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Collagen Coating on Titanium Alloy (TNTZ) with Dip Coating Method as Prosthetic Devices in Biomedical Aplications

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ABSTRACT

Metallic biomaterials have been used widely for biomedical application. One of them is titanium alloy that has proved having good biocompatibility to human body. In other hand, the materials should preferably posses a low Young's modulus in order to inhibit the stress shielding effect which would probably cause excessive bone absorption because of decreased mechanical stimulation to that part of bone replaced with metallic medical devices. One such alloy has been developed, TNTZ, is a ß-type titanium alloy and having low Young's modulus (40–60 GPa) similar to that of bone (10–30 GPa), so it is suitable as prosthetic devices especially and other biomedical devices. However, metal is considered as foreign object that may cause negative effects. It is necessary, therefore, to do surface modified with biological materials to reduce those effect. One way is surface coating with collagen, where that is one of human body components and also in human bone. Surface coating with collagen has been done with time and temperature variations. The research method was dip coating method with 5, 15, and 25 minutes for time variations, and 40C and 270C (room temperature) for temperature variations. The result showed that the best result for surface coverage is 5 minute and 40C treatment. Surface coverage is 96% with thickness was 5.2 µm. Temperature variations did not have effect significantly for surface coverage and the thickness. We concluded that the best method to coat titanium, TNTZ surface with collagen with dip coating method is 5 minutes, 40C treatment to acquire the surface coverage orderly.

KEY WORDS: *TNTZ, prostetic devices, collagen, dip coating, surface coverage.*

NOMENCLATURE

TNTZ	Titanium-29Niobium-Tantalum-4,6Zirconium
PBS	Posphat Buffer Saline
SEM	Scanning Electron Microscope
Coll	Collagen

1.0 INTRODUCTION

An increasing aging population along with the expectation of a high quality of life has led to an increase in the demand for novel metallic biomaterials and also the traffic accident [1]. Conventional metallic biomaterials such as SUS316L stainless steel (SUS316L), commercially pure titanium (CP Ti), and Ti–6Al–4V extra low interstitials (ELI) alloy (Ti64 ELI) have been widely used in the biomedical field for many years. However, there are some problems associated with such biomaterials. In the case of SUS316L, it has been reported that nickel (Ni) has an allergic frequency of around 20% toward young women and around 10% toward elderly women; further, adverse reactions caused by nickel containing orthodontic devices have been observed [2,3].

Other studies have shown that Ni ions tend to accumulate in the cells and affect cellular metabolism like DNA synthesis [4,5]. Furthermore, ferromagnetism in SUS316L hampers magnetic resonance imaging (MRI) diagnose [6]. CP Ti exhibits moderate mechanical properties, which restricts its widespread use in

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removable implants. In the case of Ti64 ELI, the toxic vanadium (V) ions released from metal implants severely affect the long-term biocompatibility of these alloys [7].In addition, it has been reported that aluminum (Al) ions are neurotoxic and inhibit bone mineralization [8]. The drawback of using removable implants in biomedical applications is that a significant difference in Young's modulus between the metallic implants and the bone tissues can introduce stress-shielding effect, which could possibly cause osteoporosis or poor osseointegration [9]. Therefore, ß-type Ti alloy with fairly low Young's moduli has attracted considerable attention from researchers in the biomedical field [10–12] because these alloys can effectively mitigate the stress shielding effect.

One such alloy developed by the authors [13], Ti-29Nb-13Ta-4.6Zr (TNTZ), is a promising candidate for a next-generation metallic biomaterial, and attempts have been made to utilize this alloy in practical applications such as the production of implant rods [14], which are a major component of spinal fixation devices. The implant rod made of TNTZ exhibits a low Young's modulus, resulting in greater springback. Thus, a low Young's modulus, which is one of the outstanding features of TNTZ as a metallic biomaterial and obviously a desirable property for patients, becomes an undesirable property for surgeons [15]. However, metal is considered as foreign object that may cause negative effects. It is necessary, therefore, to do surface modified with biological materials to reduce those effect. Also, accelerated and increased bone contact with the implant surface can be achieved by surface modifications [23]. Several attempts have been made to improve adhesion, proliferation, and differentiation of cells by coating the implant surfaces with extracellular matrix proteins [16]. One of them is collagen, that is one of human body components and also in human bone.

Collagen type I serves as a basis for the mineral scaffold. It binds mainly to the integrins α1βb1 and affects attachment and differentiation of osteoblastic cells. 20±23 Culture of fetal rat calvarial osteoblasts on collagen type I-coated dishes results in acceleration of mature osteoblast phenotype development [16].

Many of these strategies take advantage of the specific interactions between ECM protein ligands and integrin cell surface receptors. Integrin receptors play a crucial role in cell attachment and ECM-mediated cell signaling [17]. Integrin dimers, consisting of one α and one ß subunit, bind to specific sites contained within ECM proteins, thereby promoting cell attachment, migration, mechano transduction, differentiation, and numerous other cell functions [18,19].

Based on that function, researcher want to apply collagen coating on TNTZ, titanium alloy, to support its function as prosthetic devices, substitution of human joint, spinal and etc to improve life quality of human.

2.0 MATERIALS AND METHODS

2.1 Tools and Materials

TNTZ material, sandpaper, aseton, HNO_3, methanol, PBS (Posphate buffer saline), Acetic acid, Collagen, Scanning Electron Microscope (SEM), stereo microscope, OHAUS Pioneer^{TM} digital, image J software,Thickness gauge, incubator 37 ^{0}C, 1000 µl micropipette, tube centrifuge, centrifuge, tube of 15 ml and 50 ml.

2.2 Samples preparation

Material (TNTZ) was cut into disc form with the thickness was 2-3 mm and diameter wes 5-6 mm. Surface of the disc was polished with sandpaper # 500, # 800, # 1500 and ultrasonically cleaned with HNO_3 10%, aseton, and methanol (each solution 15 minuets) all samples then sterilized with autoclave and was saved until it was used.

2.3 Coating solutions preparation

The solution consist of acetic acid 10 mM , PBS 60 mM and Collagen type I powder. First, incubated at 4^{0}C 1 mg/ml collagen with acetic acid overnight then added PBS with same volumes with acetic acid into solution at 37^{0}C for 4 hours and centrifuged at 5000xx, 27^{0}C for 30 minutes. After that, supernatan was throwed and was got pellet. Added PBS into pellet with composition was about1 mg/ml collagen and centrifuged again for 15 minutes, 5000xg at 27^{0}C.

Pellet resuspended with PBS 60 mM with concentration was about 2mg/ml collagen and then incubated the disc into solutions. Time to incubate were 5, 15, and 25 minutes with temperature variations were 4^{0}C and 27^{0}C.

2.4 Microstructure Analysis

Microstructure of samples was observed with stereo optical microscop, Olympus LG-PS2 and SEM (Scanning Electron Microscope), Hitachi S 3400. The voltage of SEM was 15.0 kV with magnification was 100x, 500x, and 1000x.

2.5 Analysis of surface coverage and the thickness

Analysis of surface coverage was did with ImageJ software that gave surface area data of samples and collagen coating. The thickness of samples was measured with sanfix *thickness gauge series* type GF-280.

3.0 RESULTS AND DISCUSSIONS

Result of this research was interpretated based on morphology of the sufrace layer, measurement of surface coverage and also collagen mass that adhere to TNTZ disc surface.

3.1 Morphology of Surface Layer

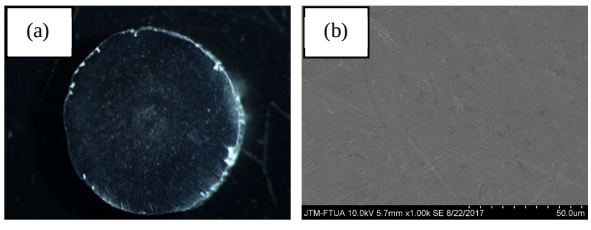

Figure 1: Morphology of sample surface. (a) observation with stereo microscope with magnifition is 1.25x (b) observation with SEM, magnification is 100x.

Samples was polished and cleaned [20] to acquire the surface of samples that ready to coat with collagen. The surface of samples before coating process are in Figure 1. Coating process with collagen has been done with some variation of time and

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temperature to obtain full coverage. Results of coating process based on stereo microscope visual are in figure 2.

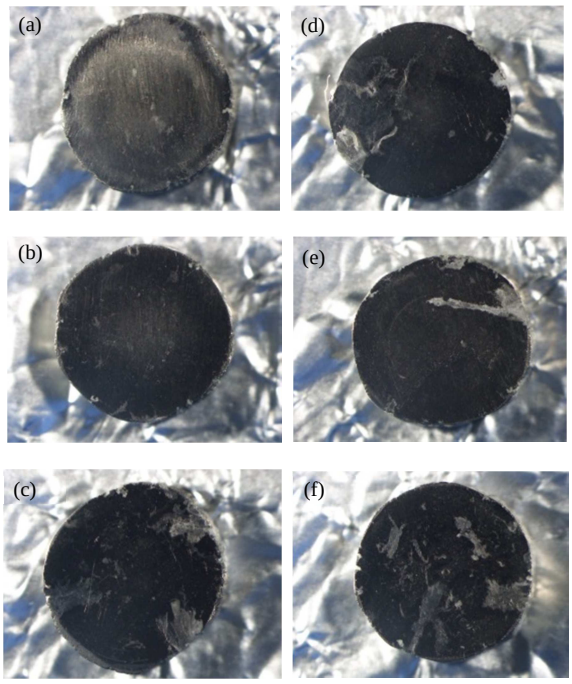

Figure 2: Observation with stereo microscope on the surface of samples. Visualization of 4^0C are (a) in 5 minutes, (b) in 15 minutes (c) in 25 minutes dip process and at 27^0C are (d) in 5 minutes, (e) in 15 minutes and (f) 25 minutes minutes dip process.

Along with the addition of time, collagen layer on samples forms clotty. There are buildup of collagen on some spot of samples surface. It causes uneven of the surface of samples. Based on the stereo microscope, the treatment with 5 minutes in 4^0C has been coated by collagen. It is also proved with SEM analysis (figure 3) to observe the surface coverage of sample. The surface of sampel is coated by fibrils and particles of collagen. Collagen as one of extracellular matrix (ECM) has immobilized [21] features than others that made it deserve to be a coating. Although it is only thin layer, but collagen is almost covered all of sample surface.

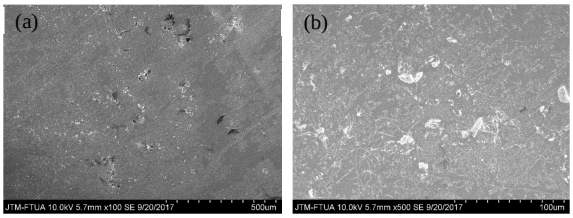

Figure 3: The result of SEM analysis with magnification are (a) 100x dan (b) 500x for 5 minutes dip coating process in 4^0C.

Furthermore, effect of time and temperature variations to surface coverage of samples surface may see on Figure 4. This result is from ImageJ analysis. The higgest value of surface coverage is belog to 5 minute, 4^0C and the lowest is belog to 25 minutes, 27^0C. Previous researches have used 15 minutes in 4^0C [21] and surface characteristics of sample have not shwon, samples was coated with fibrils of collagen. Roehlecke, *et al* [24] used 15 minutes, 25^0C (room temperature) to coat samples. Samples coated with fibrillar collagen and found that collagen type I promoted adhesion, proliferation, and differentiation of osteoblast. Becker *et al.*, [16] and Roβûler *et al.*, [25] used 15 minutes at room temperature and found that the adsorbed amount of type I fibrillar collagen ranged from 3 to 6 mg/cm^2. Becker declared that collagen type I induced proliferation and differentiation of rat calvarial osteoblasts.

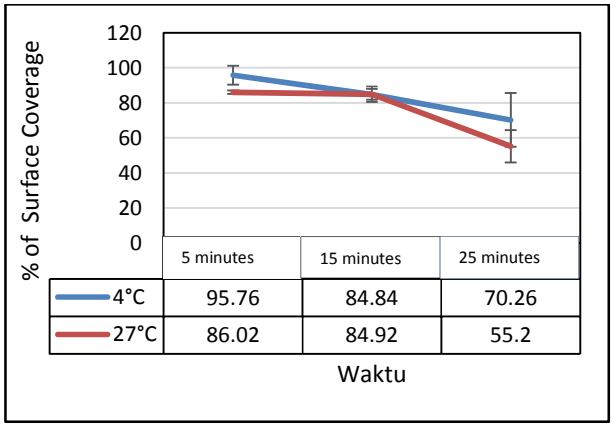

Figure 4: Graph of the effect of time and temperature variations to surface coverage of samples surface.

Result of thicknes analysis has shown in figure 5. Based on the graph, the highest value for the thicknes is 25 minutes at 4^0C treatment and the lowest is 5 minutes at 27^0C. Though, 25 minutes at 4^0C treatment is the highest values, but spread of collagen is uneven and there are collagen clotty. The best result, even the thicknesss value is low but surface coverage is excellent, is 5 minutesat 4^0C treatment.

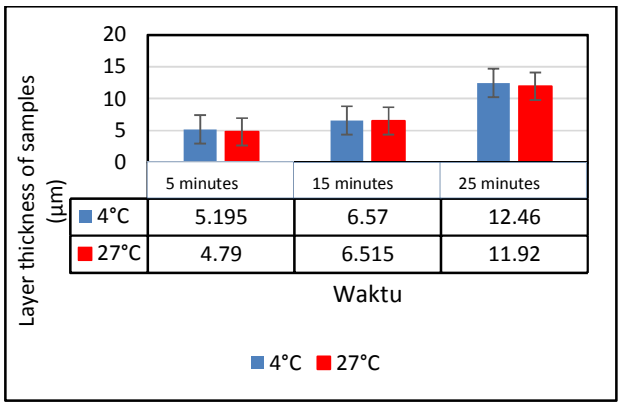

Figure 5: Graph of the coating thickness for each treatment.

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4.0 CONCLUSION

The following conclusions are reached:

1. The best method to coat titanium, TNTZ surface with collagen with dip coating method is 5 minutes, 40C treatment to acquire the surface coverage orderly.
2. Temperature variations did not have effect significantly for surface coverage and the thickness.

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