

PAPER • OPEN ACCESS

## Study of Nano-Hydroxyapatite:Poly Lactide Acid (n-HA:PLA) Composites and Their Biocompatibility, Bioactivity, and Biodegradability Characteristics

To cite this article: Yudyanto *et al* 2019 *IOP Conf. Ser.: Mater. Sci. Eng.* **515** 012034

View the [article online](#) for updates and enhancements.

# Study of Nano-Hydroxyapatite:Poly Lactide Acid (n-HA:PLA) Composites and Their Biocompatibility, Bioactivity, and Biodegradability Characteristics

Yudyanto\*, D Hardianti, Hartatiek, N Hidayat, R Kurniawan

Department of Physics, Faculty of Mathematics and Natural Sciences, Universitas Negeri Malang, Jl. Semarang 5, Malang 65145, East Java, Indonesia

\*Corresponding author's email: yudyanto.fmipa@um.ac.id

**Abstract:** A synthesis of nano-hydroxyapatite:polylactide acid (n-HA:PLA) composites based on natural materials has been performed. The synthesis was performed using sonication method, with a variation of n-HA:PLA ratio of 90:10, 80:20, 70:30, 60:40, 50:50 in %wt. The crystal structure and morphology of the sample were characterized by using X-ray diffractometer (XRD) and scanning electron microscopy (SEM). The bioactivity and biodegradable tests were performed in vitro using Krebs solution. Meanwhile, the biocompatibility test was performed by a separate bath organ method. The results show that for all compositions of n-HA:PLA exhibit a biocompatible characteristic. The sample with a ratio of 90:10 %wt shows higher bioactivity and biodegradability properties than other compositions. Our result would give a good understanding of the synthesis and biocompatibility, bioactivity, and biodegradability of n-HA:PLA composite, which is crucial for bone regeneration.

**Keywords:** PLA composite, n-HA, biocompatibility, bioactivity, biodegradability

## 1. Introduction

Bioceramics, especially calcium phosphate groups, have been widely used in the field of orthopedics and dentistry as bone replacement materials [1]. Hydroxyapatite ( $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ ) (HA) is one of the compounds widely used in the calcium phosphate group due to its attractive properties [2]. The HA is a mixture of two tricalcium phosphate salt compounds and calcium hydroxide [3], which can be obtained from natural sources or synthesis. Several studies have been conducted to produce HA from inexpensive natural sources, such as corals, algae [4], human bones/teeth, cow bones, sheep bones, and chicken bones using calcination methods. Others study reported that the HA can be obtained from mineral deposits, such as phosphate or phosphorus rock, which are sedimentary rocks with essential components of carbonate fluorapatite [5], calcite stones, and onyx stones [6,7].

The Hydroxyapatite (HA) is a bioactive ceramic that has biocompatibility, osteoconductivity, bio-resorbability, non-toxic, and can be fused to the bone [8-10], which potential to improve bone regeneration. The regeneration process of damaged bones can be performed by regenerating tissue through a framework for cell growth called scaffold. Scaffold serves as a place for tissue growth, where the scaffold must have high bioactivity, biodegradability, biocompatibility, and can repair damage in a



short time [11]. The HA has a similar chemical and crystalline structure with bone and stable in the body, but it shows a low biodegradability. In a recent clinical report on 6-7 years of follow-up studies confirmed that HA implanted in the body is not biodegradable, there is residue in the body for very long periods without biomaterial absorption signals [12].

To enhance the biodegradability of HA, we propose a composite based system, where HA is composed of synthetic polymers, polylactide acid (PLA) with a chemical formula  $(CH_3CHOHCOOH)_n$ . PLA is one of the biodegradable polymers that have superior mechanical properties, thermoplastic processability, and excellent biological properties, such as biocompatibility and biodegradable. The PLA has been widely used in the USA Food and Drug Administration (FDA) for biomedical applications [13]. The PLA has also used in wide range biomedical field, including bone fixation material, drug delivery microsphere, and tissue engineering [14], and develop 3D porous scaffolds [15]. Our result would give a good understanding of the synthesis and biocompatibility, bioactivity, and biodegradability of n-HA/PLA composite, which is crucial for bone regeneration.

## 2. Methods

The nano-hydroxyapatite/polylactide acid (n-HA:PLA) composites were synthesized in three stages. The first stage is the initial preparation of natural rock materials calcite from Druju, Malang as a source of calcium. Calcite powder was sieved with a 100 mesh and calcined at 1000 °C for 5 hrs to produce CaO. The CaO was bathed in distilled water for 24 hrs to produce  $Ca(OH)_2$ . The second stage is a synthesis of n-HA using co-precipitation method by reacting  $Ca(OH)_2$  and  $HNO_3$  to produce  $Ca(NO_3)_2$ . Furthermore,  $Ca(NO_3)_2$  was mixed with distilled  $(NH_4)_2HPO_4$  for 2 hrs at 35 °C and rotation speed of 700 rpm. During the stirring process, the pH was maintained at 9-10 by dropping  $NH_4OH$ . The solution was precipitated for 24 hrs, then washed 3 times using DI water and filtered. The precipitate was dried at 100 °C for 24 hrs to remove  $H_2O$  content. The third stage is a synthesis of n-HA: PLA composites using sonication methods with composition variations of pure n-HA, 90:10, 80:20, 70:30, 60:40, and 50:50 (in %wt.), respectively.

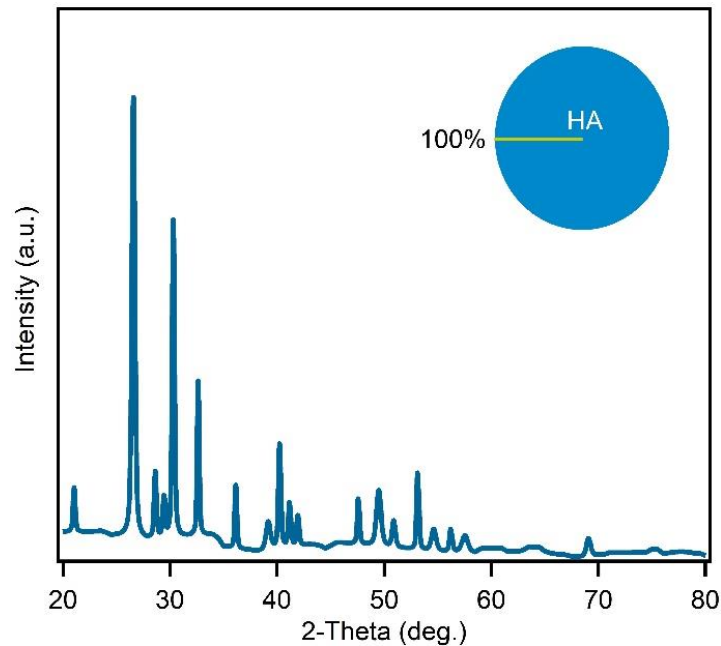
The crystallinity of the sample was characterized using XRD (PANalytical Expert-Pro). Furthermore, the crystallinity properties of the sample were obtained by using the Rietica program. The morphology of the sample was investigated by SEM-EDX (Brand FEI Type Inspect-S50). Biocompatibility properties were tested using a kymograph to determine the toxicity level of n-HA: PLA composites.

## 3. Results and Discussion

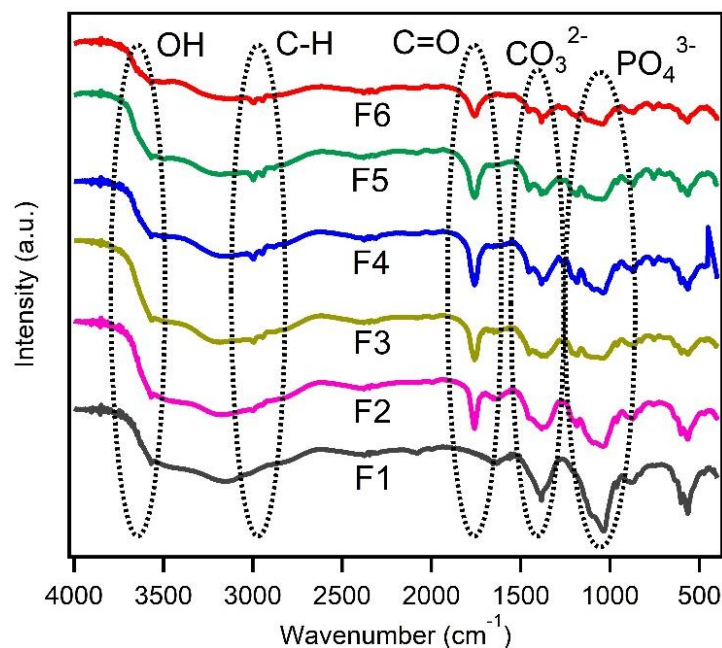
Figure 1 presents the diffraction pattern of the hydroxyapatite (HA). To obtain the information of crystal structure of HA, a Rietveld analysis using American Mineralogist Crystal Structure Database (AMCSD) was performed. The analysis confirms that the sample has a good agreement with the model (AMCSD No: 0002281). The detail of the crystalline properties of the sample is shown in Table 1. A peak around 30° indicated by arrow belongs to impurity. The result reveals that HA fit a hexagonal system with the crystallite size of 10.12 nm.

**Table 1.** List of crystalline properties of the HA calculated by Rietica.

Sample	Parameter	Value
HA	$a = b$ (Å)	$9.5180 \pm 0.0001$
	$c$ (Å)	$6.8887 \pm 0.0001$
	$V$ (Å <sup>3</sup> )	$540.4 \pm 0.001$
	Crystal size (nm)	$10.12 \pm 0.01$
	$GoF$ (%)	1.98
	$R_{wp}$	22.49
	$R_p$	17.36



**Figure 1.** The diffraction pattern of the HA sample refined using Rietica.



**Figure 2.** FTIR spectra of n-HA:PLA with PLA fraction of 0% (F1), 10% (F2), 20% (F3), 30% (F4), 40% (F5), and 50% (F6).

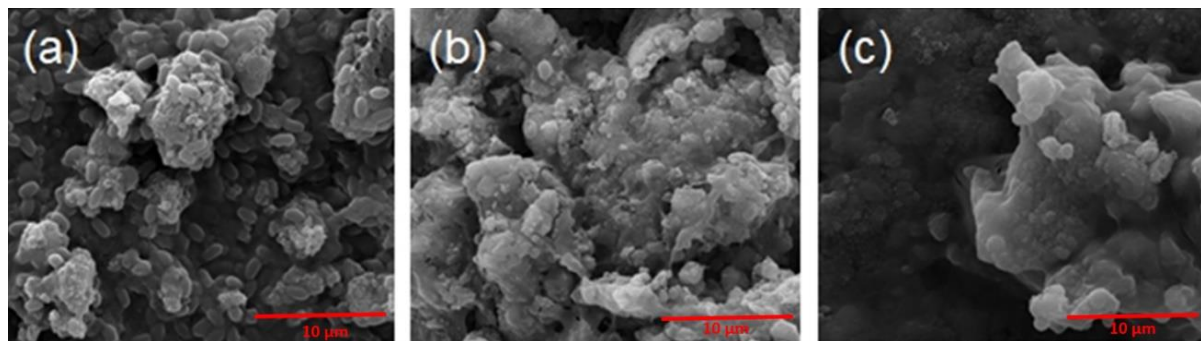
Figure 2 shows the FTIR spectra of the n-HA:PLA composites. The C-H and C=O vibration modes were observed in the sample F2 to F6. The existence of C-H and C=O modes [16-18] reveals that the n-HA:PLA composites were successfully synthesized. To obtain the biocompatibility of n-HA:PLA composites, toxicity tests are conducted using a test with a separate organ. The data is displayed in a graph of intestinal contraction of the ileum part in *Mus musculus* animals. Record time was performed between 5-15 mins due to the high contraction characteristic of ileum compared with other organs [19]. Table 2 shows a graph of the intestinal contraction given by the drug compared to intestinal contractions that received an injection of n-HA:PLA composite.

The intestinal contraction records in *Mus musculus* animals confirms that the intestine has contracted, indicated by a change of voltage. The result notes that the normal intestinal contraction is at a change of the voltage of 0.02 volts. The atropine drug introduces a significant voltage change of 0.165 volts. This result indicates the stress changes in the body, where the body cannot accept drugs. Furthermore, all concentration of n-HA:PLA samples show the change of voltages below 0.02 volts. This result confirms that the n-HA:PLA composite is accepted by the body and is non-toxic.

**Table 2.** List of intestinal contraction of *Mus musculus* animals introduced by the drug and n-HA:PLA composite.

Sample	Voltage (volts)		Difference ( $V_f - V_i$ )
	Initial ( $V_i$ )	Final ( $V_f$ )	
Drug	0.015	0.18	0.165
n-HA:PLA (90:10)	-0.0008	-0.0004	0.0004
n-HA:PLA (80:20)	-0.0007	-0.0004	0.0003
n-HA:PLA (70:30)	0	0	0
n-HA:PLA (60:40)	-0.01	-0.01	0
n-HA:PLA (50:50)	-0.0006	-0.0006	0

To determine the bioactivity of n-HA:PLA composites, the samples were bathed in Krebs solution. HA have nature bioactive properties, which are related to the role of implant material in the formation of body tissue interfaces with implants [5]. The appearance of the apatite layer after the material is bathed in the soluble Krebs reveals the bioactive properties of the n-HA:PLA composites. Here, the investigation is performed in n-HA:PLA (90:10), (70:30), and (50:50). The morphology of the bathed-n-HA:PLA composites for 7 days in Krebs solution is shown in Figure 3.



**Figure 3.** SEM images of the sample after bathing for 7 days; (a) n-HA:PLA (90:10), (b) n-HA:PLA (70:30), and (c) n-HA:PLA (50:50).

The apatite layer (white) grows in the composition of n-HA:PLA (90:10) more than the other compositions, which consistent with the previous result [20]. This result confirms that the sample with a higher number of HA leads the higher formation of apatite layers. In addition, biodegradable testing of n-HA:PLA composites are performed by bath treatment the pellet samples in Krebs solution for 7 days. The amount of dissolution of Ca can be calculated by dividing the difference percentage of Ca (in %at.) of the sample in each measurement by the number of days (7 days). The dissolution rate calculation of Ca for each sample composition is presented in Table 3.

**Table 3.** Dissolution characteristic of the n-HA:PLA composites.

Sample	The dissolution rate of Ca (%At / 7 days)
n-HA:PLA (90:10)	3.051
n-HA:PLA (70:30)	2.510
n-HA:PLA (50:50)	2.350

#### 4. Conclusion

The n-HA:PLA composites have been successfully synthesized using natural calcite. Biocompatibility, bioactivity, and biodegradation of the n-HA:PLA composites have been studied by comprehensive analyses. We found that all concentration of n-HA:PLA have biocompatibility with the human body. Moreover, the characteristic of the change of voltages below 0.02 volts and the formation of apatite layer revealed that the n-HA:PLA (70:30) composite is preferred for material scaffolds in biomedical application, which is crucial for bone regeneration.

#### References

- [1] Wenhao Wang A, B, Kelvin W.K. Yeung. (2017). Bone grafts and biomaterials substitute for bone defect repair: A review. *Bioactive Biomaterial*. 2: 224-247.
- [2] Cunniffe GM, O'Brien FJ, Partap S, Levingstone TJ, Stanton KT, Dickson GR. (2010). The synthesis and characterization of nanophase hydroxyapatite using a novel dispersant-aided precipitation method. *Journal of Biomedical Materials Research. Part (4)*:1142-9.
- [3] Yasmine Daniels, Nathalie Lyczko, Ange Nzihou, and Spiro D. Alexandratos. (2015). Modification of Hydroxyapatite with Ion-Selective Complexants: 1-Hydroxyethane-1,1-diphosphonic Acid. *Ind. Eng. Chem. Res.*, 54 (2), pp 585–596.
- [4] Kokubo, T., 2008. *Bioceramics and their clinical applications*. Elsevier.
- [5] Park, J. 2008. *Bioceramics: Properties, Characterization, and Applications*. Springer, New York.
- [6] Yudyanto, Hartatiek, (2014). *Sintesis Nano-Hidroksiapatit Berbahan Dasar Batuan Alam Calcite Druju Kabupaten Malang sebagai Biomaterial Fungsional Pengganti Tulang*. *Jurnal Foton* **1** 1-9.
- [7] Yudyanto. Sugara Y.D. Hartatiek. (2016). "Pengaruh Nanosilika terhadap KekerasanPorositas Nanokomposit HA-SiO<sub>2</sub> Berbasis Batuan Onyx Bojonegoro". *Journal of Physical Science and Engineering* **1** 1-8.
- [8] Anjarsari, A., Dahlan, K., Suptijah, P., Kemala, T. (2017). Synthesis and Characterization of Biocomposite BCP/Collagen for Bone Material Scaffold. *J. Pengolah. Has. Perikan. Indones.* 19, 356–361.
- [9] Cengiz, B., Gokce, Y., Yildiz, N., Aktas, Z., Calimli, A. (2008). Synthesis and characterization of hydroxyapatite nanoparticles. *Colloids Surf. Physicochem. Eng. Asp.* 322, 29–33.
- [10] Nayak, A.K. (2010). Hydroxyapatite synthesis methodologies: an overview. *Int. J. ChemTech Res.* 2, 903–907.
- [11] Swetha, M., Sahithi, K., Moorthi, A., Srinivasan, N., Ramasamy, K., Selvamurugan, N. (2010). Biocomposites Containing Natural Polymers and Hydroxyapatite for Bone Tissue Engineering. *Int. J. Biol. Macromol.* 47, 1-4.
- [12] Marcacci, M., Kon, E., Moukhachev, V., Lavraoukov, A., Kutepov, S., Quarto, R., et al. (2007). Stem Cells Associated with Macroporous Bioceramics for Long Bone Repair: 6 to 7-year outcome of a pilot clinical study. *Tissue Engineering* 13 (5), 947-955.
- [13] FDA. 2002. Inventory of Effective Food Contact Substance (FCS) Notifications No. 178.
- [14] Xiao, L., Wang, B., Yang, G., Gauthier, M. (2012). Poly(Lactic Acid) based Biomaterials: Synthesis, Modification, and Applications. *Biomedical Science, Engineering and Technology*.
- [15] Salerno, A., Gutierrez, M.F., Barrio, J.S., Pascual, C.D. (2014). Macroporous and Nanometre Scale Fibrous PLA and PLA–HA Composite Scaffolds Fabricated by a Bio-Safe Strategy. *RSC Adv.* 4, 61491–61502.
- [16] W. H. Hoidy, M. B. Ahmad, E. A. J. Al-Mulla and N. A. B. Ibrahim. (2010). "Preparation and Characterization of Polylactic Acid and Polycaprolactone Clay Nanocomposites," *Journal of Applied Sciences*, Vol. 10, No. 2, 2010, pp. 97-106. doi:10.3923/jas.2010.97.106
- [17] A. J. Lasprilla, G.A.R. Martinez, B.H. Lunelli, J.E.J. Figueroa, A.L. Jardini, R.M. Filho. 2011. Synthesis and Characterization of Poly (Lactic Acid) for Use in Biomedical Field. *Chemical Engineering Transactions* 24:985
- [18] Sanyang, M. L., Sapuan, S. M., Jawaid, M., Ishak, M. R., & Sahari, J. (2016). Development and

- characterization of sugar palm starch and poly(lactic acid) bilayer films. *Carbohydrate Polymers*, 146, 36–45.
- [19] Rubinstein, A., V. H. K. Li, P. Gruber, and J. P. Robinson. (1988). “Gastrointestinal-Physiological Variables Affecting the Performance of Oral Sustained Release Dosage Forms.” *Oral Sustained Release Formulations: Design and Evaluation*, Pergamon Press, 123–56.
- [20] Xiao, Y., Li, D., Fan, H., Li, H., Gu, Z., Zhang, X. (2007) Preparation of nano-HA/PLA composite by modified-PLA for controlling the growth of HA crystals. *Materials Letters* 61 (2007) 59-62.

### Acknowledgements

This work was supported by PNPB research grant UM for Y 2018.