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CALCIUM CARBONATE DEPOSITION ON Ti-6Al-6Mo

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Abstrak

Oseointegrasi adalah salah satu properti penting dalam pengembangan material untuk aplikasi implan tulang. Meskipun material logam biokompatibel seperti paduan titanium sudah memiliki properti biokompatibel bawaan yang sudah mencukupi sebagai material implan tulang, sifat oseointegrasi masih dapat ditingkatkan dengan pelapisan biokeramik. Kalsium karbonat ($CaCO_3$) dan hidroksiapatit adalah dua biokeramik utama pada tulang yang dapat dimanfaatkan untuk meningkatkan sifat oseointegrasi pada material implan. Tantangan saat ini pada pelapisan biokeramik pada material implan adalah memperoleh metode pelapisan yang mudah diterapkan dan ekonomis untuk selanjutnya diterapkan di industri. Pada penelitian ini dilakukan sebuah metode yang sederhana untuk mendeposisi kalsium karbonat pada permukaan Ti-6Al-6Mo. Pada kegiatan ini digunakan dua larutan biomimetik yang sudah secara luas digunakan, yaitu PBS *(phosphate buffer saline)* dan SCS (*supersaturated calcification solution*) untuk membuat pembentukan kalsium karbonat pada permukaan Ti-6Al-6Mo. Pengamatan struktur mikro dan elemental dengan SEM (*scanning electron microscope*) – EDS (*energy dispersive spectroscopy*) menunjukkan keberadaan deposit kalsium karbonat pada permukaan Ti-6Al-6Mo. Lebih lanjut, analisa kristalografi dengan difraksi sinar-x (XRD) juga menguatkan keberadaan deposit kalsium karbonat pada permukaan Ti-6Al-6Mo. Metode yang diajukan juga diterapkan pada Ti murni (>95%) sebagai perbandingan dan diperoleh hasil yang serupa. Pengaruh durasi perendaman juga diamati dalam penelitian ini. Hasil dari imersi dengan durasi 7 dan 10 hari tidak menunjukkan perbedaan yang signifikan.

Kata Kunci: Kalsium karbonat, implan, larutan kalsifikasi lewat jenuh, Ti-6Al-6Mo

Abstract

Osseointegration is one of important property in development of implant materials for orthopedic applications. While biocompatible metallic materials such as titanium alloys should already have adequate biocompatibility properties as implant materials, their osseointegration property could be further improved by bioceramic coating. Calcium carbonate ($CaCO_3$) and hydroxyapatite are two major bioceramics in bones that can be utilized to improve the osseointegration property of metallic implant materials. Current challenge on bioceramic coating of metallic implant materials is to obtain coating method that is facile and economically feasible for implementation in the industry. The current activity proposes a simple and straightforward method to deposit calcium carbonate on Ti-6Al-6Mo. Two common biomimetic solutions were utilized in current activity PBS (phosphate buffer saline) and SCS (supersaturated calcification solution) to induce the calcium carbonate formation on the Ti-6Al-6Mo surface. Microstructural and elemental observations by SEM (scanning electron microscope) – EDS (energy dispersive spectroscopy) has shown the presence of calcium carbonate on the surface of the Ti-6Al-6Mo. Moreover, the crystallography analysis by XRD (x-ray diffraction) also confirmed the formation of calcium carbonate on the surface of Ti-6Al-6Mo. We also studied the proposed method on pure Ti (>95%) as comparison and similar outcomes were also observed. The effect on duration of immersion was also accounted in current setting. The outcomes of immersion duration for 7 and 10 days were not significantly different.

Keywords: *Calcium carbonate, implant, supersaturated calcification solution (SCS), Ti-6Al-6Mo*

1. INTRODUCTION

As osseointegration is an important property to be considered in orthopedic implant material development. Osseointegration is defined as the capability for an implant material to integrate structurally and functionally into the bone living tissue [1]. While metallic orthopedic implant materials such as titanium alloys, cobalt alloys, and steel alloys might provide sufficient biocompatibility properties, their osseointegration property could be improved.

One way to improve the osseointegration is by coating. The coating can be done by several methods such as biomimetic, electrodeposition, chemical or physical vapor deposition, and thermal spray or HVOF (high-velocity oxygen fuel) [2]. Among these methods, comprehensive studies have been focusing on the development of solution-based or biomimetic coating method of bioceramics onto various metallic implant materials including titanium alloys [3]. This coating method is preferable due to its ease of application which requires minimum instruments compared to other methods [4].

The application of bioactive coatings on the surface of a titanium implant is one of the most important ways to improve the surface properties [5]. Moreover, the degradation of the bioactive coatings could be associated with the increased osteoconductivity and enhanced bone-implant contact [6]. Both calcium phosphate and calcium carbonate are resorbable *in vivo.* Calcium carbonate was widely known in the field of bone regeneration due to its biodegradation and osteconductivity [7]. However, the effectiveness of bioactive coating have not yet been demonstrated as the coating cannot be achieved using spray coating and spin coating since the bioactive layer will be decomposed by heat treatment [8].

Here, we propose a study about calcium carbonate deposition onto Ti-6Al-6Mo by the biomimetic route. The Ti-6Al-6Mo has been developed in previous research as a candidate to substitute the current standard implant material, Ti-6Al-4V due to its content of vanadium which might be toxic and harmful if released [9]-[12]. It is expected that the deposition of calcium carbonate would improve its biocompatibility properties.

2. MATERIALS AND METHODS

2.1. Sample Preparation

There are two variants of the sample: pure Ti (>95%) and Ti-6Al-6Mo. The pure Ti sample was commercially obtained as a rod which was cut into a 5x5x2 mm plate using a wire cut. The Ti-6Al-6Mo was alloyed using an arc furnace and rolled into a plate which was then cut into a 5x5x2 mm plate using a wire cut. The mechanical and microstructural properties of the Ti-6Al-6Mo can be found in previous publications [9]-[12]. The Ti and Ti-6Al-6Mo plates were then cleaned using ethanol, acetone, and aquadest in an ultrasonic water bath for 15 minutes each. After that, the samples were air-dried at room temperature.

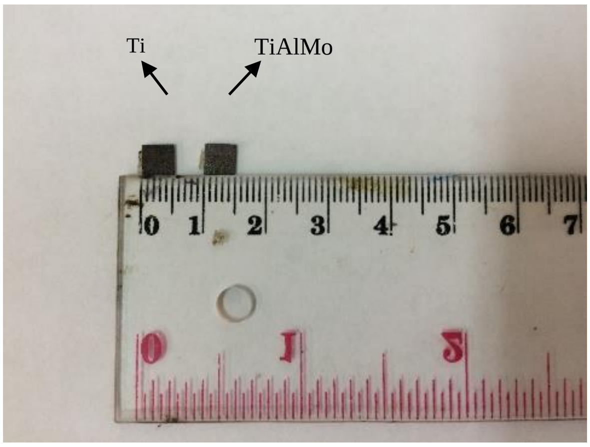


Figure 1. Titanium and Ti-6Al-6Mo plates

2.2. Solution Preparation

The PBS (phosphate buffered saline) and SCS (supersaturated aclcification solution) solutions are two among various synthetic chemical solutions that have similar ionic properties with human body fluid. They have been used in various research involving biomimetic methods and simulation tests on biocorrosion of implant materials in the human body. The PBS solution was prepared as standard stock in the laboratory. The PBS solution composition is shown in Table 1. Meanwhile, the SCS solution was prepared by mixing $CaCl_2.2H_2O$, $NaH_2PO_4.H_2O$, and $NaHCO_3$ in deionized water [13]. The SCS solution composition is shown in Table 2.

Table 1. PBS (phosphate buffered saline) solution composition

Component	Concentration (mM)
NaCl	137
KCl	2.67
Na_2HPO_4	8.10
KH_2PO_4	1.47
$CaCl_2$	0.90

Table 2. SCS (supersaturated aclcification solution) solution composition

Component	Concentration (mM)
$CaCl_2.2H_2O$	5.0
$NaH_2PO_4.H_2O$	2.5
$NaHCO_3$	1.5

2.3. PBS and SCS Immersion

Following sample and solution preparation, the pure Ti and Ti-6Al-6Mo plates were grouped depending on the solution and duration for immersion. Both pure Ti and Ti-6Al-6Mo plates were fully immersed in horizontal orientation. The immersion durations were varied for 7, 10, and 14 days in ambient temperature. The usual immersion time for the biomimetics method using SBF (simulated body fluids) is 7 days. By prolonging the duration of immersion to 10 and 14 days, the deposition of $CaCO_3$ preferably became thicker or covered the surface of the plates more evenly. The immersion solutions were replaced every two days to keep the ionic concentration stable. Table 3 shows the sample grouping with immersion solution and duration variations.

Table 3. Sample Ti and TiAlMo grouping

Sample code	Immersion solution	Immersion duration (days)
Ti SCS7	SCS	7
Ti SCS10	SCS	10
Ti SCS14	SCS	14
TiAlMo SCS7	SCS	7
TiAlMo SCS10	SCS	10
TiAlMo SCS14	SCS	14
Ti PBS7	PBS	7
Ti PBS10	PBS	10
Ti PBS14	PBS	14
TiAlMo PBS7	PBS	7
TiAlMo PBS10	PBS	10
TiAlMo PBS14	PBS	14

2.4. Cleaning and Drying

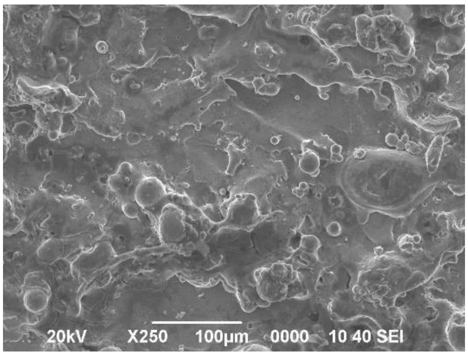
After immersion under specified duration, the pure Ti and Ti-6Al-6Mo were washed using distilled water and dried on a hotplate at 60 °C for an hour.

2.5. XRD and SEM Characterizations

All pure Ti and Ti-6Al-6Mo plates were immersed and dried before characterized using SEM-EDS (scanning electron microscope-energy dispersive spectroscopy) and XRD (x-ray diffraction). XRD characterization would provide information regarding elemental formation in the samples. XRD characterization was conducted at 2θ = 20-120° using XRD Bruker with Cobalt *x-ray* source. The morphological and chemical composition of calcium carbonate deposition was observed using SEM coupled with EDS. The SEM instrument used was JEOL JSM-6390A model with 250X and 500X magnification. The samples were coated using gold sputtering for 20 seconds to prepare the surface conductivity for SEM investigations.

3. Results and Discussions

Figures 1(a)-1(c) and 2(a)-2(c) presented the morphological of Ti and Ti-6Al-6Mo plates that were immersed in PBS (phosphate buffered saline).



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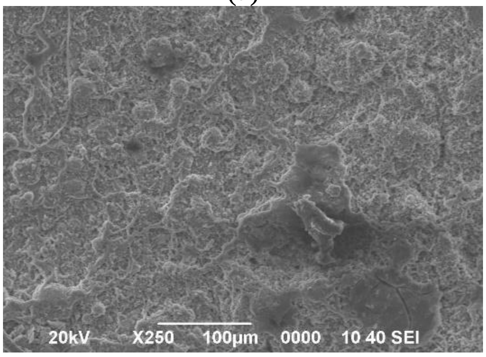

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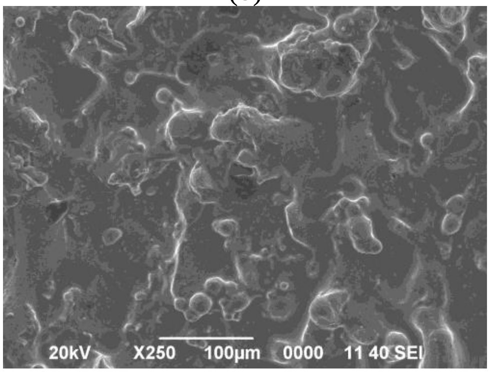

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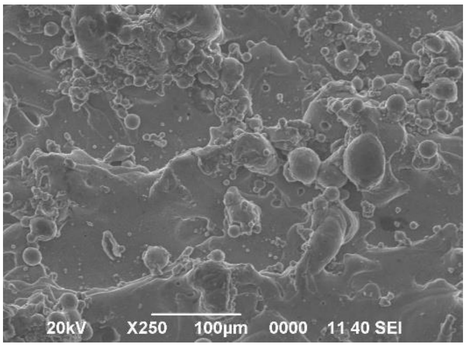
Figure 1. Surface morphological of (a) Ti PBS7, (b) Ti PBS10, and (c) Ti PBS14

The surface morphological examination of Dulbecco's PBS immersed Ti and Ti-6Al-6Mo plates show an uneven and rough surface with varied surface topography. The surface looks homogenous with no distinctive or visible deposition of calcium carbonate whether, on 7 (Ti PBS7 & TiAlMo PBS7), 10 (Ti PBS10 & TiAlMo PBS10), or 14 days (Ti PBS14 & TiAlMo PBS14) immersed plate.



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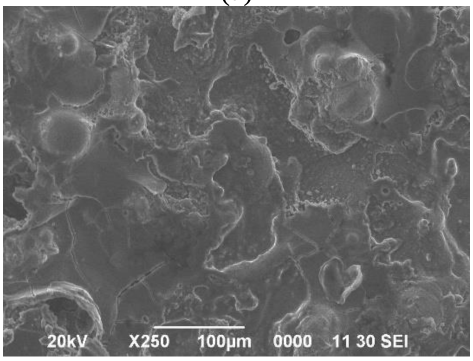

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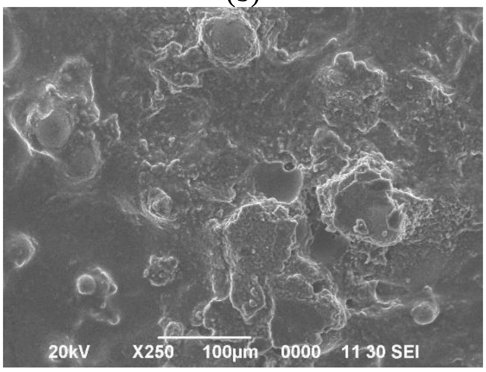

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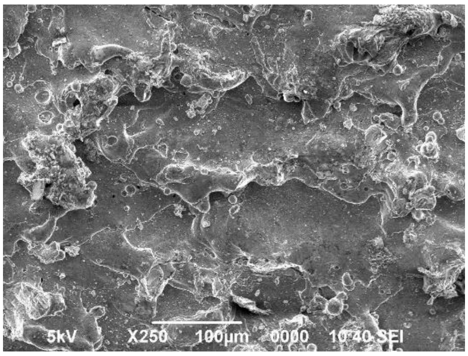
Figure 2. Surface morphological of (a) TiAlMo PBS7, (b) TiAlMo PBS10, and (c) TiAlMo PBS14

The surface morphological of SCS (supersaturated calcification solution) immersed Ti and Ti-6Al-6Mo plates were shown in Figs. 3(a)-3(c) and Figs. 4(a)-4(c). The surface morphological of SCS-immersed Ti and Ti-6Al-6Mo plates showed similar features to the PBS immersed one, which is uneven and rough. The difference with the PBS group was there is some whitish deposition of suspected calcium carbonate on the SCS immersed group. The suspected calcium carbonate deposit was not uniformly covered the surface and looked agglomerated, as shown in Figs. 3(a)-3(c) and Figs. 4(a)-4(c). All of the plate surfaces didn't have a good, uniform morphology as shown by the SEM (scanning electron microscope) results. This could be attributed to the wire cut process and no polishing treatment after cutting. The polishing process could increase the uniformity of the surface and reduce the extreme differences of the surface contour [14]. The extreme contour of the plate surfaces made it hard to characterized using AFM (atomic force microscope). The AFM probes couldn't generate the surface images due to the height of one part of the surface were too high while the other part was too low.



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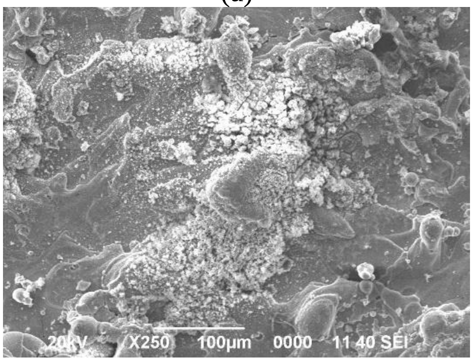

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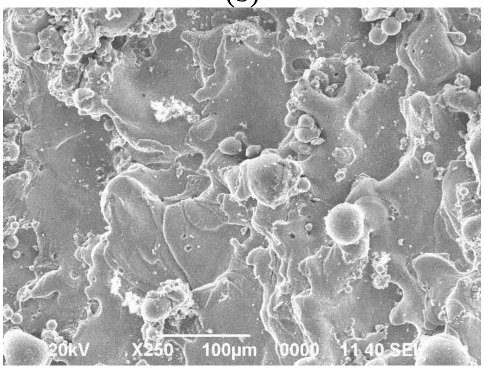

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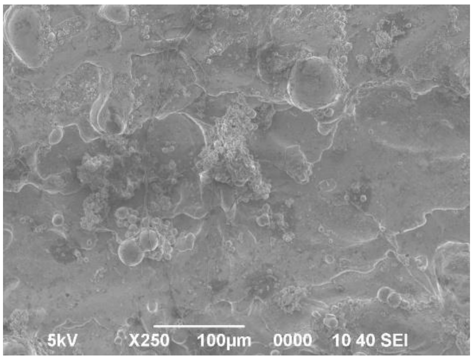
Figure 3. Surface morphological of (a) Ti SCS7, (b) Ti SCS10, and (c) Ti SCS14



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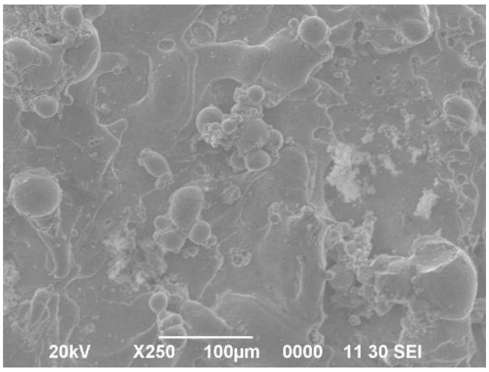

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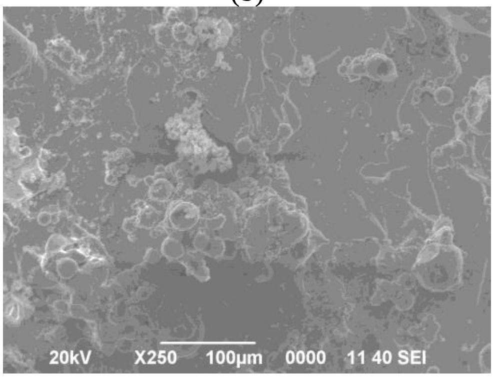


(c)

Figure 4. Surface morphological of (a) TiAlMo SCS7, (b) TiAlMo SCS10, and (c) TiAlMo SCS14

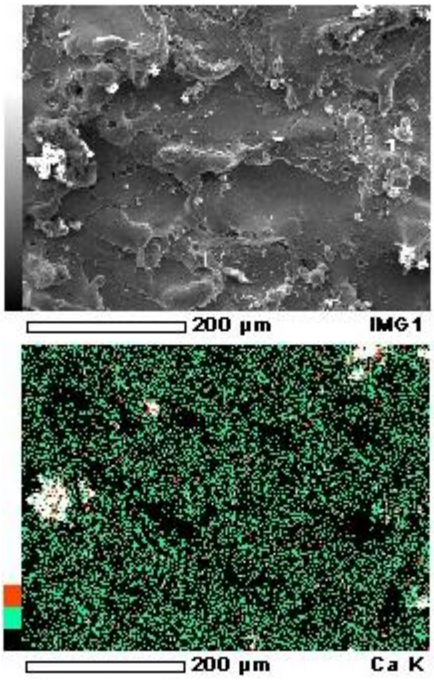


Figure 5. EDS mapping of TiSCS7 surface

The element mapping of Ti SCS7, Ti SCS10, and Ti SCS14 was shown in Figs. 5-7. The white color of EDS (energy dispersive spectroscopy) mapping results indicates the whitish compound on the plate surface as calcium, while the other color showed on the mapping results are indicating other elements. This could be an indication of calcium carbonate deposition was successfully deposited using the biomimetic route. The mapping also shows that the calcium was not uniformly covered the plate.

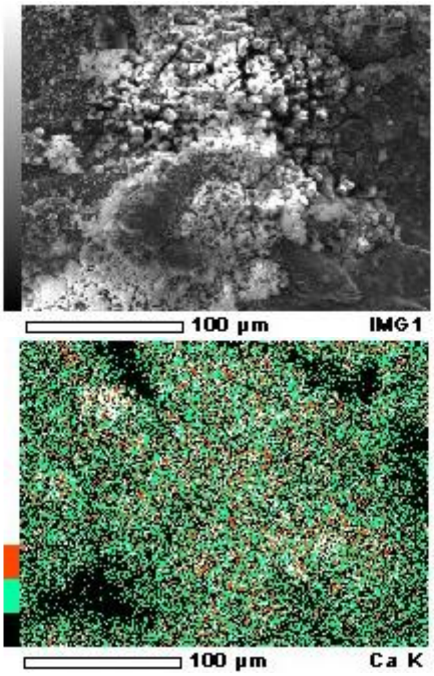


Figure 6. EDS mapping of TiSCS10 surface

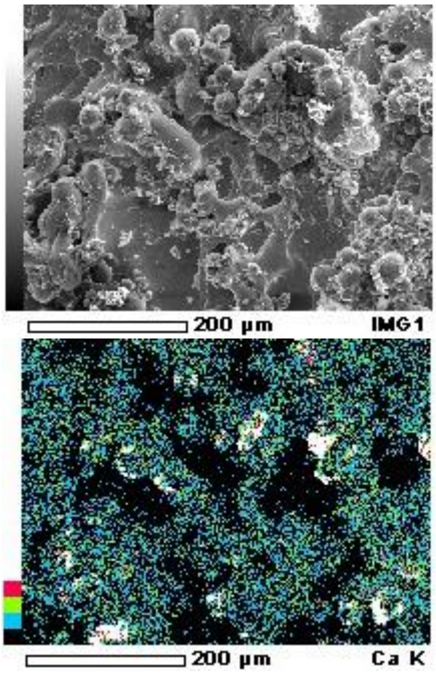
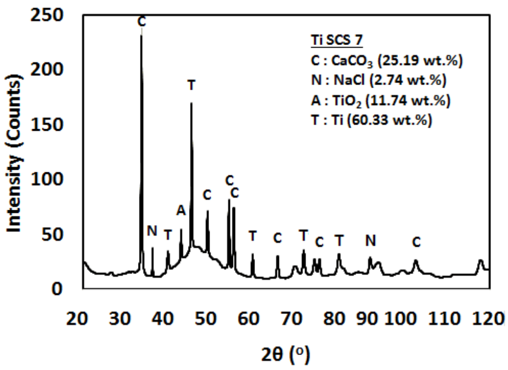


Figure 7. EDS mapping of TiSCS14 surface

Figures 8(a)-8(c) and 9(a)-9(c) show the XRD (x-ray diffraction) characterization results of the SCS immersed plate. Based on XRD results in Figure 8(a)-(c) it was confirmed that the calcium on the surface was calcium carbonate.



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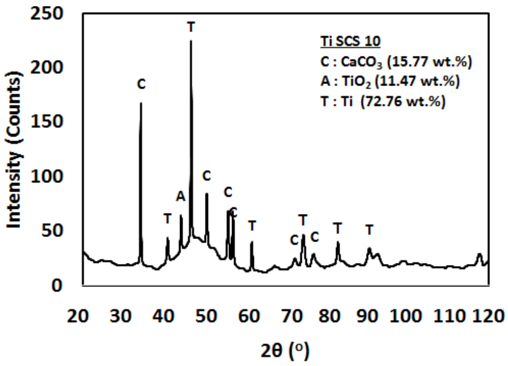
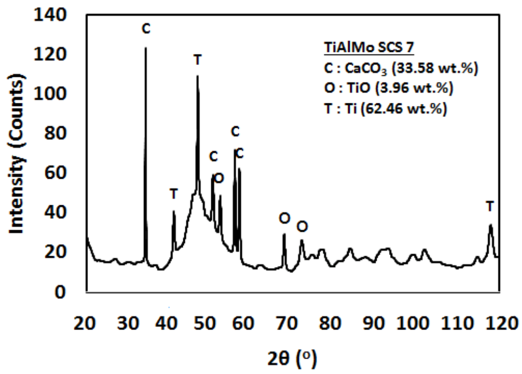


(b)

Figure 8. XRD plot of (a) TiSCS7 and (b) TiSCS10 immersed in SCS for 7 and 10 days

The Ti SCS7 was immersed for 7 days shows higher intensity than Ti SCS10 which was immersed for 10 days. This could be attributed due to the calcium carbonate on Ti SCS7 was more agglomerated than the calcium carbonate on Ti SCS10. The quantitative analysis of TiSCS7 and TiSCS10 shows a similar trend with the peak intensity. The TiSCS7 has 25.19 wt.% of $CaCO_3$ while TiSCS10 got 15.77 wt.%. This indicates the longer the immersion time doesn't always affect positively on $CaCO_3$ deposition process.

The Ti-6Al-6Mo plates which were immersed in SCS also indicated the calcium carbonate formation as shown in Figs. 9(a)-9(c). The intensity of the $CaCO_3$ XRD peak on the Ti-6Al-6Mo group increased a little along with the increasing duration of immersion. On the other hand, the quantitative analysis of the XRD shows conflicting results with the intensity of the peak. TiAlMoSCS7 has 33.58 wt% and TiAlMoSCS10 has 29.44 wt% of $CaCO_3$. This shows the higher intensity of the peak doesn't always go hand in hand with the quantity of the compounds. The thickness of $CaCO_3$ deposition on the 7^{th}, 10^{th}, and 14^{th} day couldn't be determined whether using SEM or AFM. The AFM probe couldn't generate the image of the surface due to the extreme difference of the plate's surface contour.



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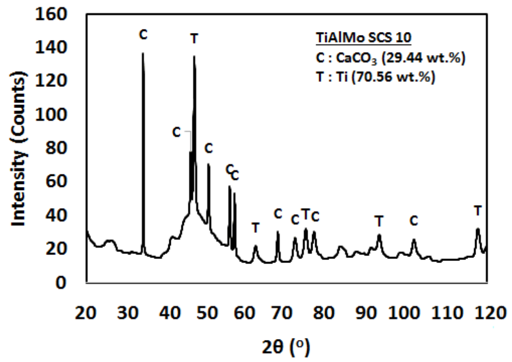

(b)

Figure 9. XRD plot of (a) TiAlMo SCS7 and (b) TiAlMo SCS10 immersed in SCS for 7 and 10 days

Molybdenum is selected due to its high capability as a beta phase stabilizer and more fair price compared to vanadium [9]. The previous experiment has shown that the Ti-Al-Mo exhibited both alpha and beta phases with hardness value ranges from 35 to 40 HRC in various thermomechanical conditions [9]. A previous study also suggested that solution-treated followed by air quenching promotes the hardness value of Ti-Al-Mo compared to water quenching due to higher alpha phase intensity compared to alpha' (α') and beta (β) phases [10].

The agglomerated and uneven deposit of calcium carbonate on the plate surface shows the deposition process was not fully complete. There is multiple causes which contributed to the incomplete deposition of calcium carbonate. First, it could be attributed to the lack of active ion on the plate surface. While using the same SCS formula, our findings differ from Ciobanu et al. This might be due to differences in the method. Here, the sample didn't expose to strong acid/base solution before immersion in SCS. In Ciobanu et al, the samples were alkaline treated using NaOH before treatment using SCS to form sodium titanate ($Na_2Ti_5O_{11}$) on the Ti-6Al-4V surface. In SCS, the sodium ions are released and exchanged with H_3O ions forming Ti-OH groups.

Later, the Ca ions merge with the Ti-OH groups forming amorphous calcium titanate. Finally, the calcium titanate merges with phosphate ions forming amorphous calcium phosphate. Their result was validated by SEM-EDS and XRD [13].

Another titanium pre-treatment to improve the calcium carbonate deposition is via heat treatment to transform the surface into rutile which proved to be highly bioactive [15]. The method to transform the inert native oxide of titanium surface into rutile consist of oxidizing the titanium plate in a furnace at 500-800 °C with 1 hour holding time, followed by furnace cooling after the plate is cleaned in an ultrasonic bath. This process will affect the zeta potential of the titanium surface. When the Ti plate had a positively charged surface, it will selectively be adsorbing the negatively charged chloride ions onto the surface. As the chloride began to accumulate the surface charge has become negative, therefore the positively charged calcium ions are adsorbed on the surface to form a calcium carbonate layer [16].

The immersion solution temperature also held an important role in the deposition process of the biomimetic route. The temperature should be around 36-37 °C to mimic human body temperature [17].

The future experiments should include the in vitro test to confirm the bioactivity and biocompatibility of the calcium carbonate coating.

4. CONCLUSIONS

The deposition of calcium carbonate on Ti and TiAlMo plate using biomimetic route were performed. Micrograph and elemental observations show the presence of calcium carbonate on the surface of the Ti-6Al-6Mo immersed in SCS. The outcomes of immersion duration for 7 and 10 days were not significantly different. Similar outcomes were also observed on the pure Ti samples.

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REFERENCES

[1] A. J. Rahyussalim, S. Supriadi, A. F. Marsetio, P. M. Pribadi, and B. Suharno, "The potential of carbonate apatite as an alternative bone substitute material," *Med. J. Indones.*, vol. 28, no. 1, pp. 92–97, 2019. Doi :10.13181/mji.v28i1.2681.

[2] E. B. Ansar, K. Ravikumar, S. S. Babu, F. B. Fernandez, M. Komath, and B. Basu, "Inducing apatite pre-layer on titanium surface through hydrothermal processing for osseointegration," *Mater. Sci. Eng. C*, vol. 105, pp. 110019(1)- 110019(7), 2019. Doi : 10.1016/j.msec.2019.110019.

[3] J. Faure, A. Balamurugan, H. Benhayoune, P. Torres, G. Balossier, and J. M. F. Ferreira, "Morphological and chemical characterisation of biomimetic bone like apatite formation on alkali treated Ti6Al4V titanium alloy," *Mater. Sci. Eng. C*, vol. 29, no. 4, pp. 1252–1257, 2009. Doi:10.1016/j.msec.2008.09.047.

[4] T. C. Lee, P. Koshy, H. Z. Abdullah, and M. I. Idris, "Precipitation of bone-like apatite on anodised titanium in simulated body fluid under UV irradiation," *Surf. Coat. Technol.*, vol. 301, pp. 20–28, 2016. Doi :10.1016/j.surfcoat.2015.11.010.

[5] Y. Liu, Y. Zhou, T. Jiang, Y. De Liang, Z. Zhang, and Y. N. Wang, "Evaluation of the osseointegration of dental implants coated with calcium carbonate: An animal study," *Int. J. Oral Sci.*, vol. 9, no. 3, pp. 133–138, 2017. Doi :10.1038/ijos.2017.13.

[6] H. Yamamoto, Y. Shibata, T. Tachikawa, and T. Miyazaki, "In vivo performance of two different hydroxyapatite coatings on titanium prepared by discharging in electrolytes," *J. Biomed. Mater. Res. - Part B Appl. Biomater.*, vol. 78, no. 1, pp. 211–214, 2006. Doi : 10.1002/jbm.b.30477.

[7] X. Li, X. Yang, X, Liu, W. He, Q. Huang, S. Li, and Q. Feng, "Calcium carbonate nanoparticles promote osteogenesis compared to adipogenesis in human bone-marrow mesenchymal stem cells," *Prog. Nat. Sci. Mater. Int.*, vol. 28, no. 5, pp. 598–608, 2018. Doi :10.1016/j.pnsc.2018.09.004.

[8] R. Shi, K. Hayashi, and K. Ishikawa, "Rapid osseointegration bestowed by carbonate apatite coating of rough titanium," *Adv. Mater. Interfaces*, vol. 7, no. 18, pp. 1–11, 2020. Doi :10.1002/admi.202000636.

[9] C. Sutowo, F. Rokhmanto, and G. Senopati, "Proses perlakuan termomekanis pada paduan α/β Ti-6Al-6Mo sebagai alternatif baru untuk aplikasi biomedis," *Widyariset*,

vol. 3, no. 1, pp. 47–54, 2017. Doi : 10.14203/widyariset.3.1.2017.47-54.

[10] I. Kartika, R. Werdaningsih, Alfirano, F. Rokhmanto, and Y. N. Thaha, “An investigation of α and α’ phases intensity and hardness of Ti-6Al-6Mo implant alloy influenced by temperature of solution treatment and quenching media,” *AIP Conf. Proc.*, vol. 2120, no. 1, pp. 050009(1)-050009(6), 2019. Doi:10.1063/1.5115685.

[11] F. Rokhmanto, I. N. G. P. Astawa, E. P. Utomo, D. P. Malau, R. I. Purawiardi, and J. Triwardono, “Characteristic the α phase in Ti-6Al-6Mo implant alloy after various pre-heating time of heavy hot rolling process,” *Proc. 3RD Int. Semin. Metall. Mater. Explor. New Innov. Metall. Mater.*, vol. 2232, no. 1, pp. 070003(1)- 070003(6), 2020.

[12] G. Senopati, I. N. G. P. Astawa, and C. Sutowo, “Synthesis and characterization of Ti-6Al-6Mo prepared by arc melting process,” *IOP Conf. Ser.: Mater. Sci.* Eng., vol. 202. no.1. pp. 012034(1)- 012034(6), 2017.

[13] G. Ciobanu, G. Carja, and O. Ciobanu, “Structural characterization of hydroxyapatite layer coatings on titanium supports,” *Surface and Coatings Technology*, vol. 202, no. 11, pp. 2467–2470, 2008. Doi :10.1016/j.surfcoat.2007.11.038.

[14] J. C. M. Souza, M. B. Sordi, M. Kanazawa, S. Ravindran, B. Henriques, F. S. SIlva, C. Aparicio, and L. F. Cooper., “Nano-scale modification of titanium implant surfaces to enhance osseointegration,” *Acta Biomater.*, vol. 94, pp. 112–131, 2019. Doi: 10.1016/j.actbio.2019.05.045.

[15] J. Forsgren, F. Svahn, T. Jarmar, and H. Engqvist, “Formation and adhesion of biomimetic hydroxyapatite deposited on titanium substrates,” *Acta Biomater.*, vol. 3, no. 6, pp. 980–984, 2007. Doi: 10.1016/j.actbio.2007.03.006.

[16] D. K. Pattanayak, T. Kawai, T. Matsushita, H. Takadama, T. Nakamura, and T. Kokubo, “Effect of HCl concentrations on apatite-forming ability of NaOH-HCl- and heat-treated titanium metal,” *J. Mater. Sci. Mater. Med.*, vol. 20, no. 12, pp. 2401–2411, 2009. Doi : 10.1007/s10856-009-3815-0.

[17] L. Jonášová, F. A. Müller, A. Helebrant, J. Strnad, and P. Greil, “Biomimetic apatite formation on chemically treated titanium,” *Biomaterials*, vol. 25, no. 7–8, pp. 1187–1194, 2004. Doi : 10.1016/j.biomaterials.2003.08.009.