

Poly (1,8 Octanediol-co-Citrate) Hydroxyapatite Composite as Antibacterial Biodegradable Bone Screw

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Abstract. The high bone fracture rates reaching up to 300-400 cases per month have been treated with surgical procedure of internal fixation. Nevertheless, the commonly used metal screw has shown several weaknesses. Therefore, it is required bone screw of which primary characteristics include being biocompatible, bio-functional, biodegradable, and anticorrosive. The study aimed to synthesize Antibacterial Poly 1,8-Octanediol-co-Citrate (POC) and investigated the effect of chitosan on the antibacterial and compatibility characteristics of POC-HA composite as antibacterial biodegradable bone screw. The characterization were conducted on POC-HA composite to assess its functional cluster, antibacterial activity, cytotoxicity, degradation capacity, and morphology. Pre-polymer POC was composited with 62% nano-HA, followed by post-polymerization treatment. The sample then coated by chitosan with composition variations of 1%, 3%, and 5%. The nano-HA marked with the appearance of phosphate cluster on the wavenumber of 872.17 cm^{-1} and 559.51 cm^{-1} , while the chitosan marked with C=O stretch cluster of ester at 1729 cm^{-1} from Fourier Transform Infra-Red (FTIR) measurement. The best result was obtained with 3% chitosan coating. The POC-HA composites showed bacterial inhibiting ability of 16.92 mm with non-toxic characteristics. These results indicated that chitosan coating Poly 1,8-Octanediol-co-Citrate (POC)-Nano Hydroxyapatite composite is a potential candidate for an antibacterial biodegradable bone screw.

Keywords: Poly-1,8-octanediol-co-citrate (POC), hydroxyapatite, chitosan, nanomaterial, condensation polymerization, antibacterial biodegradable bone screw.

1. Introduction

Fracture is cracking in a bone structure resulting from excessive force. The overall estimated annual incidence of fractures was 100 cases per 10,000 populations for males and 81 cases per 10,000 populations for females. The hip fracture incidence map of the International Osteoporosis Foundation indicates that the hip fracture rate in Indonesia is 119/100,000 per year both for men and women [1]. The orthopedic surgeon repositions the bone fragments with external and internal fixations using a bone screw. The surgical procedures for bone fractures incidents have implemented internal fixation by using bone screw [2]. Bone screw comprises special screw for bones as a method for treating fractures [3]. Sri Rahayu (2012) [4] invented a simpler design for



bone fracture fixation using screw on the bone cortex made of a mixture of titanium and stainless steel adaptive to the body. Also, Respati (2010) [5] claimed that stainless steel is mechanically adaptive for bones, yet less compatible with the body tissues. Stainless steel cannot be used for long-term application as it may cause local corrosion in body fluid environment. This disadvantage calls for an innovation of material that is not only mechanically adaptive but also compatible with body tissues, such as a combination of biocompatible polymers or composites. A commonly used polymer for medical biomaterial is polyethylene. Some studies have reported that *poly (1,8-Octanediol-co-Citrate) (POC)* biomaterial has exhibited the characteristics of being biocompatible, non-toxic, easily available and inexpensive [6].

POC was chosen for its flexible degradation time. *Hydroxyapatite (HA)* is ceramic material belonging the apatite mineral group with biocompatible and bioactive chemical formula of $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ of which mineral content chemically and physically resembles bones [5]. Nano-size hydroxyapatite may offer a significant advantage in its function as filler for it is expected to improve the mechanics and compatibility of cells in bone screw application and to accelerate the growth of osteoblast. Moreover, chitosan is a polysaccharide from crustaceans group with biocompatible and antibacterial characteristics [7, 8]. There are reported cases [9] where a metal screw had corrosion in bone tissue, and it caused neurological spasm. Besides that, metal screw needs to be taken out from the body (re-operation). Metal stainless steel bone screw is good for the bone, but less suitable for the tissue [10]. Therefore, it is necessary to study on the synthesis of *chitosan coating poly (1,8-Octanediol-co-Citrate) (POC) nano-hydroxyapatite* as a candidate for antibacterial biodegradable bone screw that would result in a biocompatible, biodegradable, antibacterial material with excellent mechanical characteristics and capability of activating osteoblast growth.

2. Experimental Method

2.1. Materials

Materials used for the study comprised of 1,8-octanediol (98%) which was obtained from Sigma-Aldrich (St. Louis, MO: USA), citrate acid (99.5%) [Mw: 210.14] from SAP, Hydroxyapatite (HA) [Mw: 502.32, assay > 90%; particle size < 100nm from PAIR-BATAN, chitosan from Biochitosan, *Ethanol for analysis* (99.99%), and *Phosphate Buffer Saline (PBS)*, with pH 7.4, at 37 °C..

2.2. Methods

Bone screw making was initiated with a synthesis of Poly (1,8-octanediol-co-citrate), mixing citric acid concentration and 1,8-octanediol by adding it to 250 ml three neck flask round-bottom equipped with inlet and outlet adapters. The mixture melts at 160-165 °C under nitrogen gas flow while stirred. The temperature of the system was later reduced to 140-145°C for 1 hour with stirring to make pre-polymers. The synthesis of this polymer pre-POC dissolves into dioxane (50% b/v).

The nano-hydroxyapatite and poly (1,8-octanediol-co-citrate) (POC) Composites (POC/HA) were synthesized with the composition of HA is 62%. Then 10% glycerol was added to the composite as crosslinker. The composite post-polymerization occurred after vacuumed at 120 °C for 1 day, 3 days, and 6 days. The synthesized composite poured and centered in a square shaped iron mold with a size of 15 cm x 15 cm and a thickness of about 0.5 mm. The purpose of the composite was so that the produced form is perfect and to keep the film thickness all across the surface. Chitosan will coat the composite. The chitosan powder dissolved in 2% (b/b) acetic acid was homogenized. The composite was then immersed into a homogeneous mixture after the coating, and the sample was subsequently dried at room temperature for 12 hours. The process was continued with drying in a vacuum oven at 50 °C.

3. Results and Discussion

The results of this study were in the form of the development of antibacterial bone screw from composite hydroxyapatite and *poly (1,8-Octanediol-co-Citrate)* (POC) that is biodegradable, biocompatible, and antibacterial.

3.1. Functional Cluster Analysis by Fourier Transform Infra-Red

The *Fourier Transform Infrared* (FTIR) data showed that a cross-combination occurred on C=O stretch cluster which was an ester cluster on 1729 cm^{-1} as a result of the following chemical reaction. The peaks at 1729 cm^{-1} indicated successful polymerization and formation of polyester groups [6,11].

Figure 2 shows the POC-HA composite absorption spectrum after polymerization. The POC molecule formation is marked with the four-wave peaks showing the functional clusters: CH_3 (wave number 2928.51 cm^{-1}), C-H (wave number 2854.9 cm^{-1}), C=O stretch (ester) (wave number 1729.55 cm^{-1}), and C-H wag ($-\text{CH}_2\text{X}$) (alkyl halide) (wave number 1184.68 cm^{-1}). Hydroxyapatite molecule formation was marked with the five wave peaks showing the functional clusters of CO_3^{2-} (wave number 1406.26 cm^{-1}), HPO_4^{2-} (wave number 872.17 cm^{-1}), and PO_4^{3-} (wave number 1015.41 , 599.74 , and 559.51 cm^{-1}). Those peaks were also present in FTIR spectra recorded for POC-HA composites and related to the phosphate groups of HA [12]. Functional cluster analysis on the POC-HA composite IR spectrum did not show a new cluster formation. This is because the composite comprising of the POC polymer as the matrix and the hydroxyapatite as the filler is not tied chemically but only physically.

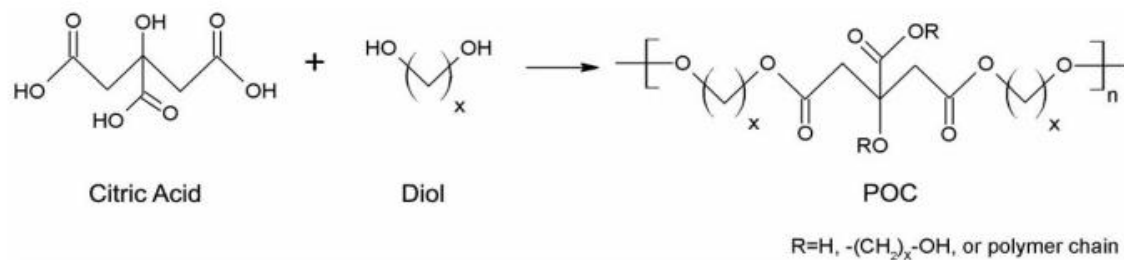


Figure 1. Chemical reaction of POC synthesis



Figure 2. POC-HA composite IR spectrum

3.2. Antibacterial Assay

The antibacterial characteristic of the sample was tested by *Staphylococcus aureus*, which is a pathogenic agent of several infectious diseases commonly detected in musculoskeletal infection [13]. The incubated samples are shown in the following Figure 3.

The observation results revealed that the 3% chitosan coating is the best concentration to inhibit *S. aureus* bacteria growth, shown by its greatest clarity zone diameter of 16.32 mm among other variations.

In 5% chitosan concentration, there has not shown the bacterial inhibition activity. It was suspected to consider that the high chitosan concentration will produce a viscous solution. A viscous solution will be difficult to diffuse. As a result, the inhibition diameter data showed a decline in the inhibitory activity. Chitosan solution which applied in a well could not diffuse. Chitosan molecules could not penetrate the bacterial cell wall. The inhibition zone diameter declined together with the increasing chitosan concentration [14].

The antibacterial effectiveness was based on Greenwood's classification of bacterial growth inhibition responses [15]. The 3% chitosan coating sample showed a medium inhibition capacity as it occurred on the bacteria inhibition zone of > 16-20 mm signifying that the sample has an antibacterial characteristic. Chitosan had a tendency to act as bacteriostatic rather than bactericidal [16], although the exact mechanism was not fully understood and several other factors might contribute to the antibacterial action [17].

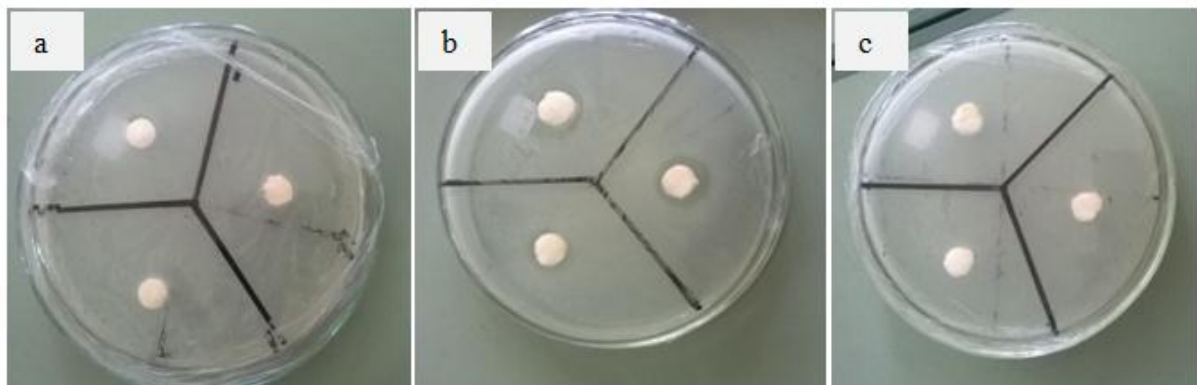


Figure 3. (a) 1% Chitosan sample, (b) 3% Chitosan sample, (c) 5% Chitosan sample

Table 1. Antibacterial test results

Sample	Clarity zone diameter (mm)	Average clarity zone diameter (mm)
1% Chitosan	8.9	9.9
	10.6	
	0	
3% Chitosan	15.93	16.32
	16.13	
	16.92	
5% Chitosan	0	0
	0	
	0	

Table 2. Classification of bacterial growth inhibition capacity [15]

Inhibition Zone Diameter	Growth Inhibition Capacity
> 20 mm	strong
16 - 20 mm	medium
10 - 15 mm	weak
< 10 mm	none

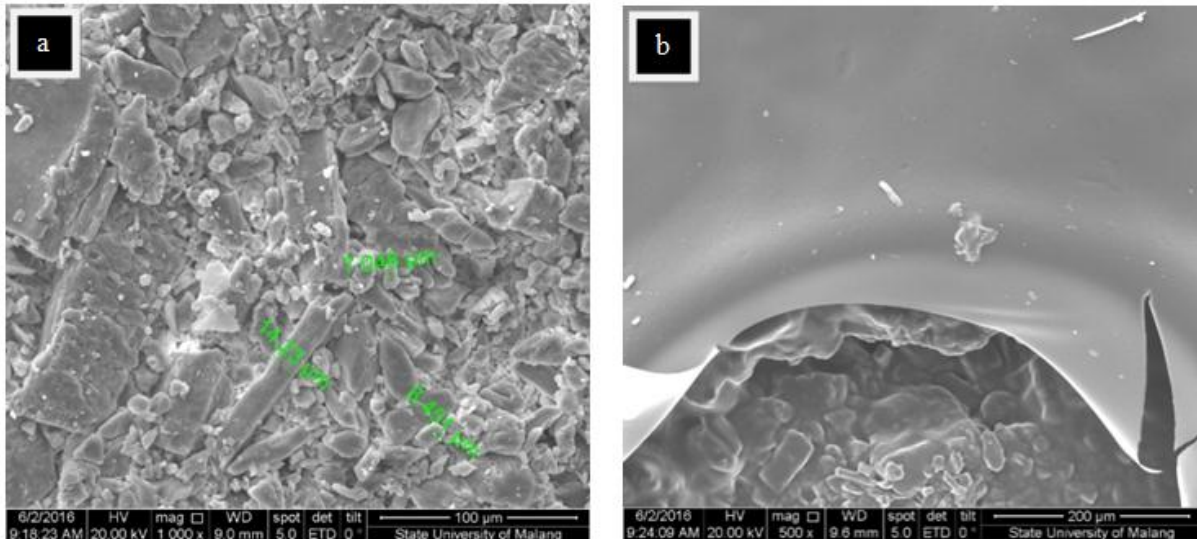


Figure 4. (a) Pre-coating sample morphology, (b) Post-coating sample morphology

Table 3. Elemental content of the pre-coating sample

Element	Wt%	At%
OK	41.22	61.58
PK	19.27	14.87
CaK	39.51	23.56
Matrix	Correction	ZAF

Table 3. Elemental content of the post-coating sample

Element	Wt%	At%
CK	39.54	49.36
NK	09.59	10.26
OK	36.70	34.39
PK	06.24	03.02
CaK	07.93	02.96
Matrix	Correction	ZAF

Notes: *Wt%* = weight percentage, *At%* = Atom percentage, *CK* = Carbon, *OK* = Oxygen, *NaK* = Natrium, *PK* = Phosphor, *CaK* = Calcium, and *NK* = Nitrogen.

The EDX spectra of HA/POC are illustrated in Figure 8 and 9. The Figures indicated the element content differences of the samples before and after coating, and the emergence of a new element, namely N, which is the chitosan amino cluster. Hence, this proves that the coating process occurred in the samples. The presence of HA in the HA/POC composite was confirmed by an appearance of calcium, phosphorous, and oxygen characteristics in the peaks, which are the main components of hydroxyapatite [18].

3.3. Degradation Assay

Degradability is a capability of being slowly broken down into simple parts or chemically degraded. The actual mass loss of the biodegradable bone screw occurs due to release of soluble

degradation products, phagocytosis by macrophages and intracellular degradation [19]. The comparison of coating variation effects on some samples for degradation speed is illustrated in a graphic presented in Figure 5.

The degradation process was conducted by incubating the samples in *Phosphate Buffer Saline* on pH 7 liquid and static temperature for four weeks. The total degradation calculation with chitosan coating samples shows a total degradation of a month. The length of total degradation on this sample is in accordance with Appley's maximum bone recovery process of 21 months [20].

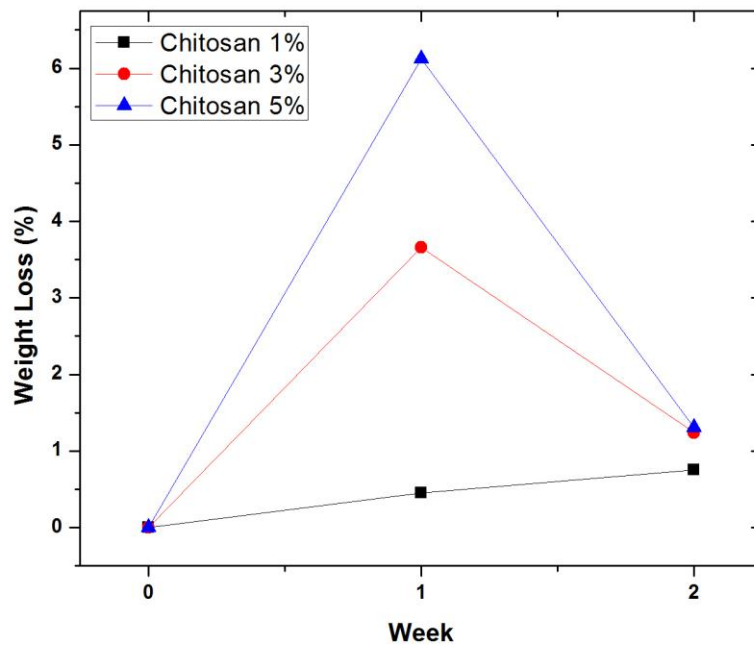


Figure 5. The result of degradation test

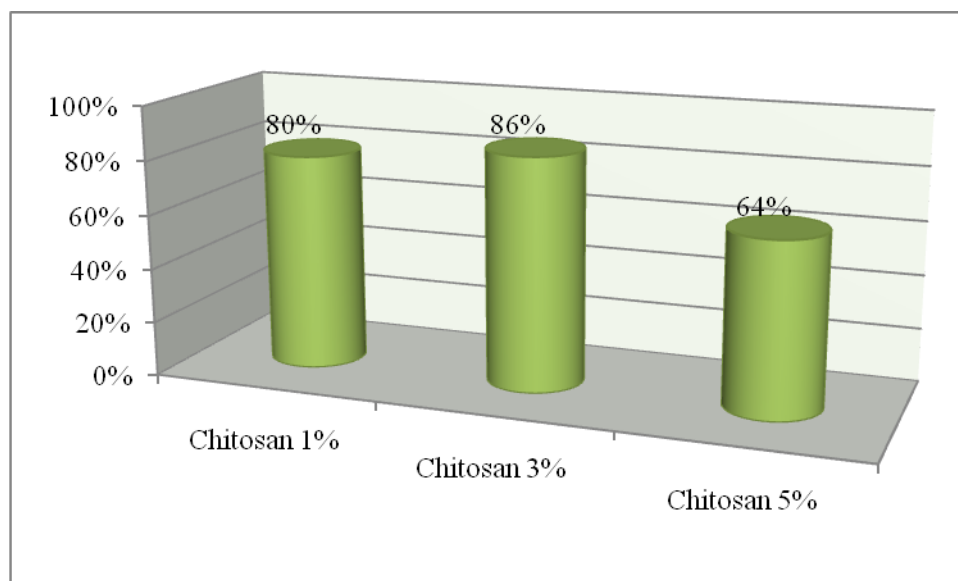


Figure 6. Cytotoxicity test graphic

3.4. Cytotoxicity Assay

The body will recognize the implanted antibacterial Biodegradable Bone Screw as a foreign matter. Therefore, the material should be compatible with the body. Accordingly, a cytotoxicity test or MTT was conducted. The MTT principle is the reduction of tetrazolium MTT (3-[4,5-dimethyliazol-2-yl]-2,5-difenil tetrazolium bromide) yellow salt by reduction system, then its absorption capacity was measured with ELISA reader [21] using the Hepatocyte cell. The cytotoxicity test results can be seen in Figure 6.

Figure 6 illustrates the percentage of living cells in each sample. The best result was presented by chitosan coating sample of 86%. All composition variations were within the non-toxic range due to the cell viability which was higher than 50% [22].

4. Conclusion

The POC-HA composite was successfully synthesized. The FTIR Result showed that The POC marked by C=O stretch ester cluster chain at 1729 cm^{-1} , while HA marked with phosphate cluster at 872.17 cm^{-1} and 559.51 cm^{-1} . The highest degradation speed in antibacterial activity was obtained in the TOC-HA Composite with 3% chitosan. The POC-HA composites showed antibacterial and non-toxic characteristics. The chitosan coating poly(1,8-Octanediol-co-Citrate) (POC) nanohydroxyapatite composite was a potential candidate for the antibacterial biodegradable bone screw. These results indicated that chitosan coating Poly 1,8-Octanediol-co-Citrate (POC)-Nano Hydroxyapatite composite is a potential candidate for an antibacterial biodegradable bone screw.

5. References

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