

Porous-structure engineering of hydroxyapatite-based scaffold synthesized from *Pomacea canaliculata* shell by using polyethylene oxide as polymeric porogen

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Abstract. Polymeric porogen leaching method was used to fabricate hydroxyapatite (HAp)-based scaffolds. The starting material was prepared from golden apple snail (*Pomacea canaliculata*) shells. The synthesized material exhibit nanostructured HAp/ β -TCP composite. Pore engineering is carried out by removing the polyethylene-oxide (PEO) as porogen from the mixture in sintering process. By varying the concentration of weight of PEO in the mixture, different porosity of scaffolds were obtained for each treatment. The weight concentration of PEO plays an important influence on the morphological porous structure of the HAp-based scaffolds. The FTIR spectra of HAp-based scaffolds did not exhibit PEO functional groups which means that the porogen was completely leached out from scaffold. The thermal analysis of porogen during heating confirms that PEO has leached in sintering process. The mechanical strength of the scaffold decreased as the pore size increased.

1. Introduction

Bioceramic materials such as calcium phosphate become a new alternative material in orthopedic applications, due to their advantages over commonly used metal materials. Calcium phosphate has excellent material properties in terms of biocompatibility as well as quality of integration with bone [1]. Bone tissue engineering has been widely developed to overcome the problem of bone defects by restoring and maintaining the natural function of bone tissues. One type of artificial bone tissue engineering developed today is the fabrication of bone scaffold both polymer-based and ceramic-based scaffold.

Ceramics hydroxyapatite (HAp) is a part of the apatite mineral compound having a $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ chemical formula with a calcium-phosphate (Ca/P) molar ratio of 1.67. Hydroxyapatite itself has biocompatibility properties similar to human bone tissue that compatible with bone and can improve bone regeneration process. Natural materials with high amounts of calcium carbonate are highly potential to be utilized as calcium source of synthetic HAp. The golden apple snail (*Pomacea canaliculata*) shell has an aragonite phase composition, which is the phase generally present in calcium carbonate (CaCO_3) [2].

Porosity is an important property that needs to be taken into account in the fabrication of bone scaffolds to allow growth and vascularization of bone or in other words enhance the properties of the bioactivity of the scaffold. By conditioning the composition and pore structure, we can make it possible to obtain a scaffold that acts as an osteoconductor in order to increase the scaffold's ability to support



bone regeneration [3]. Porosity will enhance the osteoconductivity of biomaterials, the properties that allow attachment, migration, proliferation, and phenotypic expression of bone cells that lead to new bone formation of biomaterials [4].

Bone biocompatibility is related to the material's ability to support bone growth by paying attention to the body's acceptance response to implant materials and toxic properties of implantable materials in the body. To be used as a site for the formation of new bone, the implant material must function as a media of stem cells and osteoblasts to adhere to and develop well [5]. The bone implant material should have good osteointegration with bone tissue. The biocompatibility of a material can be evaluated on the physical function of the material, including the biological response that may occur.

The porogen leaching method is a common method used to fabricate scaffolds with controlled porosity. The main concept of this method is to disperse porogens such as salts and waxes in both solvent and powder form through the process of porogen evaporation of the scaffold material [6]. Through this method, pores and interconnectivity of pores can be produced in the scaffold. The structure of pore geometry depends on the type of porogenic agent used [7]. This study were used polymeric porogen (*i.e.*, PEO) to fabricate the porous HAp scaffolds. PEO polymers has been reported to be utilized as biomedical materials due to its low toxicity and biocompatibility, and approved by the FDA for several medical application [8].

2. Experimental method

2.1. Synthesis of HAp

Pomacea canaliculata shells were used to prepare the starting material of porous HAp-based scaffold. Shells that have been identified were then cleaned from the macro impurities. Shell samples that have been cleaned were dried in air with temperature 100 °C. The dried shell was then milled using a ball mill in order to obtain a homogeneous shell powder. Shell powder was then tested for its elemental composition by EDS, and its functional group by FTIR. These test were performed to ensure that the shell is calcium carbonate then can be used as a source of calcium in the synthesis process. Shell powder was then calcined at high temperature to decompose the calcium carbonate into calcium oxide (CaO). The sample was then hydrated by reacting CaO with H₂O to obtain calcium hydroxide (Ca(OH)₂).

Ca(OH)₂ solution was reacted with H₃PO₄ by wet precipitation method, with 1 mL/min flow rate of H₃PO₄. The mixture was stirred in a temperature of 60 °C. The reaction was controlled at pH 10 by using ammonium solution (NH₄OH). The synthesis result was then characterized by using EDS to ensure that the molar ratio of Ca/P met the ideal molar ratio of HAp. In addition, the crystallinity of the synthesized HAp material was also characterized using XRD to see if the crystallinity of the material was in accordance with the standard HAp crystallinity. The functional groups of HAp were also characterized by using FTIR analysis. The synthesized material that meets the properties of HAp then used as the starting material in porous-structure HAp-based scaffold engineering.

2.2. Porous HAp-based scaffold engineering

The fabrication of HAp scaffold was carried out by using additional porogen with the intention of modifying the synthesis results to produce a controlled porous material [9,10]. To analyze the influence of the concentration of polymeric porogen to the porous structure of HAp scaffold, then the concentration of the porogen was varied. Polyethylene oxide (PEO 400000, Aldrich) was used as porogen by varying the concentration (*i.e.* 0, 5, 10, and 15% wt.). The preferred porogen does not have negative reaction with the body in case of interaction between body tissues and the materials [8].

The variation of porogen concentration was intended to observe the effect of porogen concentration treatment on the scaffold porosity. Porous-structure scaffold is expected to increase the osteoconductivity of bone tissues and support the growth of new bone tissues in the scaffold. Nonporogen HAp scaffold was prepared to compare with the porous HAp scaffold. The mixing results were heated at high temperatures to produce crystalline HAp. The scaffold was sintered at 1000 °C.

High temperatures were used to obtain the crystallinity of the materials. The morphology, crystallinity, and functional groups of HAp scaffolds were characterized by using Scanning Electron Microscope (SEM-EDS), X-ray Diffractometer (XRD), and Fourier Transform Infrared Spectrophotometer (FTIR), respectively. The thermal behavior of the porogen was characterized by using Differential Thermal Analyzer/Thermogravimetric Analyzer (DTA/TGA), and the mechanical properties by using Knoop Microhardness Tester.

3. Results and discussions

3.1. Synthesis of HAp

The SEM image shows the interconnected granular shape of HAp. Figure 1(a) shows the microstructure morphology of sintered HAp. The starting HAp powder consists of agglomerates formation. The molar ratio of synthesized HAp confirmed is 1.67 and it correspond to the molar ratio classification of HAp. The elemental composition of HAp is shown in Figure 1(b).

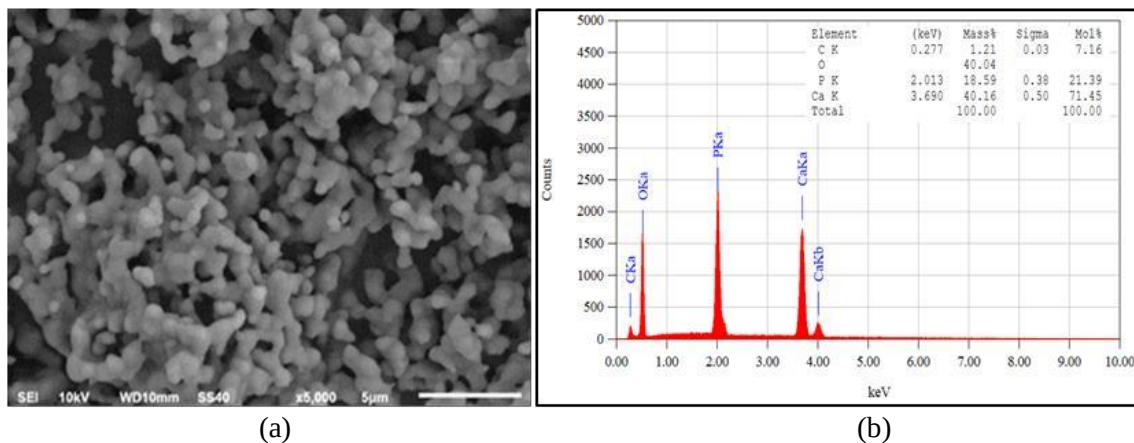


Figure 1. SEM-EDS result: (a) image of synthesized HAp (powder); (b) elemental composition of synthesized HAp (powder).

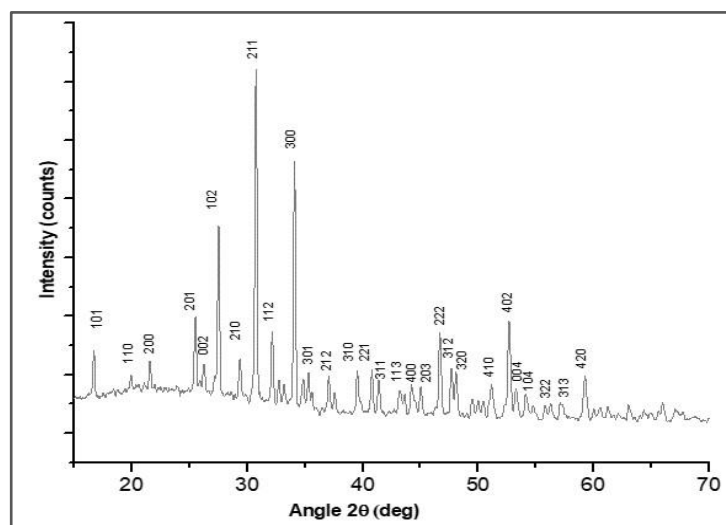


Figure 2. XRD pattern of synthesized HAp.

The X-ray diffraction peaks of the synthesized HAp is shown in Figure 2. The sharp and distinct peaks of the HAp indicate that the HAp was highly crystalline. The peaks were identified by referring

JCPDS (No. 09-0432) for HAp. The diffraction patterns of XRD correspond solely to HAp phase. The crystallite size of HAp is 49 ± 2 nm (nano-grained HAp), with lattice parameter a is equal to 0.954 nm, and lattice parameter c is equal to 0.698 nm. The XRD pattern of HAp exhibits characteristic of the highest peak at 2θ that is closed to lattice plane 211. The other peaks around 2θ is represent the HAp typical structure.

3.2. Porous HAp-based scaffold engineering

The evaporate particles of PEO from the HAp+PEO mixtures at sintering process left porous-structured morphology to HAp scaffold. From the SEM results, it is seen the dense sintered HAp has pore size distribution below $50\ \mu\text{m}$ (Figure 3a). The HAp scaffold with 5% porogen concentration (wt.) was not sufficient to make interconnect the pores. The average pore size ranged below $100\ \mu\text{m}$. The morphology of the scaffold surface is mostly crater-shaped with a non-uniform pore distribution (Figure 3b). The addition of 10% (wt.) of PEO porogen in mixture was able to create porous structure on the scaffold with macro-pore sizes ranging from about $100\ \mu\text{m}$ to $450\ \mu\text{m}$, but there appeared to be some craters that had not yet turned to pores (Figure 3c). The microporous structure of HAp scaffold shows the pore size ranges from ~ 1 to $\sim 3\ \mu\text{m}$ and it did not exhibit significant differences of pore size for each treatment.

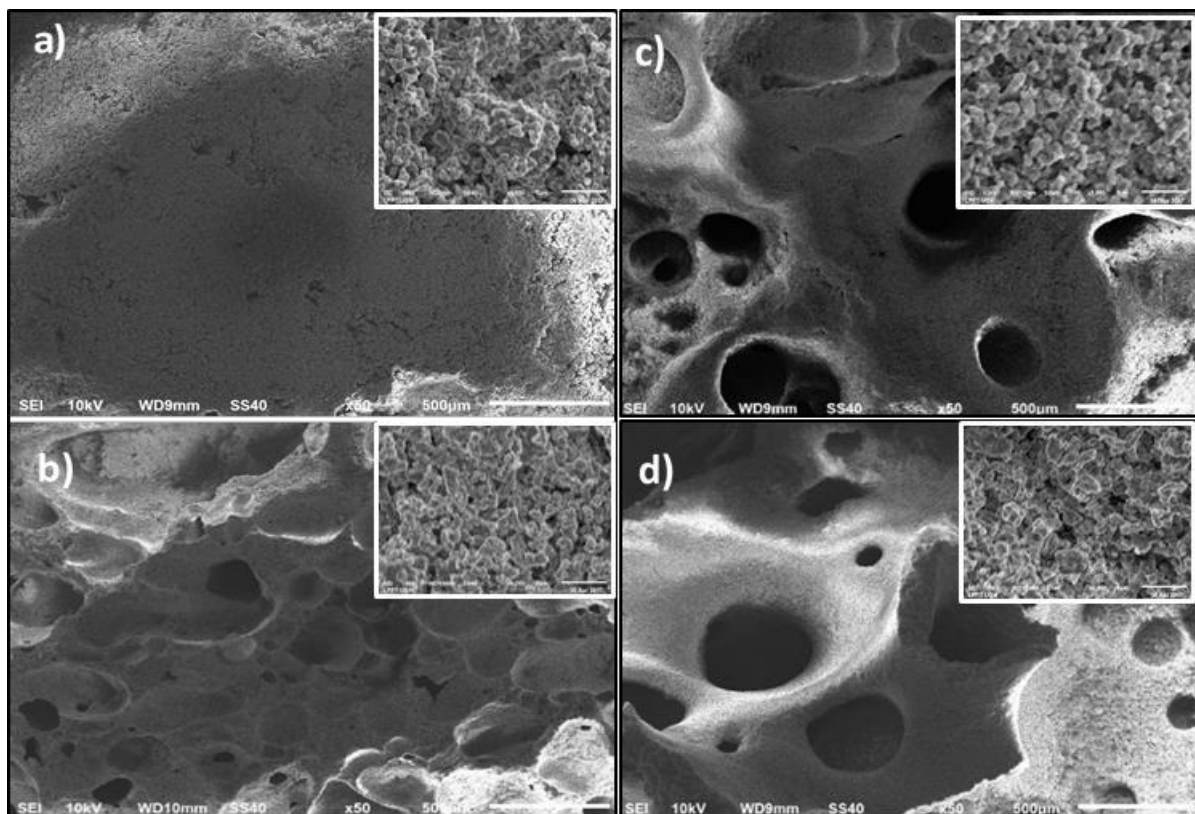


Figure 3. SEM image of HAp-based scaffold: (a) non-porogen (sintered); (b) HAp + 5 % wt. PEO (sintered); (c) HAp + 10 % wt. PEO (sintered); (d) HAp + 15 % wt. PEO (sintered). Inset: microstructure pores of HAp (magnification 5000 times).

The porogen in the suspension slowed down the densification process of the HAp particles, causing the particles to be less interconnected by the higher porogen concentrations in the suspension. Pore interconnectivity has been seen to result from using of porogen by 15% (wt.) in mixture (Figure 3(d)). The surface craters were much reduced and the pore distribution of range size more than $400\ \mu\text{m}$

μm was varied more than 5% areas of the scaffold's surface. The pore size distribution of the scaffold fulfilled the requirements of bone tissue engineering, where the 100-1000 μm pore size of the scaffold has the biological function for cell penetration, cellular growth, and bone ingrowth [3].

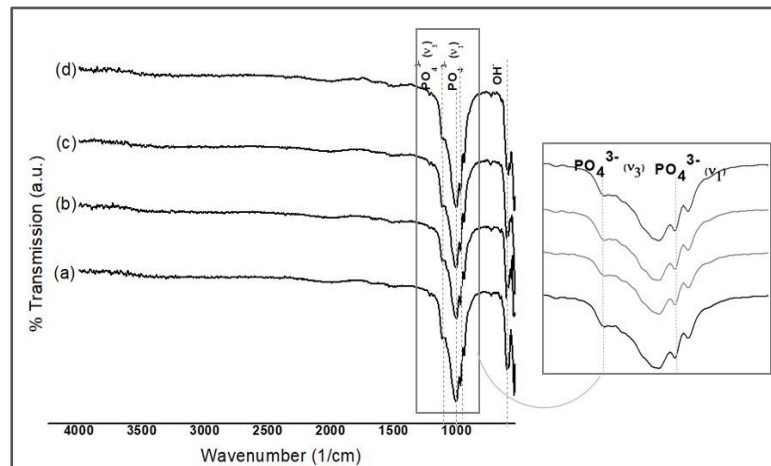


Figure 4. FTIR spectra of synthesized HAp (sintered); (a) non-porogen HAp; (b) HAp + 5 % wt. PEO; (c) HAp + 10 % wt. PEO; (d) HAp + 15 % wt. PEO.

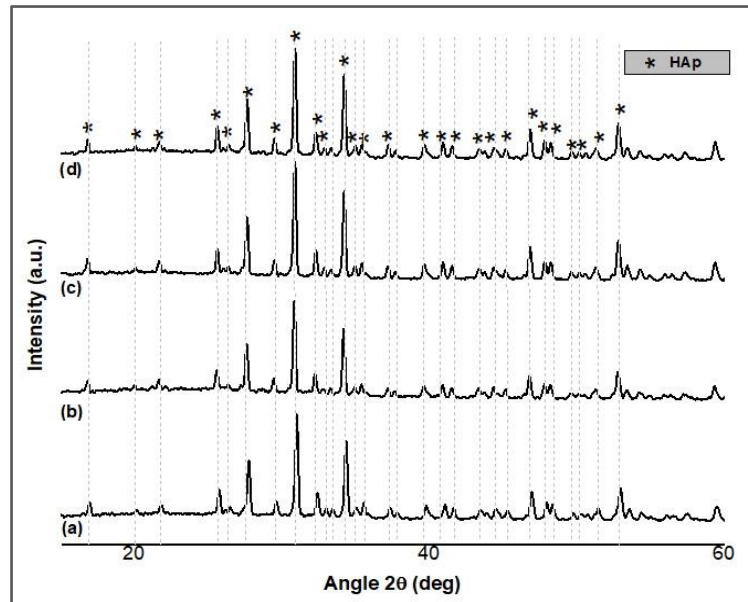


Figure 5. XRD pattern of synthesized HAp: (a) non-porogen HAp; (b) HAp + 5 % wt. PEO; (c) HAp + 10 % wt. PEO; (d) HAp + 15 % wt. PEO.

The IR spectra (Figure 4) shows the characteristic spectra of HAp. The FTIR band at wavenumber 1014.48 cm^{-1} corresponds to the presence of a PO_4^{3-} bending vibration. The band at 972.05 is attributed

to phosphate stretching vibration. The band at 551.60 cm^{-1} corresponds to vibration of hydrogel ions (OH^-) [11, 12]. The carbonate ion (CO_3^{2-}) does not appear at the spectra.

The FTIR spectroscopy also was used to study the chemical changes during the porous structure engineering process. The IR spectra of HAp scaffolds compared to the synthesized HAp confirm that no chemical decomposition occurred in the porous structure engineering process. There was no significant difference peak between the synthesized HAp and the porous-structure HAp scaffold.

The XRD patterns of HAp scaffolds treated with the addition of porogen shows that there are no significant shifting and changing of crystalline peaks (Figure 5). The peaks were also identified by referring to JCPDS data. The diffraction patterns of the scaffolds correspond solely to HAp phase with relatively small intensity of β -TCP. The crystallite size of the HAp-based scaffold tended to increase as porogen was added to the mixture (Figure 6). The addition of porogen tended to increase the agglomeration of crystallite size of the grains.

The synthesized HAp crystal occurs uniformly, the unit cell dimension change isotropically, which cause changes in lattice parameters and peak shifts. There was no peak widening indicates peak shift (211) was caused by uniform strains and changes in d -spacing values [13].

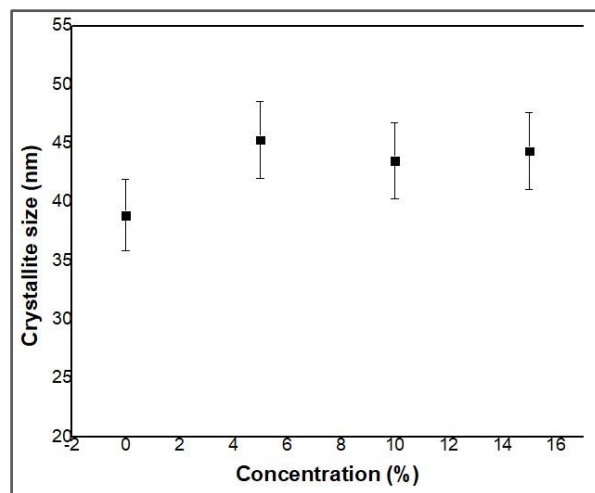


Figure 6. Crystallite size of HAp-based scaffold fabricated from various concentration of porogen PEO.

The lattice parameters a and c of the scaffold expanded compared to the lattice parameters of the HAp powder. The change of lattice parameters is likely due to the sintering process performed during the HAp densification process. This also indicates a lattice contraction of HAp. The difference in density of the HAp scaffold from each variation of porogen concentration appears to have no significant difference. However, compared to the density of the synthesized HAp powder, the density of the scaffold seemed significantly reduced.

The DTA/TGA graph shows the degradation of PEO mass in sintering process by $32\text{ }^{\circ}\text{C}/\text{min}$ heating rate, and it corresponds with the sintering rate of the scaffold. There are some weight loss regions, corresponding to the moisture evaporation, and mass degradation of PEO (Figure 7). The broader temperature range indicates the lower thermal stability of the porogen. The weight of the PEO was drastically reduced at $412\text{ }^{\circ}\text{C}$ by 99.910 % weight loss. The melting point of PEO is $68.77\text{ }^{\circ}\text{C}$. Mass degradation is associated with an exothermic event on the DTA chart. This process is followed by an endothermic event indicating that the sample in this process had decreased in enthalpy to a more stable structure. It was completely leached-out from the scaffold about $412\text{ }^{\circ}\text{C}$ for about 10 minutes after the sintering begin from the ambient temperature. The remaining temperature and time of sintering was proposed to produce crystalline material of the scaffold.

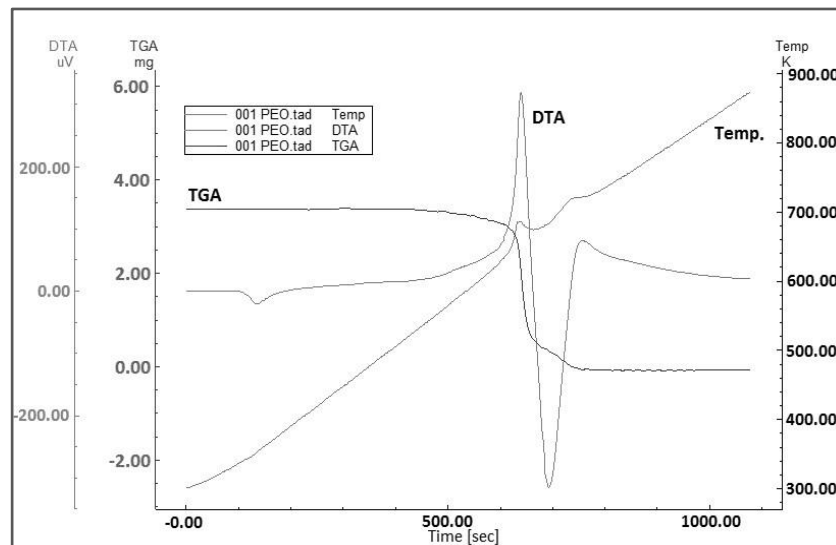


Figure 7. Thermal analysis result of PEO.

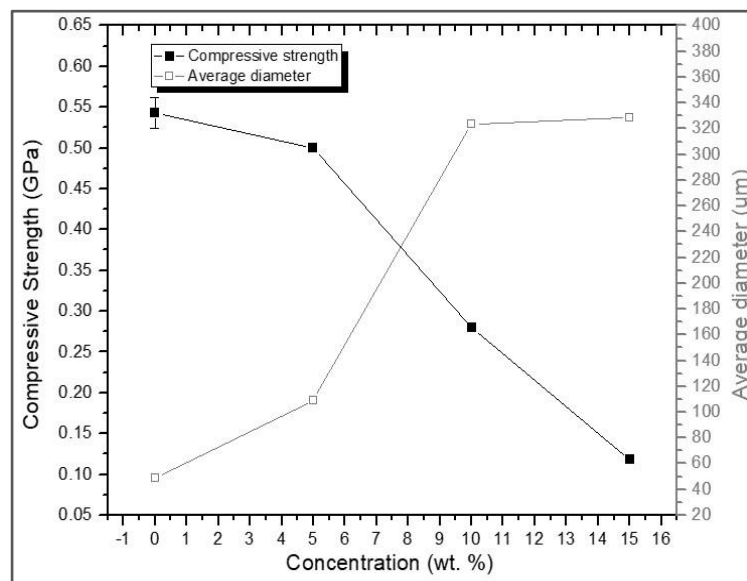


Figure 8. Effect of PEO weight concentration to the mechanical strength and pore size of HAp-based scaffold. Mechanical strength is inversely proportional to the pore size of scaffold.

The mechanical strength of the scaffold decreased with the addition of porogen (Figure 8). It was inversely proportional to the increase of pore size as shown in Figure 3. The microhardness strength of the scaffold is 0.54 GPa, 0.50 GPa, 0.28 GPa, and 0.12 GPa for concentration 0, 5, 10, and 15 % (wt.) respectively. Increase of pore distribution of scaffold caused the mechanical ability of the material to decrease. Nano-bioceramic HAp that was successfully synthesized in this study is an effective option for improving the mechanical properties of materials, where nano-sized ceramics shows better mechanical properties than micro-size bioceramics [14]. Ceramic materials with large porosity have

large exposed surface areas as well, so the risk of decreasing the mechanical strength of materials by the environment will increase.

4. Conclusion

The results showed that the golden apple snail (*Pomacea canaliculata*) shells can be utilized as a source of calcium for HAp synthesis. Hydroxyapatite with Ca/P molar ratio 1.67 was successfully fabricated. The crystallite size of synthesized HAp is nano-structure HAp. The polymeric porogen leaching method by using PEO as the porogen agent is succeeded to fabricate scaffolds with controlled porosity. Pores appear to be homogeneous at 10% (wt.). Pore interconnectivity has occurred with the addition of 15% (wt.) porogen. The microhardness of the scaffold is getting decrease while the pore size increase. The porogen was completely leached-out from the scaffold in the sintering process. The synthesized HAp that was treated using this method succeeded in fabricating porous HAp-based scaffold.

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