Effect of Bovine Collagen on Carbonate-Hydroxyapatite/Titanium Coating: Compressive Strength and Bioactivity Analysis

Diana Julaidy Patty*, Ari Dwi Nugraheni, Ika Dewi Ana, and Yusril Yusuf

***Abstract*—This study aims to analyze the effect of collagen in Titanium/carbonate-hydroxyapatite from *Pinctada maxima* shells successfully applied in Ti substrate using electrophoretic deposition (EPD) techniques. The pretreatment with HCL on the substrate reduced the compressive strength with a porous surface and excellent adhesion during the EPD process. It is proven that Ti/CHA-1, Ti/CHA-3, and Ti/CHA/Coll increase compressive strength. Furthermore, bioactivity in SBF immersion showed apatite growth on the coating surface, increasing the size of the apatite crystals. The effect collagen in Ti/CHA/Coll substrate showed lower crystallinity and mechanical strength compared to Ti/CHA substrates, but the SEM morphology of bioactivity Ti/CHA/Coll significantly showed the apatite growth on the Ti substrate surface. Ti/CHA/Coll showed excellent mechanical properties and bioactivity as a bone graft substitute.**

***Index Terms*—Collagen, carbonate-hydroxyapatite, titanium-bioactivity, compressive-strength**

I. Introduction

The elderly group has high cases of bone trauma (osteoporosis, bone cancer, and accidents), so it is necessary to engineer bone tissue in the spine, hips, to knees with biomaterials such as Hydroxyapatite (HA), Carbonate-Hydroxyapatite (CHA), apatite carbonate, and materials that have mineral components like bone constituents. Dentin and bone have many similarities, consisting of 70% by weight of nanoscale apatite crystals and organic matter, mainly collagen [1]. The application of biomaterials in bone tissue engineering requires low elastic modulus, good mechanical properties, corrosion resistance, and biocompatible and without cytotoxicity. A combination of bioceramics, metals, polymers, and composites has been developed as a bone replacement material, and more than 70% is the implantation of bioceramics-metal integration. Metals often utilized in bone tissue engineering are titanium and its alloys, stainless steel, and chromium cobalt alloys [2]

HA and CHA are bioactive materials synthesized from clamshells such as *Pinctada maxima* (*P.maxima*) [3–6], and crab shells through co-precipitation. The advantage of the *P.maxima* shell has Young's modulus of 30–40 GPa for nacre versus 20 GPa for bone, and resistance to fracture is 185–200 MPa versus 140 MPa. [7]. Previous research has developed the application of HA in the coating of Ti substrates with modifications of polymer composites by the Electrophoretic Deposition (EPD) method [8], gel sols [9], Electrophoretic Dip Deposition (EP2D) [10, 11], spin coating [12] biomimetic nano-apatite coating [13], and composite coating [14]. This study aims to analyze the effect of collagen in Titanium/carbonate-hydroxyapatite coating using electrophoretic deposition (EPD) techniques on physicochemical properties, mechanical properties, and bioactivity in SBF solution.

Manuscript received August 8, 2023; revised September 10, 2023; accepted October 26, 2023.

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II. Procedure of Coating Substrate

The CHA powder was applied to the coating Ti substrate, synthesized from the *P.maxima* shell as previously reported [3], and the CHA slurry was calcinated at 1100 ºC for two hours. Bovine collagen was purchased from a distributor co. id 116 Jakarta technoboga. Substrate Ti (ASTM B265-GR.1) was purchased from NET ARTIDAYA as Engineering and Industrial Support Company in Bekasi, Indonesia.

EPD Process. Ti substrate preparations**.** Pretreatment of substrate Ti (20×10×0.8 mm) by etching Ti metal in 12 M HCL (10 mL) while heated at 80 ºC for 1 h, then rinsed with distilled water and dried.

Preparation of suspense for EPD. The solution for EPD uses CHA (1 %, 3 %) and collagen 1%. The 0.44 g CHA was dissolved in 40 mL of distilled water; for suspense, 0.14 g collagen bovine was added in 10 mL (1 %). The EPD process used two Ti substrates as cathode and anode. Ti substrates were an immersion into CHA solutions (1% and 3 %), coded Ti/CHA-1 and Ti/CHA-3, and CHA/Coll suspension coded Ti/CHA/Coll. The solution was stirred with a magnetic stirrer (Thermo Fisher Scientific, Waltham, MA, USA) with a DC voltage source of 50 V with an immersion time of 10 min. The substrate coating was rested at room temperature, then annealed at 900 ºC for three hours.

Characteristics of samples. The Physicochemical properties of CHA and Ti substrate layers were analyzed using XRD X'Pert Pro-Japan Type devices with Cu-Ka radiation wavelengths of 0.154 nm. To identify typical diffraction peaks from CHA and Ti substrate using JCPDS data. In addition, the XRD spectra are used to analyze the crystal size and crystallinity of the sample. SEM morphology on CHA and Ti coating before and after SBF immersion was observed using Joel JSM-6510LA-1400-Japan.

Bioactivity in SBF Solution. The bioactivity of *the scaffold* was investigated by immersion in an SBF solution by

DOI: 10.7763/IJET.2023.V15.1235

Kokubo. SBF was propped up to a pH of 7.4 at 37 °C by adding 1 M HCl and Tris (hydroxymethyl) aminomethane [15]. The bioactivity test lasted 3 and 9 days, with the solution refreshed every two days. The incubation process is carried out in an incubator with a temperature of 37 °C at LPPT UGM.

Mechanical Properties. The mechanical properties of Ti substrate coating using a universal testing machine device (UTM, type TN20MD, Controllab, Paris, France).

III. Result and Discussion

Physico-Chemical Properties of CHA and Ti Substrates. The FTIR of CHA in Fig. 1a show identical band of OH^-, CO_3^{2-} and PO_4^{3-}. Based on XDR spectra (Fig. 1b) with a sharp peaks, have a grain size is 33.40 nm (using the Scherrer equation), and crystallinity of 86%, with stoichiometric (Ca/P) 1.72, and the bone was 1.71. CHA characteristics based on the morphology of SEM and EDS in Fig. 1(c) show crystalline granules with phosphate, calcium, and oxygen elements.

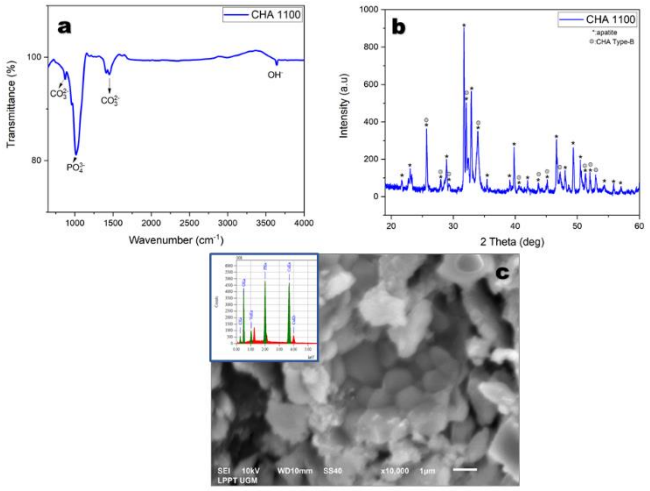

Fig. 1. (a) FTIR, (b) XRD and (c) SEM-EDX of CHA

Spectra XRD of Ti/CHA-1, Ti/CHA-3, and Ti/CHA/Coll in Fig. 2 shows a sharp peak, and a narrow band was identified phase based on Ti (JSPDS no.89-5009), and CHA type-B (JSPDS no.19-0272). The estimated crystal size in Ti/CHA-1, Ti/CHA-3, and Ti/CHA/Coll is 42.02 nm, 38.88nm, and 35.22nm, respectively, with a crystallinity of 63%, 68%, and 57%. Ti/CHA/Coll has the lowest crystallinity and a smaller crystal size due to the substitution of collagen. Collagen is amorphous and affects the crystallinity of the coating. The nanoparticle size and high crystallinity will affect the mechanical strength of the implant. The higher the crystallinity, the more the implant strength increases. The size of the nanoparticles also plays an important role in coating the Ti substrate because it can cover surfaces and small pores evenly, reduce the volume of blanks, and increase the density of the bond between CHA and Ti substrates. An evenly closed surface increases rigidity and can protect against external influences [16].

The SEM morphology in Fig. 3 shows the surface uniformly closed and without cracking after high-temperature annealing. Cross-sections (white and red box) on the substrate show a layer thickness range of 20-148 µm. Coating thickness with the EPD method shows that this process has a thickness of 10–200 µm to thousands [2]. The coating thickness on each substrate (red box) shows the level of thickness and more in Ti/CHA-3 substrate because of the CHA-3%.

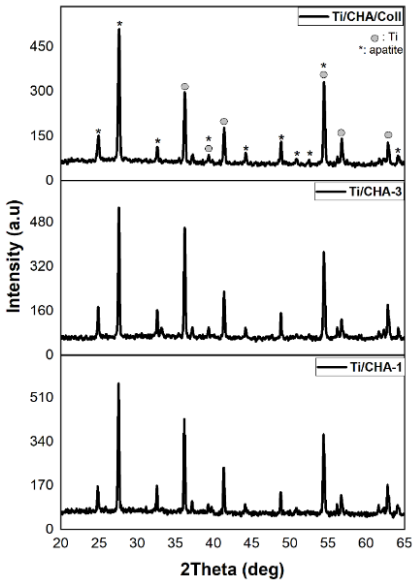

Fig. 2. XRD Spectra of CHA coating; Ti/CHA-1, Ti/CHA-3, and Ti/CHA/Coll.

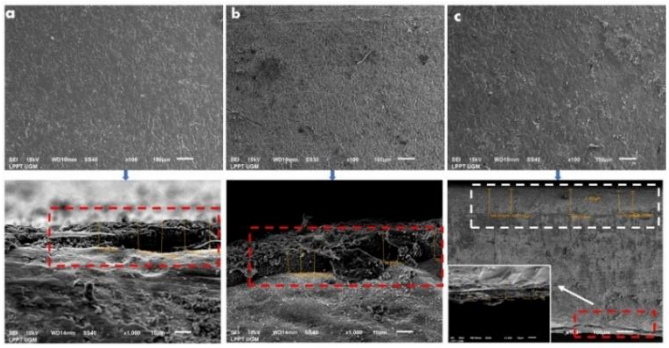

Fig. 3. SEM morphology of Ti substrate and cross-section on (a) Ti/CHA-1, (b) Ti/CHA-3, and (c) Ti/CHA/Coll

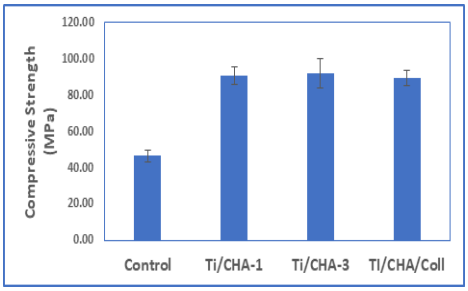

Fig. 4. Compressive strength of control and substrates coating of CHA.

Compressive Strength of Ti. Fig. 4 shows the compressive strength of Ti/CHA-1, Ti/CHA-3, and Ti/CHA/Coll was 90.84±4.11 MPa, 92.14±8.62 MPa, and 89.39±4.08 MPa, respectively, and control/uncoated-Ti 46.71±3.26MPa, were trabecular bone 0.2–80 MPa[17]. The mechanical strength of control by previous studies [18], 83.30±10.15 MPa, used the same type of Ti substrate but a different pretreatment using 1500 grit sandpaper and soaking in acetone again for one night. The test results showed Ti/CHA-3 had the most compressive strength due to CHA

(3%) high concentration with coating thickness investigated through SEM morphology. In addition, collagen substitution in the Ti/CHA/Coll reduced the substrate's compressive strength.

Bioactivity of Ti Substrates. The bone-bonding ability of a material is often evaluated by examining the ability of apatite to form on its surface in Simulated Body Fluids (SBF) with ion concentrations close to that of human blood plasma. Evaluation of apatite growth on Ti substrate coating in SBF helps predict bone bioactivity in vivo and significantly reduces the use of test animals. Bioactivity assay of Ti/CHA-1, Ti CHA-3, and Ti/CHA/Coll in SBF immersion for 3 and 9 days, and SEM-EDS analyzed the composition of the elements before and after immersion in SBF immersion. The surface morphology of Ti substrate coating before immersion in SBF in Fig. 5 (a)–(c) shows agglomeration, porous, and solid apatite crystals, particularly on Ti/CHA/Coll. In addition, the EDS spectra identified calcium, phosphorus, titanium, and oxygen. After SBF immersion for 3 to 9 days, the apatite growth significantly with a porous agglomeration surface. Collagen substitution in Ti/CHA/Coll coating significantly impacts apatite growth in SBF solution. Collagen is a major constituent of bone mass, significantly increasing bone organic matter and Bone Mineral Density (BMD) [19].

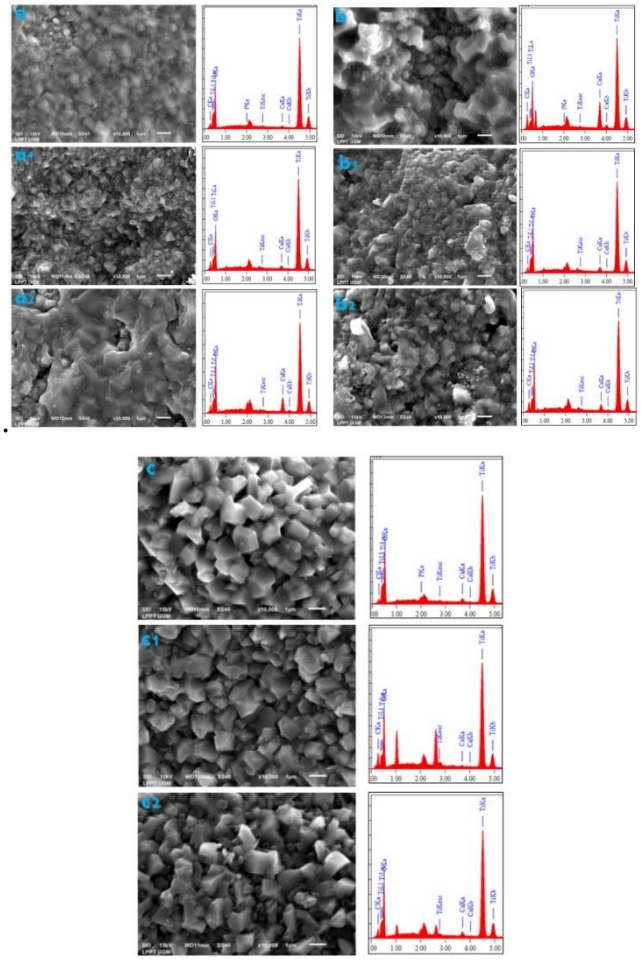

Fig. 5. SEM Morphology after SBF immersion for 3-9 days of (a1-a2) Ti/CHA-1, (b1-b2) Ti/CHA-3, and (c1-c2) Ti/CHA/Coll.

IV. Conclusion

CHA synthesized successfully applied in Ti substrate coating. The pretreatment on Ti substrate (in HCl) reduced the mechanical compressive strength by 46.71±3.26 MPa but provided a rough and pores surface and good adhesion, proven increases the compressive strength of Ti/CHA-1, TI/CHA-3, and Ti/CHA/Coll, were 90.84±4.11, 92.14±8.62, and 89.39±4.08 respectively. Bioactivity by SBF immersion (3–9 days) showed the growth of apatite on the surface, with an increase in the size of the apatite crystals, which were solid, porous, and agglomeration. EDS spectra also identified the elements of calcium and phosphorus. Ti substrate coating with CHA and Coll showed excellent mechanical properties and bioactivity as a bone graft substitute.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Conceptualization: Diana Julaidy Patty (DJP), Ari Dwi Nugraheni (AND), Ika Dewi Ana (IDA), Yusril Yusuf (YY). Data curation: DJP, AND, IDA, YY. Formal DJP, AND, IDA, YY. Investigation: DJP. Methodology: DJP, AND, IDA, YY. Project administration: DJP, YY. Resources: DJP, YY. Software: DJP. Supervision: AND, IDA, YY. Validation: DJP, AND, IDA, YY Visualization: DJP, AND, IDA, YY. Writing: original draft: DJP. Writing: review & editing: DJP, AND, IDA, YY. All authors had approved the final version.

Funding

This research was supported by Indonesian Endowment Fund for Education (LPDP KET-1569/LPDP.4/20.

Acknowledgment

The authors thank the Indonesian Endowment Fund for Education for supporting this research and Material Physics Laboratory at the Universitas GadjahMada, Indonesia.

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