**Coating Nano Hydroxyapatite Based on Rice Snail Shells (*Pila ampullacea*)/Chitosan/Hyaluronic Acid on Titanium (Ti-6Al-4V) Using Electrophoretic Deposition Method for Biomedical Applications**

Achmad Dwitama Karisma1, a), Agnes Surya Putri Anggraeni1, b), Yasmin Inayah1, c) Eva Oktavia Ningrum1, d), Fahmi Mubarok2, e), and Heri Suroto3,f)

Author Affiliations

1*Department of Industrial Chemical Engineering, Faculty of Vocational Studies, Institut Teknologi Sepuluh Nopember, Surabaya, 60111, Indonesia*

2*Department of Mechanical Engineering, Faculty of Industrial Technology And Systems Engineering, Institut Teknologi Sepuluh Nopember, Surabaya, 60111, Indonesia*

*3Orthopedic and Traumatology Department, Faculty of Medicine, Airlangga University, Surabaya, 60286, Indonesia*

*Author Emails  
a) Corresponding author: dwitama@its.ac.id*

**Abstract.** The demand for implants in Indonesia is expected to increase to US$ 36.67 million by 2026 at a CAGR of 15.2%. Most of the implants used are Ti6Al4V due to its mechanical properties and biocompatibility. However, these implants do not adhere and can leach aluminum and vanadium ions into the body, which accelerates implant failure. Therefore, in this research, the coating with Hydroxyapatite based on rice snail shell/Chitosan/Hyaluronic Acid (HAp/CS/HA) using the Electrophoretic Deposition (EPD) method was conducted. HAp (Ca₁₀(PO₄)₆(OH)₂) is a bioactive ceramic material that can improve the corrosion resistance of implants with synthetic results HAp shows good affinity, so it has the potential to be used as a coating. Hyaluronic Acid (HA) and Chitosan (CS) are biocompatible compounds that have the potential as corrosion resistance coating materials. Therefore, this study aims to investigate the possibility of developing bioactivity and corrosion-resistant coatings. The HAp synthesis process was carried out by precipitation method using 1.3 M H3PO4. The EPD process was carried out at a voltage of 40V with a carbon anode for 2, 4, 6, and 8 minutes at Hydroxyapatite concentrations of 10%, 20%, 30%, and 40% w/v. The results showed strong adhesion with slow bioactivity behavior and corrosion rate in the range of 0.006% - 0.038%.

# INTRODUCTION

Implants are one of the most needed medical devices in Indonesia, where in 2021, the market for orthopedic and prosthetic implants will reach US$ 20 million and is expected to increase to US$ 36.67 million in 2026 with a compound annual growth rate (CAGR) of 15.2% [1]. Most implant materials used in orthopedic surgery are made from Titanium and its alloys, especially Ti6Al4V, due to its mechanical properties suitable for replacing bone in vivo, good corrosion resistance, and biocompatibility [2]; [3]; [4];[5]. Titanium implants bind to bone through a morphological bond that results in the formation of a non-adherent fibrous shell around the implant [6]. These non-adherent implants can create wear debris due to friction between the implant and bone, which can trigger an inflammatory reaction that causes fluids in the body to become more corrosive[2], [4]. Although Ti-6Al-4V has good corrosion resistance, after long-term exposure to corrosive body fluids, implants can release aluminum and vanadium ions into the body, causing serious complications, toxicity problems, and accelerated implant failure [3], [5], [7]–[9]. In addition, Ti-6Al-4V cannot induce biological fixation to surrounding tissue, thereby limiting the formation of new bone tissue around the implant, and is susceptible to fibrous tissue encapsulation, which causes bone-implant interface failure and implant loosening [3], [10]–[12].

Implant coating is a simple and effective technique to improve the biocompatibility, bioactivity, and osseointegration of implants without changing implant properties [13]–[16]. Hydroxyapatite (HAp) is the right material to be used as a coating [17]. Hydroxyapatite (Ca₁₀(PO₄)₆(OH)₂) is a bioactive ceramic material with high affinity, biocompatibility, and osteoconductivity. Applying HAp as a coating will combine the strength and toughness of Ti-6Al-4V with the bioactive characteristics of HAp, which can induce the growth of surrounding bone tissue and increase implant fixation through the formation of an apatite layer. In addition, HA coating can improve the corrosion resistance of coated implants, which can reduce the release of metal ions. HAp can be produced from the synthesis of natural materials that contain a lot of calcium, one of which is rice field snail shells with a CaCO3 content reaching 93.438% [19]. The abundant presence of rice snail shells in rice fields makes it a potential material for the synthesis of hydroxyapatite [20], [21]. The synthetic HAp results show good affinity, namely that it can chemically bond with bone [22]. This shows the potential for hydroxyapatite synthesis to be applied as a coating on metal implants [18], [22].

On the other hand, the combination of several materials as coatings has been researched. Chitosan and Hyaluronic acid have potential as corrosion resistance coating. Chitosan (CS) is a natural, non-toxic, biocompatible, and biodegradable polymer compound that can be modified with other compounds to improve their properties [23]. Hyaluronic acid (HA) is a highly hydrophilic, biocompatible, and biodegradable compound, making it highly suitable as a biomaterial. By combining these biomaterials, the resulting coating can exhibit enhanced bioactivity and corrosion resistance, making it an attractive solution for biomedical applications[25][26]. The combination of Hyaluronic Acid (HA) and Chitosan (CS) shows promising potential as a highly biocompatible and corrosion-resistant coating material[27][25][8]. This combination can also improve and reduce the risk of peri-implant bone resorption, ultimately enhancing the long-term performance and success of titanium implants. In addition, CS can improve the mechanical strength of HAp, which is easily detached from the substrate due to its low intensity and high brittleness[28].

Various coating techniques have been investigated, starting from thermal spraying, physical vapor deposition, and sol-gel. However, these methods have disadvantages, namely poor adhesion to the substrate, formation of amorphous structures, coarse grains, inability to create composite layers, and are prone to cracking, have a high price, require a lot of time, and low deposition rate [29]. Meanwhile, the electrophoretic deposition method comes as a very promising alternative technique, with a simple procedure, low cost, uniform thickness, fast deposition time, the ability to coat substrates with complex geometries, and the possibility of combining with reinforcing agents [30].

Therefore, the focus of this study was to investigate the possibility of developing bioactive and corrosion-resistant coatings on Ti-6Al-4V surface using Hydroxyapatite based on Rice Snail Shells/Chitosan/Hyaluronic Acid (HAp/CS/HA) mixture by Electrophoretic Deposition (EPD) method. The effects of HAp concentration parameters and deposition time on bioactivity (by degradation test) and corrosion resistance were evaluated.

# MATERIALS AND METHOD

## Materials

The material used in this research was Rice Snail Shell, Phosphoric Acid Precursor (H3PO4) (Merck, Kenilworth, NJ, USA) Grade: ACS, ISO, Reag. Ph Eur 85%, Distilled water, Nitrogen Gas, Acetic acid (CH3COOH) (glacial) 100% CAS 64-19-7 anhydrous for analysis EMSURE® ACS, ISO, Reag. Ph Eur, Ethanol 96% CAS number 64-17-5 Grade Reag. Ph Eur, Grade-5 titanium metal (Ti-6AL-4V), Chitosan (MW 100 Da), Hyaluronic Acid 99.7%, carbon anode, *S. aureus* bacteria, *E. coli* bacteria, Nutrient Broth (Merck) HS Code: 3821 00 00, Nutrient Agar (Merck) HS Code 3821 00 00, and simulated body fluid.

## Methods

### **Hydroxyapatite Synthesis**

The hydroxyapatite synthesis procedure was based on the previous research by Karisma et al. (2023)[31]. Starting with pre-treatment for the rice snail shell, then the calcination process at the temperature of 1000℃ for 8 hours to form the calcium carbonate into calcium oxide and carbon dioxide. The calcium oxide was mixed with distilled water and H3PO4 1.3 M, 100 mL solution. The hydroxyapatite particle was sintered for 6 hours at 800℃.

### **Suspension Preparation**

Hyaluronic Acid (HA), Chitosan (CS), and hydroxyapatite (HAp) were prepared to make the suspension. HA solution was made by dissolving 0.05 grams of HA in 25 ml of hot distilled water. CS solution was made by dissolving 0.05 grams of CS in 25 mL of 1% acetic acid, and HAp solution was prepared by dissolving HAp in 50 mL Ethanol. Each solution was stirred for 20 minutes. The mixture of CS and HA solutions that had been stirred for 10 minutes was added to the HAp solution and stirred for 30 minutes. In this study, the various HAp concentrations were 10%, 20%, 30%, and 40% w/v.

### **Electrophoretic Deposition**

A diagram of a battery

Description automatically generated

**FIGURE 1.** Schematic of Hydroxyapatite/Chitosan/Hyaluronic Acid Coating Equipment on Ti-6Al-4V Using The Electrophoretic Deposition Method

The initial step begins with the pre-treatment of Ti-6Al-4V, which is cleaned with ethanol and water using ultrasonication before the coating operation. The metal was connected to a 40-volt direct current power supply, where Ti-6Al-4V was the cathode (the substrate) and carbon was the anode, as shown in **FIGURE 1**. The distance between the two metal electrodes was set at 20 mm. EPD was carried out with variable deposition times of 2, 4, 6, and 8 minutes. The coated Ti-6Al-4V was dried at room temperature for 24 hours and then sintered at a temperature of 120 °C for 1 hour.

### **Characterization of Hydroxyapatite Synthesis**

To determine the characteristics of Hydroxyapatite made from Rice Snail Shells, XRD was carried out at the Department of Materials and Metallurgy, Institut Teknologi Sepuluh Nopember, with a scanning range of °2θ = 10 - 100° and a step size of 0.017° to determine crystal structure and degree of crystallinity and FT-IR at the Department of Industrial Chemical Engineering, Institut Teknologi Sepuluh Nopember with the wavenumber from 800 - 4000 cm-1 to determine the chemical structure.

### **Corrosion Behaviour Analysis**

The potentiodynamic polarization was carried out at the Department of Materials and Metallurgy, Institut Teknologi Sepuluh Nopember, with the potential range from -0,3 to 0.2 V at a scan rate of 0.5 mV and simulated body fluid as an electrolyte. To determine the corrosion rate on Ti-6Al-4V with coating to compare with the corrosion rate on Ti-6Al-4V without coating. These results are to provide an understanding of the effect of HAp/CS/HA coating against corrosion resistance in the body environment.

### **Degradation Analysis**

Samples were immersed in SBF at 37 ± 1℃ for 14 days. The SBF solution was replaced with a new one every 72 hours. The surface inspection using visual analysis before and after immersion. The loss of weight was determined by measuring their weight before and after immersion with a high-precision scale. These results are to evaluate the biological response of the coatings in a physiological solution.

# result and DISCUSSION

## Visual Analysis

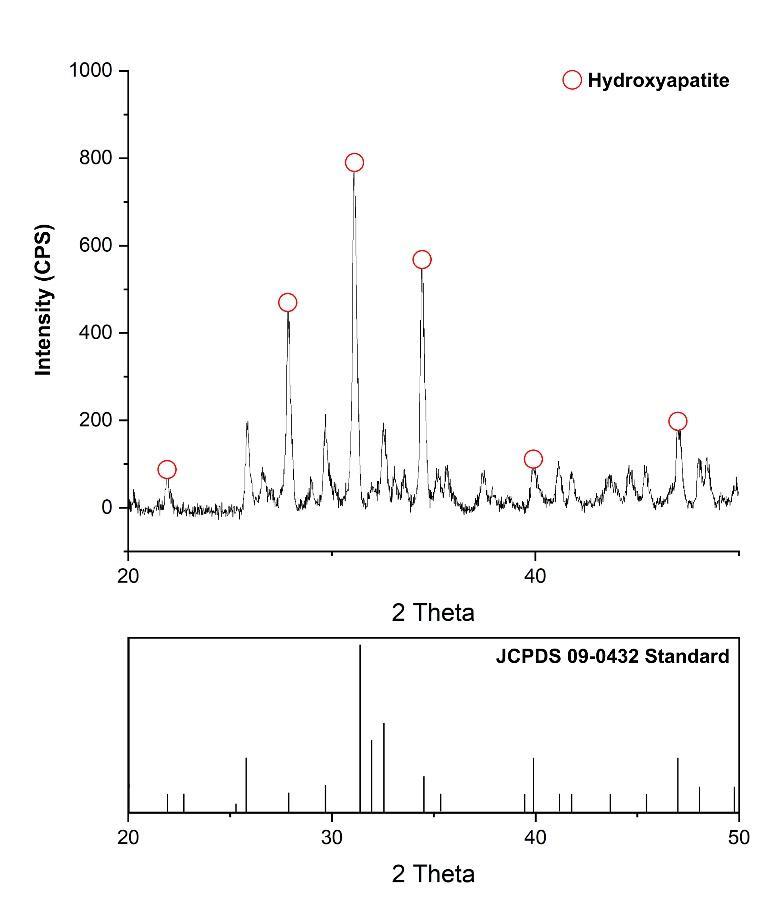
A comparison of metal bars

Description automatically generated with medium confidence

**FIGURE 2.** Results of Hydroxyapatite/Hyaluronic Acid/Chitosan Coating with Electrophoretic Deposition Method with Variables (a) Deposition Time (2, 4, 6, and 8 Minutes) (b) Concentration Hap / CS-HA (1, 2, 3, 4% w/v)

Analysis was carried out on the deposition time variable first to find out the best deposition time for thin and even coating results. In **FIGURE 2,** it can be seen that the best results are obtained at a deposition time of 4 minutes, where the coating results show the most uniform and thin results compared to minutes 2, 6, and 8. For the HAp concentration variable, uniform coating results were obtained at concentrations of 2% and 4% w/v HAp, although in variable 4% w/v HAp, the coating formed was thick so that the best variable was the variable 2% w/v HAp 4 minutes that show thin and uniform coating results compared to other variables.

## X-Ray Diffraction (XRD) Analysis



**FIGURE 3.** XRD Pattern of (a) Hydroxyapatite Synthesis (b) JCPDS 09-0432 standard

The XRD pattern of HAp synthesis was compared with the JCPDS 09-0432 standard. Based on the JCPDS standard, the three main peaks of HAp at intensities of 211, 112, and 300 are at 2θ = 31.85°, 32.31° and 32.95°[32]. In **FIGURE 3.**, the main peaks are at 2θ = 32.55°, 35.24°, and 34.59°, which means that the synthesized results are hydroxyapatite based on the similarity of the intensity of the synthesized peak with the JCPDS 09-0432 standard. Percent crystallinity was calculated by the Landi method and obtained a percent crystallinity of 84%, which means the synthesized HAp can be used for medical applications based on the FDA percent crystallinity standard for medical HAp is 62%[33]. In addition, the crystallinity of human bone is in the range of 60-87%, so the percentage of crystallinity resulting from the synthesis is by the crystallinity of the original bone.

## Fourier Transform Infrared Spectroscopy (FTIR) Analysis

FTIR is used to determine the functional group present in the synthesis result and to investigate coating chemical composition and HAp/HA/CS ion interaction. The FTIR spectra of the raw material and coating are shown in **FIGURE 4**. The main characteristic absorption bands of hydroxyapatite are the bending vibration of O-P-O fragment deformation at 500 - 670 cm-1 and the asymmetric stretching vibration of P-O bond at 800 - 1200 cm-1 of PO43- functional group as well as the stretching vibration of O-H bond at 3850 - 3800 cm-1 of OH- functional group [35]. The presence of PO43- and OH- functional groups in the FTIR results shows that the synthesis result is hydroxyapatite, which means the synthesis of hydroxyapatite from rice snail shells had been successful[32]. The presence of CO32- -functional groups in the synthesis result is because of the CaO content, which can bind free CO2 in the air during the synthesis process to form CaCO3[35], [36].

**A graph of a graph of a graph

Description automatically generated with medium confidence A graph of a graph

Description automatically generated**

**(a) (b)**

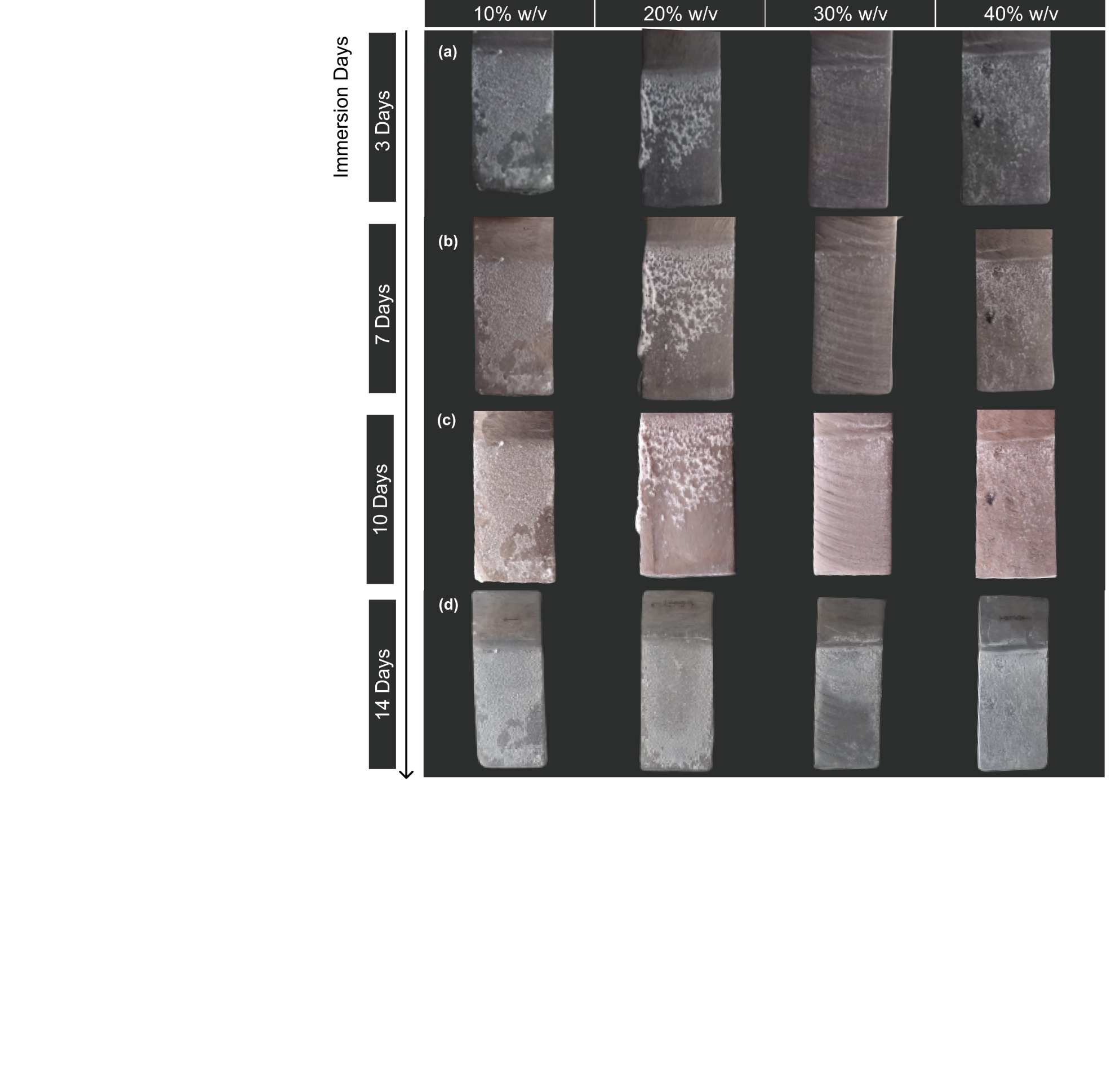
**FIGURE 4.** FTIR Spectrum of (a) raw HAp, Chitosan, and Hyaluronic acid, (b) Ti-6Al-4V coated by 10% w/v HAp, 20% w/v HAp, 30% w/v HAp, and 40% w/v HAp

**TABLE 1.** The IR bands absorption of HAp/HA/CS coating

| **Component** | **Functional Group** | **HAp Concentration** | | | |
| --- | --- | --- | --- | --- | --- |
| **10%** | **20%** | **30%** | **40%** |
| HAp | CO32- | 722.4 | 727.2 | 727.6 | 727.6 |
| PO43- | 1018.4 | 1018.6 | 1015.8 | 1021.1 |
| OH- | 3727.5 | 3753.3 | 3745.8 | 3798.2 |
| HA & CS  (Overlapping) | CO32- | 1461.2 | 1465.0 | 1461.2 | 1461.2 |
| -CH2- | 2878.6 | 2879.2 | 2883.9 | 2883.9 |
| OH- | 3365.9 | 3368.4 | 3363.3 | 3363.3 |

The absorption bands of chitosan and hyaluronic acid overlap because they have the same functional groups and bonds. The absorption bands of chitosan and hyaluronic acid overlap because they have the same functional groups and bonds. The main characteristics of CS and HA are a stretching O-H bond and stretching vibration N-H bond of the OH- functional group at 3250-3420 cm-1, a stretching symmetric methyl C-H bond of -CH2-functional group at 2850-2990 cm-1, and amide I group of C=O carboxyl and stretching C-N bond of the primary aromatic amine of CO32- functional group at 1309-1680 cm-1 [37], [38]. Based on **TABLE 1**, the presence of HAp, HA, and CS content in the coating was confirmed by the presence of their functional groups. These results indicate that the three components were successfully coated on Ti6Al4V.

## Degradation Analysis



**FIGURE 5.** Degradation observation after immersed in SBF for (a) 3 days, (b) 7 days, (c) 10 days, (d) 14 days

The degradation analysis was carried out to understand their biological capabilities further. **FIGURE 5** shows the degradation process of the coating after immersion in SBF for 14 days. Visually, the degradation process is not apparent. Therefore, the degradation behavior of the coating was observed through the weight loss analysis of the samples[41], [42]. The rate of weight loss can be calculated by,

where :

: weight loss rate;

M : the mass of the sample before immersion (gram);

: the mass of the sample after immersion (gram).

**TABLE 2.** The degradation rate of coating before and after immersion in SBF Solution

| **No** | **Variable (w/v HAp)** | **M (gram)** | **M1 (gram)** | ***Weight Loss Rate* (%)** |
| --- | --- | --- | --- | --- |
| 1. | 1% | 8.364 | 8.354 | 0.124 |
| 2. | 2% | 9.434 | 9.413 | 0.220 |
| 3. | 3% | 6.889 | 6.881 | 0.116 |
| 4. | 4% | 7.272 | 7.262 | 0.143 |

From **TABLE 2.**, the average total weight loss rate was only 0,15%, which means that the HAp/HA/CS coating has less reactivity. This is because reactivity and degradation are linearly correlated, where the less reactive the coating, the less degraded the coating [41]. To strengthen the results of this analysis, ion release analysis is required to get a better understanding of the degradation behavior of the coating.

The results of this degradation analysis can be used to confirm the bioactivity results of the coatings where the ability of the coatings to form apatite layers will faster when the coatings are more reactive, but the greater bond strength is produced by, the less reactive coatings, so the results of this analysis indicate that the HAp/HA/CS coatings have strong adhesion with slow bioactivity behavior[41].

## Corrosion Behavior Analysis

The corrosion behavior of bare Ti-6Al-4V and coated Ti-6Al-4V was analyzed using a potentiodynamic polarization test. From the polarization curves obtained, the corrosion potential (Ecorr) and corrosion current density (Icorr) can be determined using the Tafel extrapolation method. The Ecorr and Icorr values of Ti-6AL-4V obtained with and without coating are presented in **TABLE 3**. The corrosion current value (Icorr) and the corrosion potential value (Ecorr) determine how fast or slow the corrosion rate is, where the lower the Icorr value, the slower the corrosion rate, and the more positive the Ecorr, the lower the corrosion tendency[43].

**TABLE 3.** The Corrosion Parameters Derived from Potentiodynamic Curves

| **No** | **%w/v HAp** | **Ecorr (Volt)** | **Icorr (A/cm2)** | ***Corrosion Rate* (mmpy)** |
| --- | --- | --- | --- | --- |
| 1. | Ti-6AL-4V | -0.551 | 3.3546×10-6 | 0.038897 |
| 2. | 10% | -0.260 | 2.064×10-7 | 0.015796 |
| 3. | 20% | -0.264 | 1.873×10-7 | 0.014334 |
| 4. | 30% | -0.333 | 1.253×10-7 | 0.009591 |
| 5. | 40% | -0.311 | 8.848×10-8 | 0.006771 |

**FIGURE 6.** The Effect of HAp/CS/HA Concentration on Corrosion Rate

**FIGURE 6** shows the corrosion rate decreased with the increase in HAp concentration. The corrosion rate value obtained is in accordance with European medical application standards, where the requirement for a corrosion rate value is less than 0.475 mmpy[44]. The more hydroxyapatite used, the higher the layer thickness will be, thereby increasing the corrosion resistance of the implant[45].

# CONCLUSION

This research succeeded in synthesizing hydroxyapatite from rice field snail shells (*Pila ampullacea*) using ultrasound-assisted precipitation method that has been confirmed by FTIR analysis of the presence of hydroxyl (OH-) and phosphate (PO4³⁻) functional groups, which are characteristic of HAp. and XRD analysis the present of the three main peaks of HAp. This study has also successfully coated HAp, HA, and CS on Ti-6Al-4V, confirmed by FTIR analysis the presence of HAp, HA, and CS functional group with a thin and uniform coating was obtained at 20%w/v with a deposition time of 4 minutes.

The corrosion rate was found to be in the range of 0.006% - 0.038%, which is by the European medical standard for implant safety (<0.475 mmpy). Degradation analysis indicated the coatings have strong adhesion and slow bioactivity behavior with an average degradation rate of 0.1513% within 14 days of immersion. These results indicate that the coating of Nano Hydroxyapatite Based on Rice Snail Shells (*Pila ampullacea*)/Chitosan/Hyaluronic Acid has potential as a coating material for biomedical applications.

# Acknowledgments

Our gratitude goes to the relevant parties who have helped this research run smoothly, the research grant for the innovation and downstream research program of the ADB HETI fund – ITS (Grant no. 2160/PKS/ITS/2022), Department of Industrial Chemical Engineering, Faculty of Vocational Studies ITS, and Instalasi Pusat Biomaterial dan Bank Jaringan RSUD Dr. Soetomo, Indonesia.

# References

[1] S. H. Setiawan, R. A. Rahadi, and E. Sumirat, “Financial business feasibility of biomedical implant production using additive manufacturing process,” *COSTINGJournal Econ. Bus. Account.*, vol. 7, pp. 2709–2714, 2023.

[2] R. Drevet, N. Ben Jaber, J. Fauré, A. Tara, A. Ben Cheikh Larbi, and H. Benhayoune, “Electrophoretic deposition (EPD) of nano-hydroxyapatite coatings with improved mechanical properties on prosthetic Ti6Al4V substrates,” *Surf. Coatings Technol.*, vol. 301, pp. 94–99, 2016, doi: 10.1016/j.surfcoat.2015.12.058.

[3] S. V. Harb *et al.*, “Hydroxyapatite and β-TCP modified PMMA-TiO2 and PMMA-ZrO2 coatings for bioactive corrosion protection of Ti6Al4V implants,” *Mater. Sci. Eng. C*, vol. 116, no. May, p. 111149, 2020, doi: 10.1016/j.msec.2020.111149.

[4] M. Bajt Leban, T. Kosec, and M. Finšgar, “The corrosion resistance of dental Ti6Al4V with differing microstructures in oral environments,” *J. Mater. Res. Technol.*, vol. 27, pp. 1982–1995, 2023, doi: 10.1016/j.jmrt.2023.10.082.

[5] Y. C. Lin *et al.*, “Study on surface hydrogenated Ti6Al4V alloy for orthopedic implants,” *J. Mater. Res. Technol.*, vol. 28, no. December 2023, pp. 1504–1513, 2024, doi: 10.1016/j.jmrt.2023.12.066.

[6] S. Sanyal, M. Shukla, N. Dandapat, and S. Ghosh, “In vitro evaluation of bioactive glass ceramic coating for application on Ti6Al4V based biomedical implants,” *J. Non. Cryst. Solids*, vol. 500, no. October 2017, pp. 22–29, 2018, doi: 10.1016/j.jnoncrysol.2018.04.043.

[7] J. Andrade del Olmo *et al.*, “Hyaluronic acid-based hydrogel coatings on Ti6Al4V implantable biomaterial with multifunctional antibacterial activity,” *Carbohydr. Polym.*, vol. 301, no. September 2022, 2023, doi: 10.1016/j.carbpol.2022.120366.

[8] J. Andrade del Olmo *et al.*, “Multifunctional antibacterial chitosan-based hydrogel coatings on Ti6Al4V biomaterial for biomedical implant applications,” *Int. J. Biol. Macromol.*, vol. 231, no. January, 2023, doi: 10.1016/j.ijbiomac.2023.123328.

[9] M. Moradi, R. Saidi, B. Hoomehr, and K. Raeissi, “The effect of bioactive glass nanoparticles on corrosion barrier performance and bioactivity of zinc oxide coatings electrodeposited on Ti6Al4V substrate,” *Ceram. Int.*, vol. 49, no. 6, pp. 9239–9250, 2023, doi: 10.1016/j.ceramint.2022.11.088.

[10] A. Yanovska, V. Kuznetsov, A. Stanislavov, S. Danilchenko, and L. Sukhodub, “Synthesis and characterization of hydroxyapatite-based coatings for medical implants obtained on chemically modified Ti6Al4V substrates,” *Surf. Coatings Technol.*, vol. 205, no. 23–24, pp. 5324–5329, 2011, doi: 10.1016/j.surfcoat.2011.05.040.

[11] V. Müller and E. Djurado, “Microstructural designed S58 bioactive glass/ hydroxyapatite composites for enhancing osteointegration of Ti6Al4V-based implants,” *Ceram. Int.*, vol. 48, no. 23, pp. 35365–35375, 2022, doi: 10.1016/j.ceramint.2022.08.138.

[12] D. Stojanovic, B. Jokic, D. Veljovic, R. Petrovic, P. S. Uskokovic, and D. Janackovic, “Bioactive glass-apatite composite coating for titanium implant synthesized by electrophoretic deposition,” *J. Eur. Ceram. Soc.*, vol. 27, no. 2–3, pp. 1595–1599, 2007, doi: 10.1016/j.jeurceramsoc.2006.04.111.

[13] B. Beig, U. Liaqat, M. F. K. Niazi, I. Douna, M. Zahoor, and M. B. K. Niazi, “Current challenges and innovative developments in hydroxyapatite-based coatings on metallic materials for bone implantation: A review,” *Coatings*, vol. 10, no. 12, pp. 1–29, 2020, doi: 10.3390/coatings10121249.

[14] S. A. I. Jariya, K. Ravichandran, and T. S. N. S. Narayanan, “Development of novel multi-functional composite coatings on titanium: Evaluation of structural characteristics, bioactivity and corrosion behaviour,” *J. Alloys Compd.*, vol. 855, p. 157290, 2021, doi: 10.1016/j.jallcom.2020.157290.

[15] H. Ye, X. Y. Liu, and H. Hong, “Cladding of titanium/hydroxyapatite composites onto Ti6Al4V for load-bearing implant applications,” *Mater. Sci. Eng. C*, vol. 29, no. 6, pp. 2036–2044, 2009, doi: 10.1016/j.msec.2009.03.021.

[16] J. Li, T. Zhang, Z. Liao, Y. Wei, R. Hang, and D. Huang, “Engineered functional doped hydroxyapatite coating on titanium implants for osseointegration,” *J. Mater. Res. Technol.*, vol. 27, pp. 122–152, 2023, doi: 10.1016/j.jmrt.2023.09.239.

[17] J. Park *et al.*, “Improving hydroxyapatite coating ability on biodegradable metal through laser-induced hydrothermal coating in liquid precursor: Application in orthopedic implants,” *Bioact. Mater.*, vol. 25, no. July 2022, pp. 796–806, 2023, doi: 10.1016/j.bioactmat.2022.06.020.

[18] M. Javidi, S. Javadpour, M. E. Bahrololoom, and J. Ma, “Electrophoretic deposition of natural hydroxyapatite on medical grade 316L stainless steel,” *Mater. Sci. Eng. C*, vol. 28, no. 8, pp. 1509–1515, 2008, doi: 10.1016/j.msec.2008.04.003.

[19] M. Sundalian, S. G. Husein, and N. K. D. Putri, “Review: Analysis and benefit of shells content of freshwater and land snails from gastropods class,” *Biointerface Res. Appl. Chem.*, vol. 12, no. 1, pp. 508–517, 2022, doi: 10.33263/BRIAC121.508517.

[20] R. Ridha, H. Manalip, and M. R. I. A. J. Mondoringin, “Sebagai Substitusi Parsial Semen Terhadap Nilai Modulus Elastisitas,” *J. Statik*, vol. 8, no. 5, pp. 655–664, 2020.

[21] E. Edrizal and E. Desnita, “Pengaruh Cangkang Keong Sawah (Pila Ampullacea) Terhadap Pembentukan Tulang Baru (Remodeling Tulang),” *Heal. Med. J.*, vol. 2, no. 2, pp. 42–51, 2020, doi: 10.33854/heme.v2i2.559.

[22] H. B. Ardhiyanto, “Peran hidroksiapatit sebagai material,” *Stomatognatic*, vol. 9, no. 16–18, pp. 13–15, 2012.

[23] H. Krawiec *et al.*, “Corrosion Rate and Mechanism of Degradation of Chitosan/TiO2 Coatings Deposited on MgZnCa Alloy in Hank’s Solution,” *Int. J. Mol. Sci.*, vol. 25, no. 10, 2024, doi: 10.3390/ijms25105313.

[24] A. Valverde *et al.*, “Antibacterial hyaluronic acid/chitosan multilayers onto smooth and micropatterned titanium surfaces,” *Carbohydr. Polym.*, vol. 207, no. July 2018, pp. 824–833, 2019, doi: 10.1016/j.carbpol.2018.12.039.

[25] J. A. del Olmo *et al.*, “Antibacterial catechol-based hyaluronic acid, chitosan and poly (N-vinyl pyrrolidone) coatings onto Ti6Al4V surfaces for application as biomedical implant,” *Int. J. Biol. Macromol.*, vol. 183, pp. 1222–1235, 2021, doi: 10.1016/j.ijbiomac.2021.05.034.

[26] X. Chen, J. Zhou, Y. Qian, and L. Z. Zhao, “Antibacterial coatings on orthopedic implants,” *Mater. Today Bio*, vol. 19, no. December 2022, p. 100586, 2023, doi: 10.1016/j.mtbio.2023.100586.

[27] A. V. Bansod, N. N. Khobragade, K. V. Giradkar, and A. P. Patil, “Effect of concentration of hyaluronic acid and NaCl on corrosion behavior of 316L austenitic stainless steel,” *Mater. Res. Express*, vol. 4, no. 11, 2017, doi: 10.1088/2053-1591/aa94da.

[28] F. Xing *et al.*, “Hyaluronic acid as a bioactive component for bone tissue regeneration: Fabrication, modification, properties, and biological functions,” *Nanotechnol. Rev.*, vol. 9, no. 1, pp. 1059–1079, 2020, doi: 10.1515/ntrev-2020-0084.

[29] H. Maleki-Ghaleh and J. Khalil-Allafi, “Characterization, mechanical and in vitro biological behavior of hydroxyapatite‑titanium‑carbon nanotube composite coatings deposited on NiTi alloy by electrophoretic deposition,” *Surf. Coatings Technol.*, vol. 363, no. December 2018, pp. 179–190, 2019, doi: 10.1016/j.surfcoat.2019.02.029.

[30] B. Priyadarshini, M. Rama, Chetan, and U. Vijayalakshmi, “Bioactive coating as a surface modification technique for biocompatible metallic implants: a review,” *J. Asian Ceram. Soc.*, vol. 7, no. 4, pp. 397–406, 2019, doi: 10.1080/21870764.2019.1669861.

[31] A. D. Karisma, O. N. Kriswanto, and R. Rachmaningtrias, “Sintesis Nanohidroksiapatit Berbahan Cangkang Keong Sawah (Pila ampullacea) dengan Variasi Konsentrasi H3PO4 Menggunakan Metode Ultrasound Assisted Precipitation,” *Tek. Kim. FTI UPN Veteran Yogyakarta*, pp. 4–8, 2023.

[32] E. Hartati, D. Setiawan, and Y. B. Yuliyati, “Sintesis Dan Karakterisasi Hidroksiapatit (Hap) Untuk Bahan Pengikat Tungstat Dalam Sistem Generator 188w/188re,” *J. Sains dan Teknol. Nukl. Terap.*, vol. 15, no. 2, pp. 55–68, 2014.

[33] D. P. Utami, D. J. Indrani, and Y. K. Eriwati, “Peran metode modifikasi permukaan implan terhadap keberhasilan osseointegrasi,” *J. Kedokt. Gigi Univ. Padjadjaran*, vol. 31, no. 2, pp. 95–101, 2019, doi: 10.24198/jkg.v31i2.17967.

[34] Siswanto, D. Hikmawati, N. Benecdita, and S. Nurmala, “Synthesis of Hydroxyapatite Based on Nano Coral Using precipitation Method for Bone Substitution,” *J. Phys. Conf. Ser.*, vol. 1445, no. 1, 2020, doi: 10.1088/1742-6596/1445/1/012015.

[35] A. M. Kurniawan, S. Hartini, and M. N. Cahyanti, “The effect of Phosphate Concentration on Ca/P Ratio of Hydroxyapatite from Ceramic Industrial Gipsum Waste,” *Eksakta J. Ilmu-Ilmu MIPA*, vol. 19, no. 1, pp. 46–56, 2019.

[36] A. Haris, Ahmad Fadli, and S. R. Yenti, “Sintesis Hidroksiapatit dari Limbah Tulang Sapi menggunakan Metode Presipitasi dengan Variasi Rasio Ca/P dan Konsentrasi H3PO4,” *JOM FTEKNIK*, vol. 3, no. 2, 2016.

[37] A. A. Mohammed and A. K. Niamah, “Identification and antioxidant activity of hyaluronic acid extracted from local isolates of Streptococcus thermophilus,” *Mater. Today Proc.*, vol. 60, pp. 1523–1529, 2022, doi: 10.1016/j.matpr.2021.12.038.

[38] P. Upadhyay and A. Ullah, “Enhancement of mechanical and barrier properties of chitosan-based bionanocomposites films reinforced with eggshell-derived hydroxyapatite nanoparticles,” *Int. J. Biol. Macromol.*, vol. 261, no. P2, p. 129764, 2024, doi: 10.1016/j.ijbiomac.2024.129764.

[39] F. Baino and S. Yamaguchi, “The use of simulated body fluid (SBF) for assessing materials bioactivity in the context of tissue engineering: Review and challenges,” *Biomimetics*, vol. 5, no. 4, pp. 1–19, 2020, doi: 10.3390/biomimetics5040057.

[40] T. Kokubo and H. Takadama, “How useful is SBF in predicting in vivo bone bioactivity?,” *Biomaterials*, vol. 27, no. 15, pp. 2907–2915, 2006, doi: 10.1016/j.biomaterials.2006.01.017.

[41] B. Garrido, A. Martin-Morata, S. Dosta, and I. G. Cano, “Improving the bond strength of bioactive glass coatings obtained by atmospheric plasma spraying,” *Surf. Coatings Technol.*, vol. 470, no. July, p. 129837, 2023, doi: 10.1016/j.surfcoat.2023.129837.

[42] M. Supernak-Marczewska and A. Zielinski, “Effects of the origin and deacetylation degree of chitosan on properties of its coatings on titanium,” *Coatings*, vol. 10, no. 2, pp. 1–13, 2020, doi: 10.3390/coatings10020099.

[43] Z. N. Jofalo and P. H. Tjahjanti, “Analysis of Corrosion Breakdown Rate in Low Carbon Steel with Aluminum Coating,” *Procedia Eng. Life Sci.*, vol. 1, no. 1, 2021, doi: 10.21070/pels.v1i1.837.

[44] N. Susanto, E. Putri, A. Indriani, U. Himawati, and A. Aminatun, “Sintesis Paduan Kobalt melalui Teknik Peleburan dan Karakterisasinya sebagai Implan Tulang Prosthesis,” *Pekan Ilm. Mhs. Nas. Progr. Kreat. Mahasiswa-Penelitian 2013*, 2013.

[45] N. Mulya, A. Fadli, and A. Amri, “Pengaruh Penambahan Hidroksiapatit dan Waktu Pencelupan Terhadap Pelapisan Logam Stainless Steel 316L Dengan Metode Dip Coating,” *Jom FTeknik*, vol. 3, no. 1, pp. 1–7, 2016.