 0.6M CaO: 0.4M H₃PO₄ had characteristics resembling β -TCP (Sigma-Aldrich).

Keywords: β -Tricalcium phosphate; dissolution precipitation; green mussel shell; whitlockite

INTRODUCTION

Bone is hard tissues that can regenerate due to bone loss and damage caused by trauma, infection, tumors, resection, and bone abnormalities.¹ Bone defects can be overcome by surgical procedures, or bone grafting.² According to the source of material, bone grafts are divided into four types: autograft, allograft, xenograft, and alloplastic graft.³ Alloplastic grafts are the only type of bone graft that comes from synthetic materials such as ceramics and polymers.⁴ β -tricalcium phosphate is a commonly used alloplastic graft material and has a biodegradable level that corresponds to the rate of bone growth. Meanwhile, because hydroxyapatite has high stability in the bones, it is difficult to be absorbed by the body and will remain in the body for a long time.⁵

β -TCP can be synthesized by reacting calcium and phosphate compounds. Natural materials such as clam shells can be used as a source of calcium and phosphoric acid, from which phosphate is derived.⁶ Based on statistical data from the Ministry of Marine Affairs and Fisheries, shellfish production in Indonesia reached 148,381.02 tons in 2020.⁷ One shellfish species with high economic value in Indonesia is green mussels. Green mussel shells contain calcium carbonate of 95-99%, so they can be used as a source of calcium. They also have a higher calcium content than other types of shells, such as kepa shells and abalone shells.^{8,9} There are several methods that can be used for calcium phosphate synthesis: dissolution precipitation, sol-gels, hydrothermal, and various emulsions.^{10,11}

The dissolution precipitation method is widely used today because this method is relatively cheaper, does not use organic solvents, and the process is easier and faster than other methods.¹²

In dentistry, bone graft material can be used for periodontal regeneration therapy, guided bone regeneration procedures before implant placement, and to fill bone defects caused by peri-implantitis.^{13,14} The use of bone graft material is becoming increasingly popular. However, the price of bone graft material in Indonesia is expensive and not affordable for the lower to the middle class. Therefore, it is necessary to utilize natural materials and efficient methods to synthesize bone graft material. This study aims to determine the characteristics of β -TCP synthesized from calcium sources of green mussel shells and phosphoric acid solutions with variations in molarity to produce optimal β -TCP.

MATERIALS AND METHODS

The samples for this study were green mussel shells as source of calcium and phosphoric acid solution, from which phosphate was derived, to fabricate β -TCP. Sample preparation began with cleaning the green mussel shells to remove dirt and mud. The samples were then left to dry at

room temperature for 24 hours and heated in an oven (UN55, Memmert, Büchenbach, Germany) for 5 hours at 110 °C. The shells were ground into powder and sieved to achieve 0.35-0.6 mm size. Furthermore, the powder was characterized using the XRD (Rigaku Minifex, Tokyo, Japan) to determine the formation of CaCO_3 . Sintering was performed using a furnace (Ney Vulcan D130, Merck Dentsply, Woodbridge, Ontario, Canada) at 1,000 °C for 5 hours with an increase in temperature of 10 °C /min to convert CaCO_3 to CaO.

CaO powder was reacted to phosphoric acid solution with four types of molarity ratios of CaO and H_3PO_3 (0.6 M and 0.4 M; 1.2 M and 0.8 M; 1.8 M and 1.2 M; 2.4 M and 1.6 M). Ca/P ratio of 1.50 was required to form β -TCP (Table 1). However, before the CaO was immersed into the H_3PO_4 solution, the phosphoric acid (85% w/w) solution was diluted by adding aquadest to the solution to obtain the appropriate molarity (Table 2). Dissolution precipitation method was carried out using a magnetic stirrer at 300 rpm at a temperature of 80 °C for 1 hour. The precipitate was then filtered and dried at 37 °C for 24 hours. Furthermore, the samples were sintered at 1,000 °C for 3 hours with an increase in temperature

Table 1. The composition of calcium and H_3PO_4 solution

Comparison of CaO : H_3PO_4	Massa CaO (gr)	Volume H_3PO_4 (ml)
0.6 M : 0.4 M	3.36	100
1.2 M : 0.8 M	6.72	100
1.8 M : 1.2 M	10.08	100
2.4 M : 1.6 M	13.44	100

Table 2. pH of H_3PO_4 solution after been diluted

H_3PO_4		Volume of aquades (ml)	pH
Molarity (M)	Volume (ml)		
0.4 M	2.7	97.3	1.4
0.8 M	5.4	94.6	1.1
1.2 M	8.1	91.9	1.0
1.6 M	10.8	89.2	0.7

of 5 °C/min. The β -TCP samples were subjected to characterization tests using XRD, SEM, and FTIR and compared with β -TCP (Sigma-Aldrich) as standard. Ethical approval was not applicable for this article since the experiment did not involve humans or animals. This experiment used the waste of green mussels.

RESULTS

Figure 1 shows that green mussel shell powder contains calcium carbonate (CaCO_3). Following sintering, CaO powder was produced from green mussel shell powder, as shown in Figure 2.

Samples produced after sintering were subjected to XRD characterization tests and compared with the characteristics of β -TCP (Sigma-Aldrich) powder. The graph of the results of the XRD characterization of β -TCP can be seen in Figure 3 and Table 3.

The FTIR test was performed to identify the chemical functional groups of the samples. Characterization with FTIR (Prestige 21, Shimadzu, Japan) was performed at a frequency range of 400-4000 cm^{-1} to see the molecular bonds of β -TCP. The spectra of the FTIR characterization results can be seen in Figure 4.

Table 3. The peaks of the XRD results

Peak	2θ (degrees)				
	β -TCP <i>Sigma Aldrich</i>	β -TCP 0.6 M : 0.4 M	β -TCP 1.2 M : 0.8 M	β -TCP 1.8 M : 1.2 M	β -TCP 2.4 M : 1.6 M
1	27.79	28.15	28.18	27.98	27.89
2	31.03	31.39	31.39	31.19	31.14
3	34.37	34.69	34.71	34.56	34.48

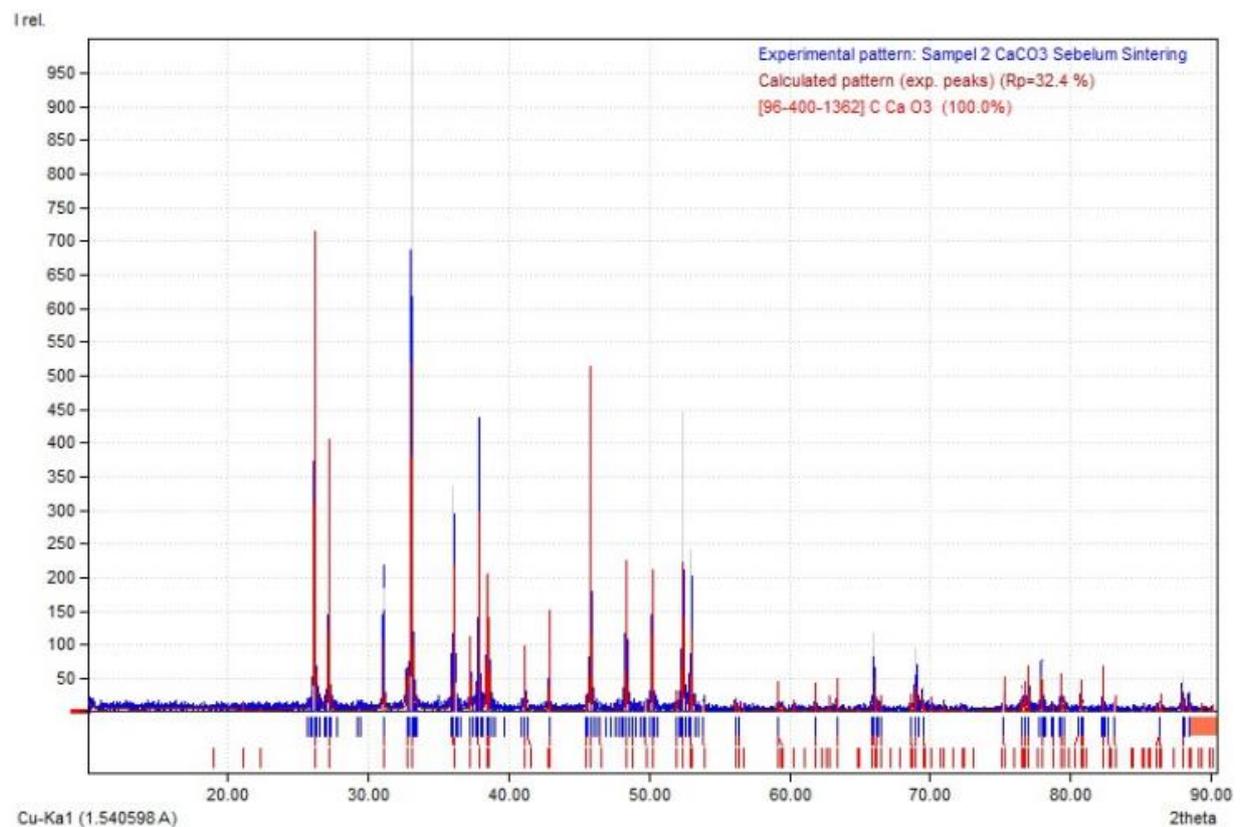


Figure 1. XRD graph of green mussel shell powder

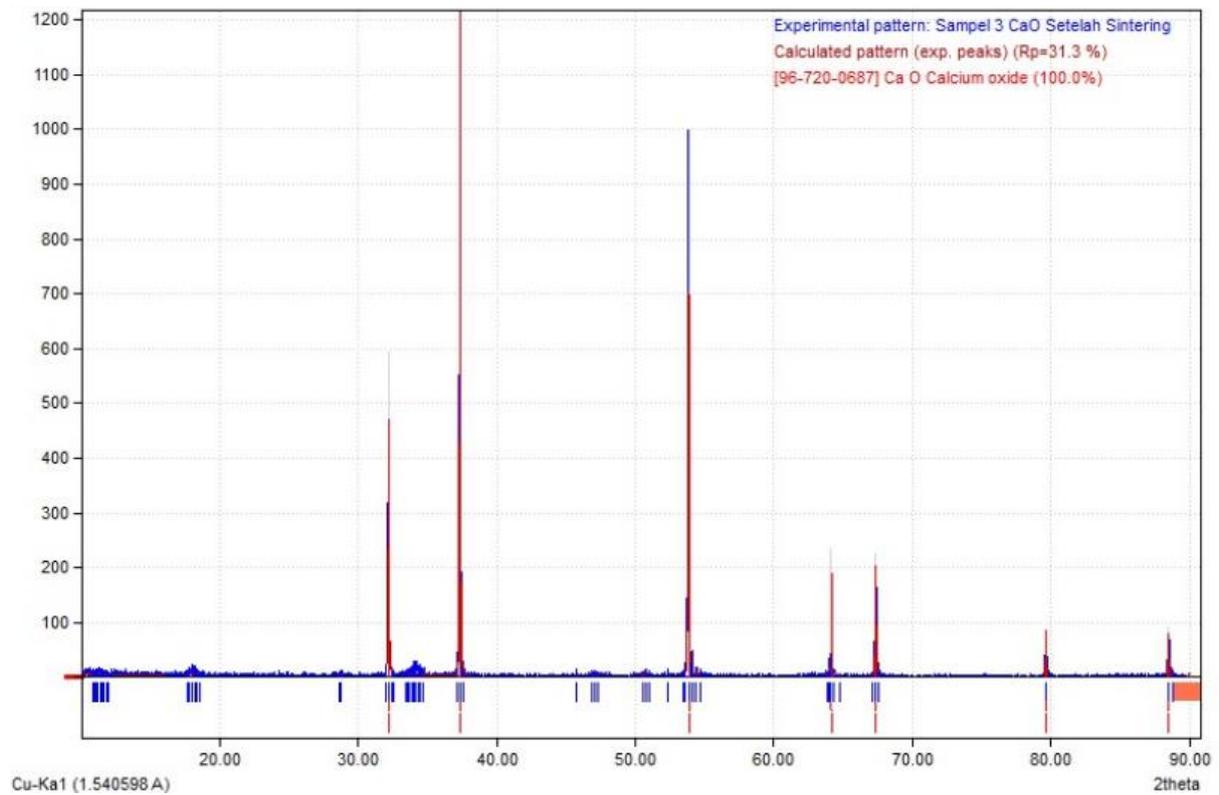


Figure 2. XRD graph of CaO powder from green mussel shells

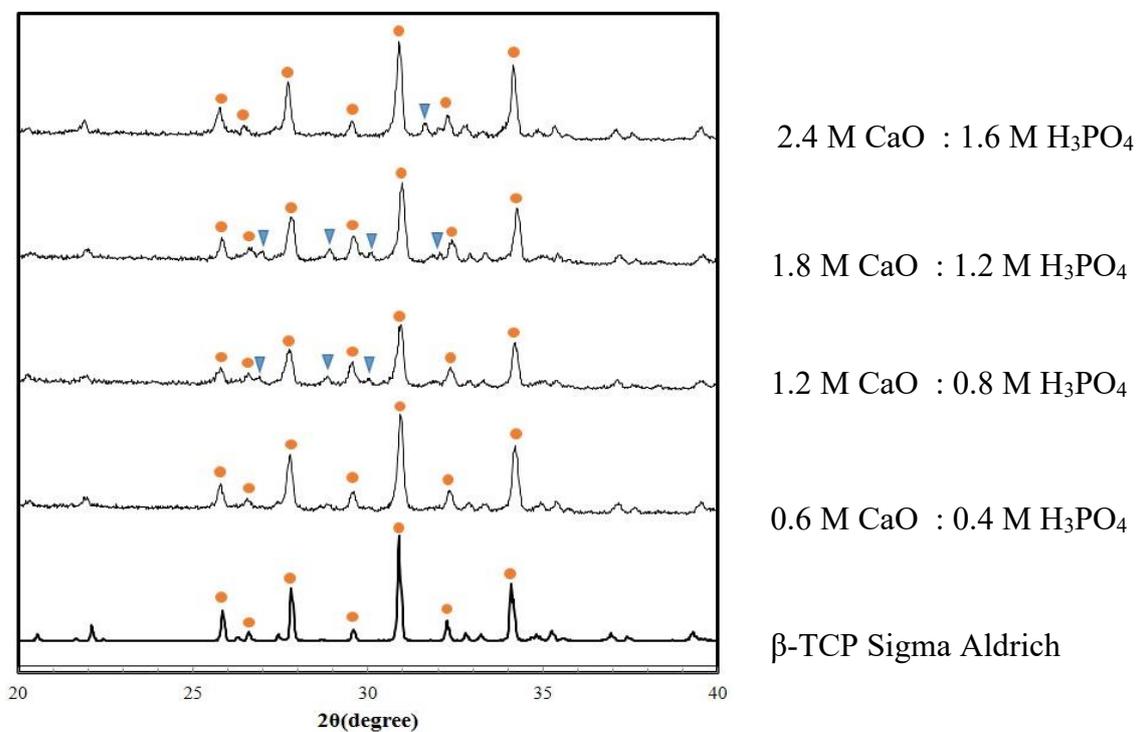


Figure 3. XRD characterization graph of β -TCP samples synthesized from green mussel shells with molarity ratio of CaO and H_3PO_4 of 0.6 M and 0.4 M; 1.2 M and 0.8 M; 1.8 M and 1.2 M; 2.4 M and 1.6 M. Sigma Aldrich β -TCP powder. The sign (●) indicates the formation of a β -TCP diffraction pattern, while the sign (▼) indicates the formation of a whitlockite diffraction pattern

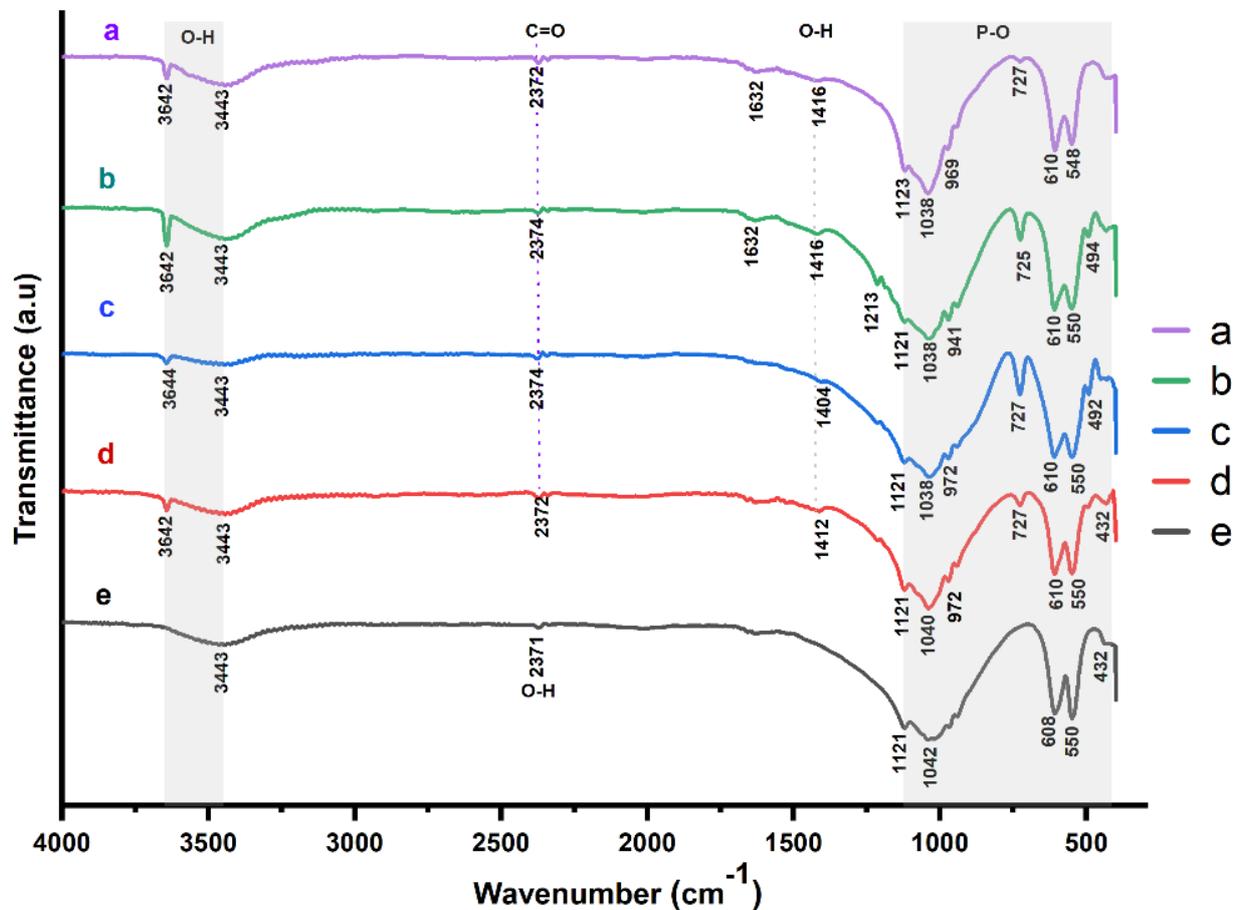


Figure 4. Graph of β -TCP FTIR spectrum analysis results TCP (a) 2.4 M CaO : 1.6 M H_3PO_4 ; (b) 1.8 M CaO : 1.2 M H_3PO_4 ; (c) 1.2 M CaO : 0.8 M H_3PO_4 ; (d) 0.6 M CaO : 0.4 M H_3PO_4 ; (e) β -TCP Sigma Aldrich

Table 4. The range of particle sizes and micropores formed in the samples

Sample	Range of particle sizes (μm)	Range of micropores (μm)
β -TCP Sigma Aldrich	1.0 – 5.7	0.4 – 10.8
0.6 M CaO : 0.4 M H_3PO_4	0.8 – 5.6	0.2 – 10.0
1.2 M CaO : 0.8 M H_3PO_4	0.6 – 4.2	0.4 – 7.1
1.8 M CaO : 1.2 M H_3PO_4	0.4 – 5.4	0.3 – 4.1
2.4 M CaO : 1.6 M H_3PO_4	0.2 – 2.7	0.2 – 3.1

SEM characterization was carried out to determine the morphological shape and size of the pores that could give β -TCP osteoinductive properties. Figure 5-7 and Table 4 show the result of SEM characterization with a magnification of 1.000x, 3.000x, and 5.000x.

DISCUSSION

Green mussel shell powder contains calcium carbonate ($CaCO_3$), which can be used as a source of calcium to synthesize β -TCP. Liemawan's research revealed that green mussel shells have $CaCO_3$ content of 95.7%.¹⁵ $CaCO_3$ has 3 phases: (a) calcite, a stable phase at room temperature and can form at low temperatures, (b) aragonite, a metastable phase and can form at high temperatures, (c) vaterite, a metastable phase at room temperature and can form at high saturation levels.^{16,17} Ismail's research found that green mussel shell powder crystals are rich in $CaCO_3$ as an aragonite phase.¹⁸

Green mussel shell powder was sintered at 1,000 °C for 5 hours to remove organic components and convert $CaCO_3$ into CaO by releasing carbon dioxide (CO_2).^{19,20} $CaCO_3$ is a stable phase with low solubility, so the reaction

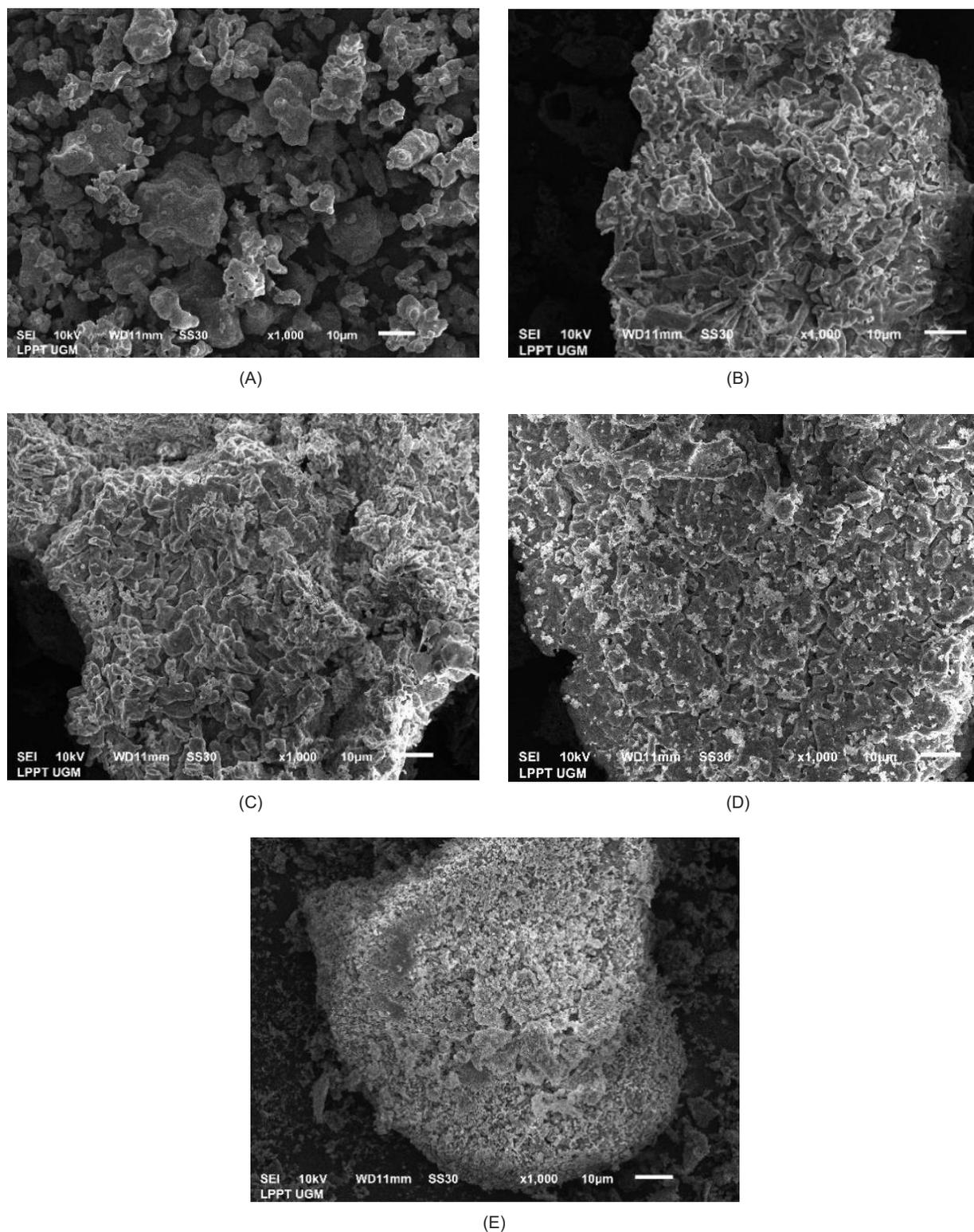
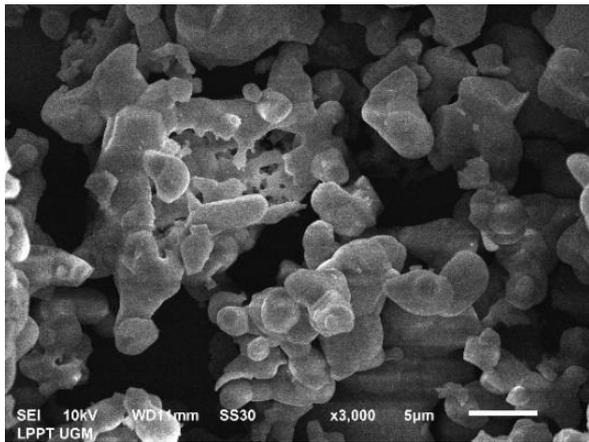
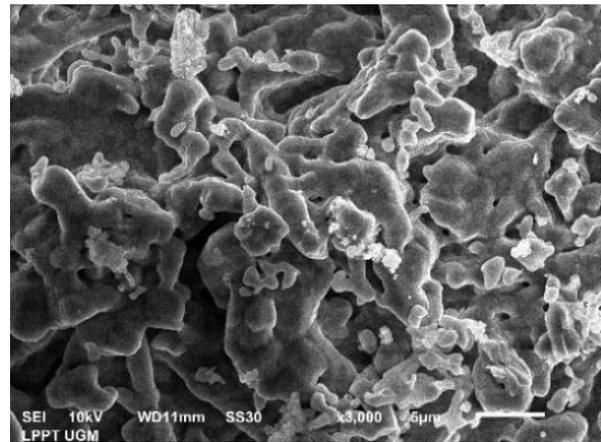


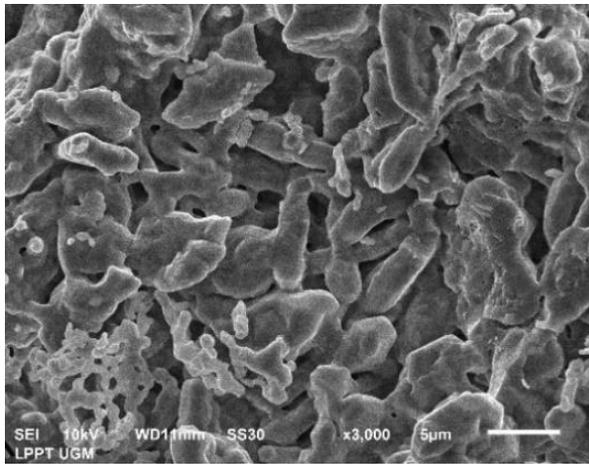
Figure 5. SEM characterization test results of sample β -TCP with 1.000x magnification. (A) β -TCP sigma aldrich; (B) 0.6 M CaO : 0.4 M H₃PO₄; (C) 1.2 M CaO : 0.8 M H₃PO₄; (D) 1.8 M CaO : 1.2 M H₃PO₄; (E) 2.4 M CaO : 1.6 M H₃PO₄



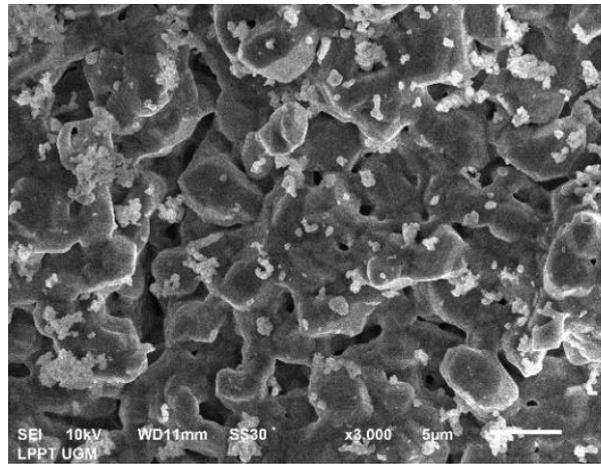
(A)



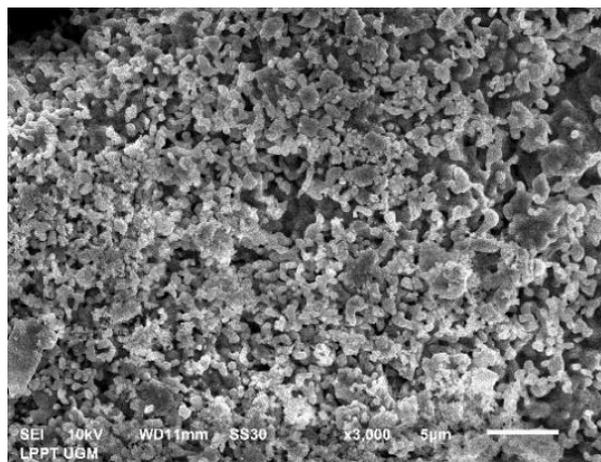
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(C)

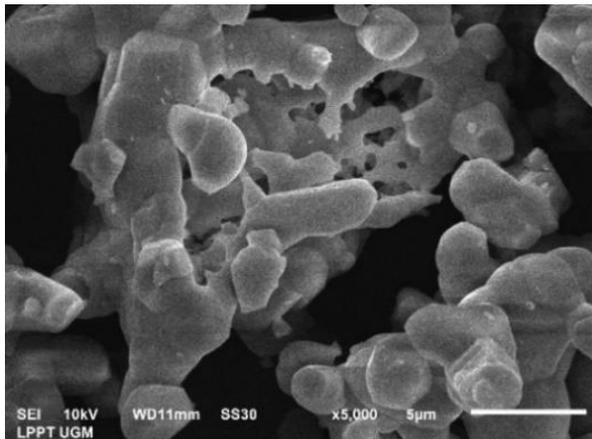


(D)

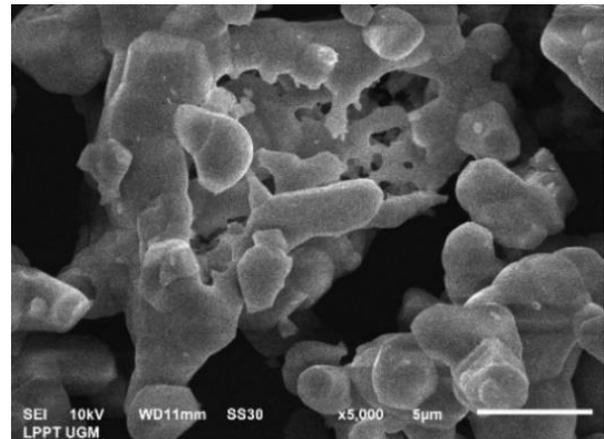


(E)

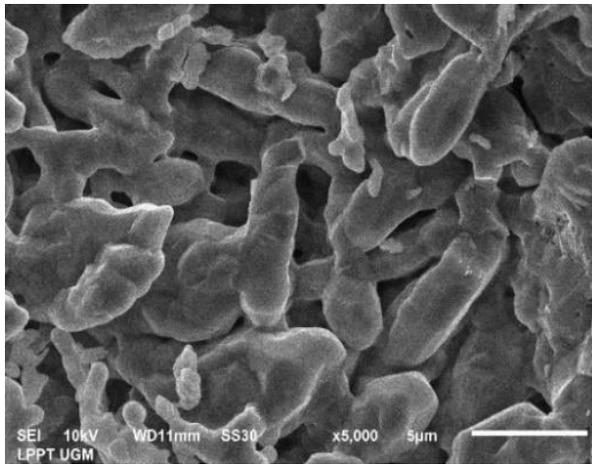
Figure 6. SEM characterization test results of sample β -TCP with 3.000x magnification. (A) β -TCP sigma aldrich; (B) 0.6 M CaO : 0.4 M H_3PO_4 ; (C) 1.2 M CaO : 0.8 M H_3PO_4 ; (D) 1.8 M CaO : 1.2 M H_3PO_4 ; (E) 2.4 M CaO : 1.6 M H_3PO_4



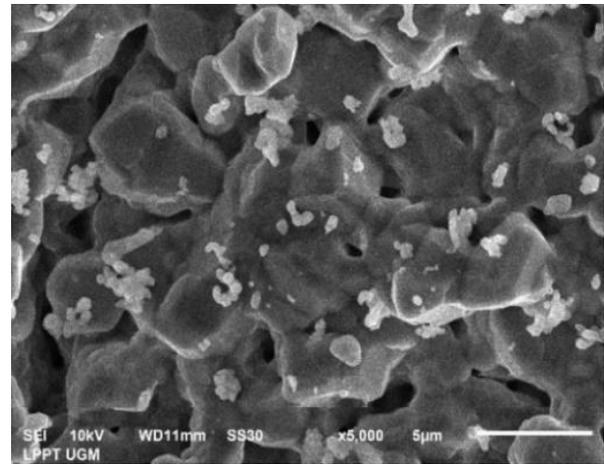
(A)



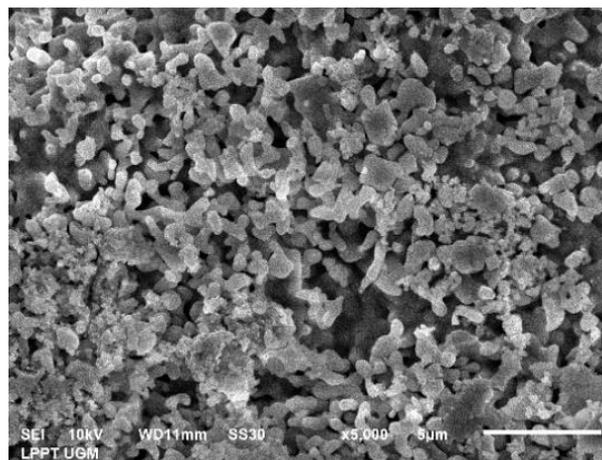
(B)



(C)



(D)



(E)

Figure 7. SEM characterization test results of sample β -TCP with 5.000x magnification. (A) β -TCP sigma aldrich; (B) 0.6 M CaO : 0.4 M H_3PO_4 ; (C) 1.2 M CaO : 0.8 M H_3PO_4 ; (D) 1.8 M CaO : 1.2 M H_3PO_4 ; (E) 2.4 M CaO : 1.6 M H_3PO_4

between calcium and phosphate compounds are more complex and longer.²¹ Therefore, in our study CaCO_3 was converted to CaO to accelerate the dissolution precipitation reaction. Green mussel shell powder that had been sintered produced a slightly greenish-white powder.

Synthesis of β -TCP was carried out using the dissolution precipitation method, forming a precipitate in a solution due to a chemical reaction. This precipitate is formed when the concentration of dissolved ions has reached the solubility limit and forms a salt compound.²² The material for establishing β -TCP is CaO synthesized from green mussel shells and phosphoric acid solution, which has a Ca/P ratio used of 1.50.^{23,24}

This precipitate produced white and fine powder. The white color of the sample indicated calcium content in the compound. Sintering process was then performed with a temperature of 1,000 °C to fuse two different substances, remove CO_2 elements, and determine the mechanical properties and porosity of the resulting sample.^{25,26} The success of β -TCP synthesis from green mussel shells was confirmed by conducting characterization tests using XRD, FTIR and SEM.

The results of the XRD analysis showed that the composition of β -TCP synthesized from green mussel shells was qualitatively similar to β -TCP (Sigma-Aldrich). The β -TCP graph pattern with a molarity ratio of 0.6 M CaO : 0.4 M H_3PO_4 was the most similar to β -TCP (Sigma-Aldrich). The results of the XRD analysis found that whitlockite phase ($\text{Ca}_{18}\text{Mg}_2(\text{HPO}_4)_2(\text{PO}_4)_{12}$), a type of calcium phosphate material, was also formed. Whitlockite had the same XRD pattern and Ca/P molarity ratio close to β -TCP, which was 1.43. The different Ca/P ratio and solubility could release different free Ca^{2+} , Mg^{2+} , and P^+ ions. Whitlockite continuously released Mg^{2+} and P^+ ions at higher levels than β -TCP under physiologically relevant conditions. In the research by Jang et al., the synthesis of whitlockite can be carried out by the dissolution precipitation method, namely by reacting the compounds $\text{Ca}(\text{OH})_2$, $\text{Mg}(\text{OH})_2$, and H_3PO_4 at 70 °C under acidic conditions.²⁷⁻²⁹ Based on the XRD characterization results, this whitlockite has a

chemical structure of $\text{Ca}_9(\text{Fe}_{0.63}\text{Mg}_{0.37})\text{H}_{0.37}(\text{PO}_4)_{17}$. Magnesium (Mg^{2+}) and iron (Fe^{2+}) ions may come from green mussel shells. According to Ismail's research, light elements produced from oxidation processes, such as oxygen and carbon, could possibly bind carbonates. In this case, various additional Ca-, Mg-, Mn-, and Fe-rich carbonate minerals can be found in green mussel shells.¹⁸ Research by Su Yeon Kwon showed that whitlockite could be used as a bone graft material due to its conductivity and biocompatibility properties comparable to hydroxyapatite and β -TCP,^{27,30} considering whitlockite has magnesium ions which can act as a cofactor for many enzymes and stimulate new bone growth, adhesion, and proliferation of osteoblastic cells to increase biocompatibility and bone formation.^{28,31}

In the FTIR analysis spectra, the phosphate groups' sharp peaks indicate a better crystallinity growth to improve the quality of β -TCP. The phosphate group has four vibrational modes consisting of stretching symmetry (ν_1), bending symmetry (ν_2), stretching asymmetry (ν_3), and bending asymmetry (ν_4).^{26,32} β -TCP (Sigma-Aldrich) and β -TCP synthesized from green mussel shells have three vibration modes: bending symmetry, stretching asymmetry, and bending asymmetry. In the FTIR analysis results, hydroxyl groups (OH^-) indicate that the sample still contains H_2O (water).³³

In addition, the results of the FTIR characterization of the study sample found peaks at wave numbers 727 cm^{-1} dan 725 cm^{-1} , which were not present in β -TCP (Sigma-Aldrich) sample. A study by Ruiz-Aguilar et al. reported that this group is the residual amount of P_2O_7 resulting from an incomplete transformation process from TCP to β -TCP phase. Several studies have found that differences in Ca/P ratio lead to the formation of β -calcium pyrophosphate ($\beta\text{-Ca}_2\text{P}_2\text{O}_7$) as the residual phase in the TCP synthesis process.³⁴ $\beta\text{-Ca}_2\text{P}_2\text{O}_7$ is a calcium phosphate material that has the potential as a bone graft material with a Ca/P ratio 1.00. $\beta\text{-Ca}_2\text{P}_2\text{O}_7$ has biocompatible, biodegradable properties and can be absorbed biologically.³⁵ This indicates that $\beta\text{-Ca}_2\text{P}_2\text{O}_7$ is not

toxic to the human body and has almost similar properties to β -TCP.

The results of SEM characterization showed that the β -TCP sample consisted of small irregularly shaped particles, and some of them were bound together like aggregates. Particle size and crystallinity are essential factors in making biomaterials because the particle size affects the properties of calcium phosphate, such as mechanical strength, degradation rate, protein adsorption, and biocompatibility.^{36,37} The result of SEM characterization (Table 4) suggests that the greater the concentration value of CaO and H_3PO_4 in the sample, the smaller the particle size. The SEM images also showed the presence of micropores with varying shapes and size ranges. Bohner's research found that micropores positively affect the biological response of β -TCP to increase resorption and osteoinduction. In addition, the formation of pores in β -TCP can also help the circulation and exchange of body fluids, nutrient supply, osteoblast penetration, ion diffusion, and vascularization.²⁵

CONCLUSION

This study has shown that β -tricalcium phosphate could be synthesized by reacting calcium from green mussel shells with a phosphoric acid solution. Based on the XRD, FTIR, and SEM results, samples with a molarity ratio of 0.6 M CaO : 0.4 M H_3PO_4 had the characteristics that were more similar to β -TCP (Sigma-Aldrich) compared to other molarity comparisons.

CONFLICT OF INTEREST

The authors declare no conflict of interest with the data contained in the manuscript.

REFERENCES

1. Ansari M. Bone tissue regeneration: biology, strategies and interface studies. *Prog Biomater*. 2019; 8: 223-237. doi: 10.1007/s40204-019-00125-z.
2. Wang W, Yeung KWK. Bone grafts and biomaterials substitutes for bone defect repair: A review. *Bioact Mater*. 2017; 2(4): 224-247. doi: 10.1016/j.bioactmat.2017.05.007
3. Kumar P, Vinitha B, Fathima G. Bone grafts in dentistry. *J Pharm Bioallied Sci*. 2013; 5(1): 125–127. doi: 10.4103/0975-7406.113312
4. Eddy. Kalsium sulfat sebagai bone graft. *JKGT*. 2021; 3(2): 4–5. doi: 10.25105/jkgt.v3i2.12612
5. Hillary A.S, Eddy. Potensi penggunaan β -tricalcium phosphate sebagai bahan substiusi tulang *JKGT*. 2023; 5(1): 54-57. doi :10.25105/jkgt.v5i1.16759
6. Suprianto K, Nilam C D. Hidroksiapatit dari cangkang telur sebagai bone graft yang potensial dalam terapi periodontal. *Clin Dent J*. 2019; 5(3): 76–87. doi: 10.22146/mkgk.65729
7. Kementerian Kelautan dan Perikanan (KKP) RI. Data Produksi Kelautan dan Perikanan Indonesia (Statistik Sektor). statistik.kkp.go.id. 2020.
8. Permatasari HA, Sari M, Aminatun, Suciati T, Dahlan K, Yusuf Y. Nano-carbonated hydroxyapatite precipitation from abalone shell (*Haliotis asinina*) waste as the bioceramics candidate for bone tissue engineering. *Nanomater Nanotechnol*. 2021; 11: 1–9. doi: 10.1177/18479804211032851
9. Ningsih RP, Wahyuni N, Destiarti L. Sintesis hidroksiapatit dari cangkang kerang kepah (*Polymesoda erosa*) dengan variasi waktu pengadukan. *J Kim Khatulistiwa*. 2014; 3(1): 22–26.
10. Cahyaningrum SE, Herdyastuty N, Devina B, Supangat D. Synthesis and characterization of hydroxyapatite powder by wet precipitation method. *IOP Conf Ser Mater Sci Eng*. 2018; 299(1): 12039–12043. doi: 10.1088/1757-899X/299/1/012039
11. Fadli A, Reni Yenti S, Zultiniar Z, Fifiyana R. Isotherm study on the adsorption of cadmium (II) onto hydroxyapatite from sea shells synthesized by low temperature hydrothermal method. In: *Proceeding of the First International Conference on Technology, Innovation and Society*.

- ITP Press; 2016. 45–52. doi: 10.21063/ICTIS.2016.1008
12. Pangestu TO, Damayanti SF, Santi SS, Muljani S. Sintesis dan karakterisasi kalsium fosfat dari cangkang bekicot dengan metode presipitasi. *CHEESA Chem Eng Res Artic.* 2021; 4(2): 82–90. doi: 10.25273/cheesa.v4i2.8931.82-90
 13. Fukuba S, Okada M, Nohara K, Iwata T. Alloplastic bone substitutes for periodontal and bone regeneration in dentistry: Current status and prospects. *Materials (Basel).* 2021; 14(5): 1–28. doi: 10.3390/ma14051096
 14. Angelis NDe, Benedicenti S, Zekiy A, Amaroli A. Current trends in bone augmentation techniques and dental implantology: an editorial overview. *J Clin Med.* 2022; 11: 1–2. doi: 10.3390/jcm11154348
 15. Liemawan AE, Tavio, Raka IGP. Pemanfaatan limbah kerang hijau sebagai bahan campuran kadar optimum agregat halus pada beton mix design dengan metode substitusi. *J Tek ITS.* 2015; 4(1): F128–133. doi: 10.12962/j23373539.v4i1.8927
 16. Wardhani S, Miralda S, Darjito, Danar P. Pengaruh temperatur sintesis precipitated calcium carbonate (PCC) dengan modifier terhadap ukuran dan jenis kristal. *J Integr Proses.* 2021; 10(1): 1–6.
 17. Gavryushkin PN, Belonoshko AB, Sagatov N, Sagatova D, Zhitova E, Krzhizhanovskaya MG, et al. Metastable structures of CaCO_3 and their role in transformation of calcite to aragonite and postaragonite. *Cryst Growth Des.* 2021; 21(1): 65–74. doi: 10.1021/acs.cgd.0c00589
 18. Ismail R, Fitriyana DF, Santosa YI, Nugroho S, Hakim AJ, Al Mulqi MS, et al. The potential use of green mussel (*Perna Viridis*) shells for synthetic calcium carbonate polymorphs in biomaterials. *J Cryst Growth.* 2021; 572(126282): 1–8. doi: 10.1016/j.jcrysgro.2021.126282
 19. Fadhilah R, Kurniawan RA, Icha MM. Synthesis of hydroxyapatit from ale-ale (*Meretrix Spp*) shell as bone graft material. *Majalah Ilmiah Al Ribaath, Universitas Muhammadiyah Pontianak.* 2015; 12(1): 44–60. doi: 10.29406/br.v12i1.79
 20. Afriani F, Mustari, Tiandho Y. Pengaruh lama pemanasan terhadap karakteristik kristal kalsium dari limbah cangkang kerang. *J EduMatSains.* 2018; 2(2): 189–200. doi: 10.33541/edumatsains.v2i2.606
 21. Hariyanto A, Sari VK, Pujiastuti C. Kinetika reaksi pembentukan kalsium fosfat dari asam fosfat dan cangkang kerang darah. *ChemPro.* 2020; 1(02): 32–38. doi: 10.33005/chempro.v1i2.48
 22. Wahyusi KN, et al. Precipitation method in calcium phosphat synthesis from blood clamshells (*Anadara Granosa*). *J. Phys.: Conf. Ser.* 2021; 1-5. doi: 10.1088/1742-6596/1899/1/012057
 23. Tavares D dos S, Castro LDO, Soares GD de A, Alves GG, Granjeiro JM. Synthesis and cytotoxicity evaluation of granular magnesium substituted β -tricalcium phosphate. *J Appl Oral Sci.* 2013; 21(1): 37–42. doi: 10.1590/1678-7757201302138
 24. Wulandari AD, Yulkifli. Studi awal rancang bangun colorimeter sebagai pendeteksi pada pewarna makanan menggunakan sensor photodiode. *Pillar Phys.* 2018; 11(2): 81–87.
 25. Bohner M, Santoni BLG, Döbelin N. β -tricalcium phosphate for bone substitution: Synthesis and properties. *Acta Biomater.* 2020; 113: 23–41. doi: 10.1016/j.actbio.2020.06.022
 26. Rahayu S, Kurniawidi DW, Gani A. Pemanfaatan limbah cangkang kerang mutiara (*Pinctada Maxima*) sebagai sumber hidroksiapatit. *J Pendidik Fis dan Teknol.* 2018; 4(2): 226–231. doi: 10.29303/jpft.v4i2.839
 27. Jang HL, Lee HK, Jin K, Ahn HY, Lee HE, Nam KT. Phase transformation from hydroxyapatite to the secondary bone mineral, whitlockite. *J Mater Chem B.* 2015; 3(7): 1342–1349. doi: 10.1039/C4TB01793E
 28. Guo X, Liu X, Gao H, Shi X, Zhao N, Wang Y. Hydrothermal growth of whitlockite coating on β -tricalcium phosphate surfaces for

- enhancing bone repair potential. *J Mater Sci Technol.* 2018; 34(6): 1054–1059.
doi: 10.1016/j.jmst.2017.07.009
29. Qi C, Zhu YJ, Chen F, Wu J. Porous microspheres of magnesium whitlockite and amorphous calcium magnesium phosphate: microwave-assisted rapid synthesis using creatine phosphate, and application in drug delivery. *J Mater Chem B.* 2015; 3(39): 7775–7786. doi: 10.1039/C5TB01106J
 30. Kwon SY, Shim JH, Kim YH, Lim CS, An SB, Han I. Efficacy for whitlockite for augmenting spinal fusion. *Int J Mol Sci.* 2021; 22(12875): 1–14. doi: 10.3390/ijms222312875
 31. Zhou D, Qi C, Chen YX, Zhu YJ, Sun TW, Chen F, et al. Comparative study of porous hydroxyapatite/chitosan and whitlockite/chitosan scaffolds for bone regeneration in calvarial defects. *Int J Nanomedicine.* 2017; 12: 2673–2687. doi: 10.2147/IJN.S131251
 32. Avifah LN. Optimasi pembuatan β -Tricalcium phosphate berbasis cangkang telur ayam ras dengan variasi molaritas. Institut Pertanian Bogor; 2014.
 33. Hardyanti. Sintesis dan karakterisasi β -tricalcium phosphate dari cangkang telur ayam dengan variasi suhu sintering. *J Biofisika.* 2013; 8(2): 42–48.
 34. Ruiz-Aguilar C, Olivares-Pinto U, Aguilar-Reyes EA, López-Juárez R, Alfonso I. Characterization of β -tricalcium phosphate powders synthesized by sol-gel and mechanosynthesis. *Bol la Soc Esp Ceram y Vidr.* 2018; 57(5): 213–220.
doi: 10.1016/j.bsecv.2018.04.004
 35. Akbar F. Sintesis $\text{Ca}_2\text{P}_2\text{O}_7$ dari limbah kerang sebagai bahan baku limbah cangkang kerang dengan metode solvothermal. *J Fis dan Apl.* 2019; 15(3): 110.
doi: 10.12962/j24604682.v15i3.4707
 36. Buasri A, Chaiyut N, Loryuenyong V, Worawanitchaphong P, Trongyong S. Calcium oxide derived from waste shells of mussel, cockle, and scallop as the heterogeneous catalyst for biodiesel production. *Sci World J.* 2013; 1–7. doi: 10.1155/2013/460923
 37. Grigoraviciute-Puroniene I, Tsuru K, Garskaite E, Stankeviciute Z, Beganskiene A, Ishikawa K, et al. A novel wet polymeric precipitation synthesis method for monophasic β -TCP. *Adv Powder Technol.* 2017; 28(9): 2325–2331.
doi: 10.1016/j.apt.2017.06.014