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The effect of hydroxyapatite concentration on the mechanical properties and degradation rate of biocomposite for biomedical applications

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Abstract. Biocomposite is a material that have potential to heal injured bones and teeth due to their biocompatible, non-toxic, non-inflammation, and bioactive properties which can prevent infections that occurs frequently during surgical processes. Biocomposites made of PLA, PCL, and HA from bovine bone as a substitute for metal materials in medical applications have been widely studied. However, there are limited studies on the biocomposites made of PLA, PCL, and HA from green mussel shells. Therefore, this study aims to produce biocomposites from Polylactic Acid (PLA), Polycaprolactone (PCL), and Hydroxyapatite (HA) from green mussel shells and to determine the effect of HA concentration on the mechanical properties and degradation rate of the resulting biocomposite. 80 ml of chloroform was used to dissolve 16 grams of a PLA/PCL mixture with a composition of 80% and 20%. After 30 minutes, the solution was agitated for 30 minutes with a magnetic stirrer at 50ºC and 300 rpm. After obtaining a homogenous solution, hydroxyapatite was added in percentages of 5%, 10%, 15%, and 20% of the total weight of the PLA/PCL mixture. The resulting mixture is poured into a glass mold in accordance with ASTM D790. Three-point bending, density, and biodegradable test were performed to investigate the effect of HA content on the mechanical properties and degradation rate of the biocomposite. The results of this study indicate that the mechanical properties of the biocomposite improved with the HA concentration increases. However, the more HA content used, the faster the biocomposite degrades.

1. Introduction

Indonesia is an agricultural country with a variety of abundant biological resources. Clove is a plant that grows a lot in Indonesia. Indonesia is one of the suppliers of clove production in the world, which is 63% [1]. The quantity of clove production in Indonesia is increasing every year, in 2020 the number of clove production produced is 140,806 tons and there is a growth of 0.14% in 2021 to 140,997 tons [2]. These cloves are processed into clove oil which is one of the types of essential oils that has many uses, including aromatherapy with a distinctive fragrance, natural pesticides, sunscreen, food additives, and in the cigarette industry.

The Biomaterial is a synthetic or natural material used to carry out body functions or replace body parts. Biomaterial is made so that they are able to interact with the body's biological systems. Biocomposite is a biomaterial made by combining several types of materials to obtain the desired properties in meeting the criteria as a biomaterial application. Biocomposite for implant materials made of bioceramic and biopolymer are concerned to be developed along with their biocompatible, non-toxic, non-inflammatory, and bioactive properties that can prevent infections that often occur in the surgical process and as a promising material to heal damage to bones and teeth [1].

The main causes of bone fractures are accidents, degenerative processes, and pathology [2]. The incidence of fractures in Indonesia is 1.3 million cases per year and is the largest in Southeast Asia [3]. According to the Ministry of Health, in Indonesia about 8 million people experience bone fractures, 46.2% of them are caused by traffic accidents [4]. Implant materials from metal that are widely used in the orthopedic world today. It happens due the price of the material is relatively cheap and has good mechanical properties, but this material is not biodegradable so a second surgery is needed for implant removal which has an impact on increasing treatment time, recovery time and treatment costs [5]. Research on biocomposite has been developed to replace the role of metal biomaterial for medical applications. PLA can be used commercially to control drug release, implant composites, and repair bone parts [6]. There are several advantages to using PLA, including being environmentally friendly, biodegradable, recyclable, and compostable [7], [8]. In addition to that, the biocompatibility of PLA is very suitable to be used as a biological material for living things. Besides PLA, the use of PCL (Polylacaprolactone) as a biomaterial is able to cover the mechanical properties of brittle PLA. PCL has high biodegradability. It can be hydrolyzed by lipase and esterase enzymes which are widely distributed in plants, animals, and microorganisms [9]. Due to its ductility, PCL has been used as a component of mixtures to develop new materials that are tougher and more ductile [9], [10]. Hydroxyapatite (HA) is calcium phosphate with the chemical formula $Ca_{10}(PO_4)_6(OH)_2$, is widely used as a biomaterial for bone replacement. HA is appealing because of its wide application potential as bone fillers, bone tissue engineering scaffolds, implant coatings, soft tissue repair, and drug delivery systems [11], [12]. Hydroxyapatite accounts for the inorganic component of bone tissue by 60-70%. The mineral phase in human bone is composed of molecules consisting essentially of phosphorus and calcium with the molecular formula $Ca_{10}(PO_4)_6(OH)_2$ and belongs to the group of calcium phosphate compounds [13].

The development of biocomposite made of PCL, PLA and HA as a substitute for bone material has been widely studied. The characterization of biocomposite in a study conducted by Moncal, et al., [14] aims to determine the mechanical properties of the resulting biocomposite. It was found that the addition of hydroxyapatite in the bicomposite had an effect on the mechanical properties and the rate of biodegradation of the biocomposite. A mixture of PLA/PCL with added HA has been studied by Moura, et. al., [15]. The results of this study showed that the addition of HA (10%) resulted in faster degradation of the biocomposite. In another study conducted by Hassanajili, et al., [16] used polylactic-acid / polycaprolactone / hydroxyapatite (PLA / PCL / HA) as a biocomposite material. To evaluate the morphology, functional groups, and biocomposite analysis, scanning electron microscope (SEM), Fourier transform infrared (FTIR) and energy dispersive spectropy (EDS) tests were used. Porosity is measured by liquid replacement technique, whilst the mechanical strength was tested by compression test. The biocomposite with the composition of PLA/PCL 70/30 and 35% HA was the best result of this study. PLA / PCL / HA with a composition of 70/30/35 had porosity, pore size and young modulus of 77%, 160 m, and 1.35 MPa. The resulting biocomposite was also tested in vitro using MG63 cells. Cytotoxicity assessment shows that cells can live and reproduce in the human body. The results showed that the composite scaffold with a weight ratio of PLA/PCL/HA with a composition of 70/30/35 had better properties in terms of biocompatibility, viability, and osteoinductivity. Research conducted by Azzaoui, et al., [17] focused on developing biocomposite membranes using PCL/PLA/HA. The use of PCL has been shown to increase the resistance and stiffness of the resulting composite material. The morphological analysis that has been carried out shows that the composite has good compatibility between the organic matrix and the inorganic matrix, thus enabling the production of very fine particles. The presence of the mineral phase (HA) in the polymer matrix (PLA/PCL) provides the desired bioactive character for bone growth. The use of polymer matrices provides convenience for the process of making biocomposite. In addition, the use of polymer matrices can produce biodegradable biocomposite.

It can be seen that many studies have been developed the synthesis of biocomposites from PLA, PCL, and HA. However, most studies still use HA from bovine bone. The use of green mussel shells as waste utilization is still rarely done. This study discusses the synthesis of biocomposites derived from PLA, PCL, and HA from green mussel shells. Furthermore, it is expected to produce biodegradable biocomposites that can also have good mechanical properties and meet the standard characteristics of human bones.

2. Materials and Methods

This study used PLA filaments produced by 3D Zaiku Indonesia, whilst the PCL filament used is manufactured by SUNLU Industrial CO., LTD. The PLA and PCL filaments are shown in Figure 1a-1b. Hydroxyapatite made from green mussel shells was obtained from the CBIOM3S lab of Diponegoro University, Semarang. XRD test results on hydroxyapatite are shown in Figure 2. XRD test results show that the hydroxyapatite synthesis obtained from the CBIOM3S Lab is a single phase without any impurities such as dicalcium phosphate, dibasic phosphate, tricalcium phosphate, and amorphous phase of calcium phosphate found [11].

(a) (b)

Figure 1. Filament (a) Polylactid Acid (PLA) (b) Polycaprolacton (PCL)

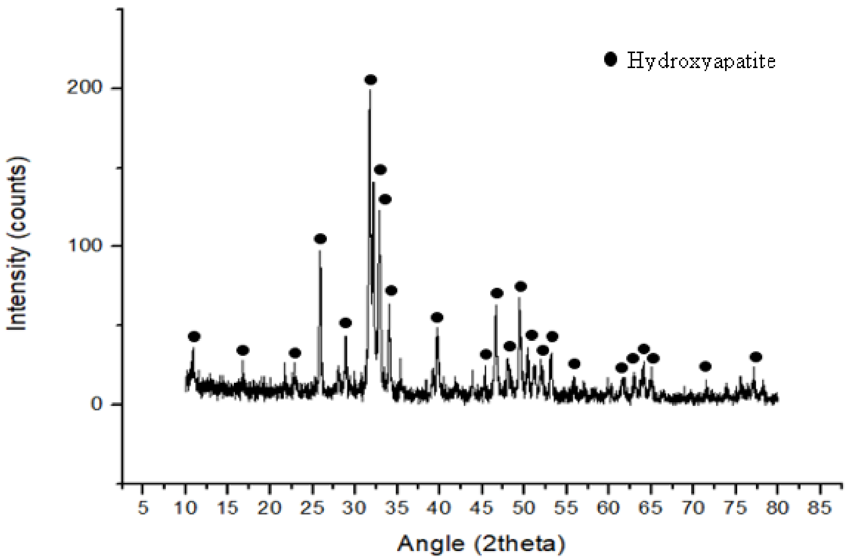

Figure 2. XRD Diffraction on The Hydroxyapatite of Green Mussel Shells

PLA and PCL filaments of 13.6 grams (85%) and 2.4 grams (15%) were cut to produce filaments with a length of ± 1 cm. The cut filaments were soaked with 80 ml of chloroform. After 30 minutes, a magnetic stirrer was used to stir the PLA/PCL solution for 30 minutes at a temperature of 50°C and 300 rpm. This process is carried out to produce a homogeneous mixture of PCL and PLA.

Hydroxyapatite with various compositions of 5%, 10%, 15%, and 20% of the total weight of the biopolymer mixture (PLA/PCL) was added to the PLA/PCL solution. The stirring process was carried out for 1 hour at a temperature of 65°C and 100 rpm in order to produce a homogeneous mixture of PLA/PCL/HA. After 1 hour, the resulting PLA/PCL/HA mixture was poured into a glass mold according to ASTM D790. The biocomposite drying process was carried out at room temperature for 2 days until the chloroform evaporated completely. Specimens that have been removed from the mold are soaked with distilled water for 24 hours (immersion process) to remove pores in the specimen before testing. Figure 3 shows the resulting biocomposite specimen.

Figure 3. Biocomposite Specimens Produced with Variations of HA

Biocomposites characterization was carried out using the density test, biodegradable test, and three point bending test. Density testing was carried out to determine the density of the resulting biocomposite. Density testing was carried out in accordance with ASTM 792-08 using a Vibra Densitymeter. Three point bending test is used to determine the flexural strength and flexural modulus of the biocomposite. The three point bending test is carried out in accordance with ASTM D790, whilst the degradable test was carried out to determine the biodegradable nature of the resulting biocomposite. The biodegradability test was carried out by immersing the biocomposite in a solution made of NaCl and aquades of 8.75 grams and 250 ml. The biodegradable test was carried out with an immersion time of 6 days [18].

3. Result and Discussion

Figure 4 shows the results of density testing on biocomposites synthesized from PLA, PCL and HA from green mussel shells. The addition of 5% HA resulted in the smallest density. Meanwhile, the highest density of biocomposite was obtained by adding 20% HA. The addition of HA can cause the biocomposite specimen to become denser. This causes the density of the biocomposite to increase [16]. This study produced a biocomposite material with a density that corresponds to the density value in human cortical bone, which is 1.1 – 1.3 g/cm3 [19]. The results of this study are in accordance with research conducted by Hassanajili, et. al., where the more concentration of hydroxyapatite mixed, the biocomposite has a low porosity value. The lower the porosity, the higher the density of the biocomposite material [16]. Research conducted by Aydin, et al., [20] showed that the porosity of the biocomposite will decrease with the increase in the amount of hydroxyapatite. This means that more hydroxyapatite in the biocomposite will increase the density of the material.

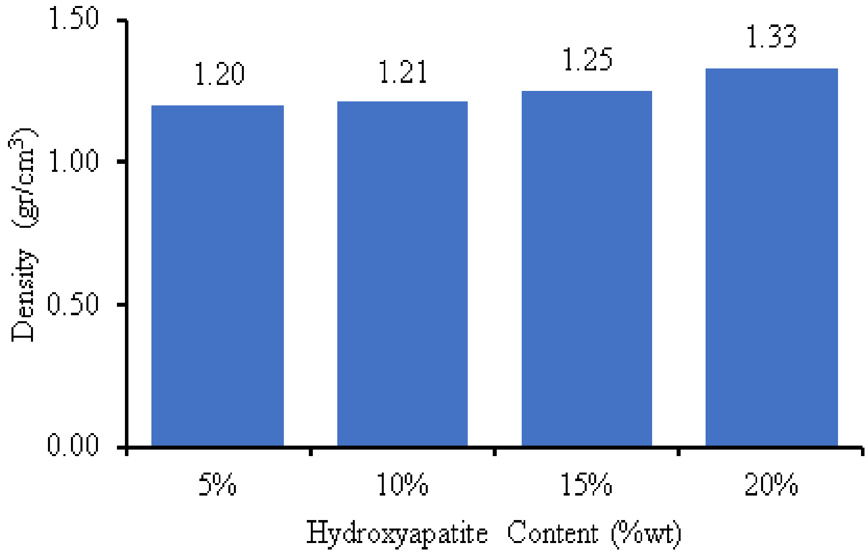

Figure 4. Density of Biocomposite with Different Concentration of HA

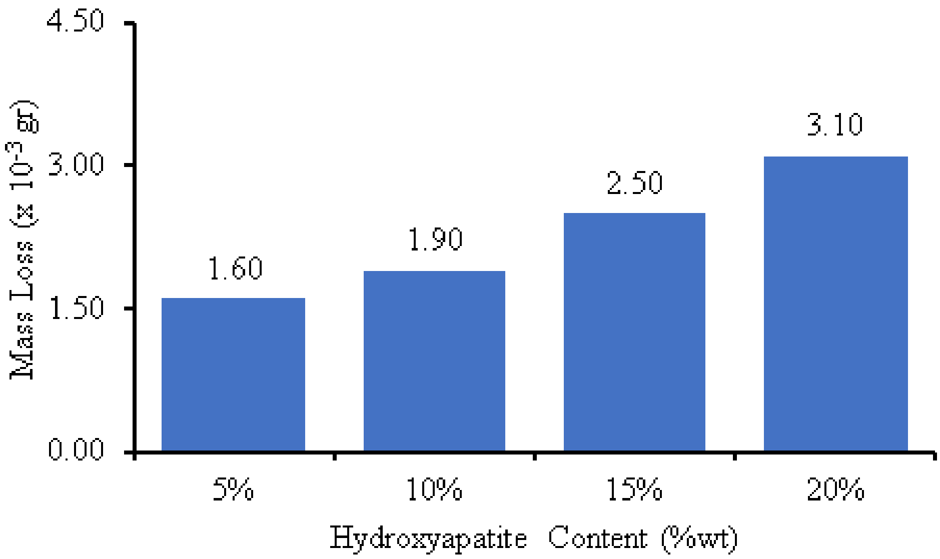

Figure 5. Mass Loss of Biocomposites with Different Concentration of HA

Comparison Figure 5 shows the results of biodegradable testing on biocomposites synthesized from PLA, PCL and HA from green mussel shells. The addition of 5% HA producesd the smallest mass loss. Meanwhile, the largest mass loss of composites was obtained by adding 20% HA. The results showed that biocomposites with more HA will be degraded faster than biocomposites with less hydroxyapatite content. These results are consistent with the research conducted by Torres, et al., who made biocomposites made from PLA, PCL, and HA. The results showed that biocomposites with 20% HA content degraded faster than a mixture of PCL/HA and PLA/PCL. This proves that mixing PLA and PCL produces hydrophilic units and reduces the overall crystallinity of PCL, so that the accessibility of water and ester molecules increases, and increased the rate of hydrolysis. The addition of hydroxyapatite provides a surface roughness that promotes interaction with water molecules. Thus, it caused the hydrolytic to crack so that the biocomposite degrades faster [21]. Moura, et al., mixed PLA/PCL/HA to produce biocomposite materials. The results of the study showed that the more addition of HA resulted in more biocomposite being degraded [15].

Figure 6 shows the results of the thee point bending test on biocomposites synthesized from PLA, PCL, and HA from green mussel shells. The addition of 5% HA resulted in the smallest flexural strength and modulus. Meanwhile, the largest flexural strength and modulus in the bio-composite was obtained with the addition of 20% HA. The results of this study indicate that the addition of hydroxyapatite to the biocomposite material resulted in an increase in the flexural modulus. This study produced a biocomposite material with a flexural modulus whose criteria for flexural modulus in cortical bone were 0.6-2.69 GPa [22]. In addition to that, the flexural modulus of the biocomposite produced in this study is greater than the flexural modulus of cancellous bone, which is 0.05 - 0.5 GPa [23].
The results of this study are in accordance with research conducted by Charles, et al., who made biocomposites made of PLA/PCL/HA. From the results of the bending test carried out, the value of the flexural modulus increased with the increase in the hydroxyapatite composition [24]. The addition of HA powder in the matrix reduces the movements of matrix phases in the surrounding area of each particle, contributing to the overall increase in modulus [25].

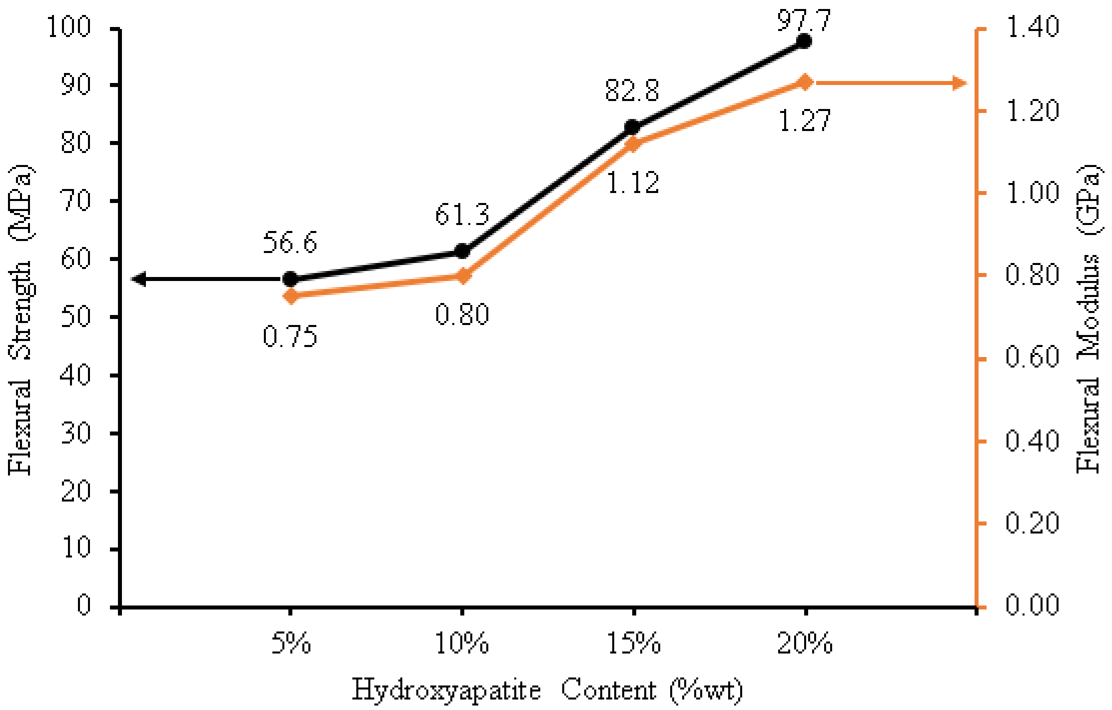

Figure 6. Flextural Strength and Modulus of Biocomposites with Different Concentration of HA

The greater the HA in the biocomposite material, the greater the flextrual strength produced. The results of this study indicate that the flexural strength of the resulting biocomposite material meets the criteria for flexural strength in cortical bone, which is 50-150 MPa [23]. The increase in flexural strength caused by HA addition can be attributable to the fact that the well-dispersed particles lengthen the crack propagation path, absorb more energy, and improve the plastic deformation. As a result, the surface fracture energy and composite strength improved [25].

The result this study indicate that the addition of HA from green mussel shells has an effect on the mechanical properties and biodegradable rate of the resulting biocomposite material. The addition of 5% HA is a candidate for biocomposite material for medical applications which is better than the other variations. This is because the more HA used will cause a faster rate of degradation in the biocomposite material. Although the mechanical properties of biocomposites with 5% HA concentration were the smallest compared to other variations, the mechanical properties of biocomposites with 5% HA had met the criteria for cortical bone. The high degradation rate of PLA/PCL/HA resulted in the decrease of its mechanical strength after implantation [26]. A faster decrease in mechanical strength after implantation results in inadequate healing because the immobilization of bone fragments does not occur completely [27].

4. Conclusion

The Biocomposite materials made from PLA, PCL, and HA from green mussel shells have been successfully made using the chemical blending method. The results show that the PLA/PCL/HA composite materials have great potential to be used as medical materials because they have mechanical properties that match cortical bone criteria.

The greater the HA composition used in the biocomposite material, the higher the density, flexural strength, flexural modulus and biodegradable rate values. The highest density, flexural strength, flexural modulus and mass loss were found in biocomposites using 20% HA with each weight of 1.33 gr/cm^3, 97.7 MPa, 1.27 GPa, and 3.10 x 10^{-3} grams. Biocomposite with 5% HA mixture is the best biocomposite. Although the mechanical properties of this biocomposite are the lowest, it has met the criteria for human cortical bone. The advantage of biocomposite in the PLA80/PCL20 mixture with the addition of 5% HA is that it has the lowest degradation rate. So that the decrease in mechanical strength occurs more slowly after the implantation process. This results in maximum healing because the immobilization of bone fragments occurs completely.

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