IMPROVEMENT OF OSTEOCONDUCTIVITY OF VALVE METALS AND THEIR ALLOYS BY HYDROPROCESSING

ZULDESMI

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by

ZULDESMI

Supervisor

Associate Professor Kensuke Kuroda

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ABSTRACT

Anti-corroded valve metals (Ti, Nb, Ta, and Zr) and their alloys are widely used as biomedical materials because of their excellent biocompatibility, excellent corrosion resistance and appropriate mechanical properties. They also have recognized as non-toxic elements and do not contribute any harmful reaction in human body. However, they show a low direct bone bonding (osseointegration) because of bioinertness. Thus, many researchers tried to concentrate on surface modification to increase their bioactivity, thereby shortening the healing period and increasing clinical success of valve metals and their alloys, especially in areas of poor bone quality. Surface roughness and hydrophilicity are important factors of biomaterials which related with protein adsorption and cell adhesion after implantation into the body, consequently their osseointegration. In recent studies, various methods such as, anodizing, UV light irradiation, plasma irradiation and hydrothermal treatment in order to create hydrophilic surface in improving implants osseointegration and osteoconductivity has been investigated.

For this purpose, I tried to improve the osteoconductivity of valve metals and their alloys by applying above methods. *In vivo* test was conducted to evaluate the osteoconductivity of valve metals and their alloys. Firstly, four surface modification methods (anodizing, UV light irradiation, plasma irradiation, and hydrothermal treatment) were applied to pure Ti. Although UV light irradiation and plasma irradiation processes created the hydrophilic surfaces on pure Ti as well as anodizing and hydrothermal treatment, they are difficult to apply for complex-shaped substrates of implant materials with complex topographies, because of the line-of-sight nature of the methods. Super-hydrophilic surface (water contact angle lower than 10 degree) with osteoconductivity higher about 50% than as-polished Ti was provided on hydrothermaled pure Ti after stored in ×5 PBS(−).

Subsequently, I continued my research to improve other valve metals (such as, Zr, Nb, and Ta), which pure Ti was used as a reference. Anodizing and hydrothermal treatment were applied to those valve metals. The surface hydrophilicity of Zr, Nb, and Ta samples were not enhanced by anodizing as well as pure Ti. Their surfaces were still in hydrophobic conditions with water contact angle higher than 60 degree. In contrast,

the improvement of surface hydrophilicity was detected after the hydrothermal treatment was implemented to all valve metals. This condition became more valuable when the hydrothermaled valve metals stored in ×5 PBS(−) where their osteoconductivity increased up to 55 % compared than those of as-polished samples. It means that, hydrothermal treatment that continue by storing in ×5 PBS(−) is not only effective to improve the osteoconductivity of pure Ti, but also effective for Zr, Nb, and Ta.

Hydrothermal treatment was conducted to Ti alloys (such as, Ti64, Ti67, TNTZ, and TCFA) in order to improve their osteoconductivities. Anodizing was used for comparison. It was found that anodizing only successful to produce hydrophlic surfaces on Ti, Ti64, and Ti67 and improved their osteoconductivities, but it was not useful for TNTZ and TCFA alloys. The hydrophobic surfaces were still formed on the surface of these two alloys. Consequently, the osteoconductivities of anodized TNTZ and TCFA were low and almost similar to those of as-polished conditions. On the other hand, all the Ti alloys surfaces became hydrophilic after hydrothermal treatment. When the hydrothermal Ti alloys were continue stored in ×5 PBS(−), the superhydrophilic surface were formed on all Ti alloys and improved their osteoconductivities significantly. It can be said that a combination process between hydrothermal treatment and storing in ×5 PBS(−) became a simple and effective way of enhancing the osteoconductivity of not only for pure Ti, but also for Ti alloys.

Then, the effect of hydrothermal treatment on osteoconductivity of Zr alloy (Zr-9Nb-3Sn) was examined. Anodizing was also used as comparison. Similar to its pure metal alloys (Zr and Nb), anodizing did not provide a significant enhancement of the hydrophilicity of Zr-9Nb-3Sn alloy. However, this condition became different when hydrothermal treatment was applied to Zr-9Nb-3Sn alloy. Its surface became hydrophilic after hydrothermal treatment. In addition, higher osteoconductivity was produced on hydrothermaled Zr-9Nb-3Sn alloy after stored in ×5 PBS(−). This suggests that hydrothermal treatment become one of the promising surface treatments which improve the osteoconductivity of implant materials such as Zr, Nb, and Zr-9Nb-3Sn alloy.

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CHAPTER 1

Introduction

1.1 Metal used for biomedical applications

Metals have been used first as implants when Lane presented metal plate for bone fracture fixation in 1895 since more than 100 years ago [1-1]. In the beginning, corrosion and insufficient strength became serious problems which had experienced by metal implants [1-2][1-3]. However, by introducing the 18Cr-8Ni stainless steel in 1920s with its superior corrosion resistance, it was getting more attention for the clinicians. Afterwards, metallic implants faced huge progress in clinical application.

Table 1.1 shows the usage of common metals for any implants devices. Specific requirements of metals and alloys depend on the specific implant applications. Superior mechanical properties, such as strength, elasticity, rigidity, toughness, and fracture resistance are needed for orthopedic implants. On the other hand, wear resistance becomes more essential for metals used as total joint replacement to avoid debris formation from friction. Similar to the shape memory effect, rigid and strong metals are required properties for dental restoration.

Until nowadays, the most used metallic implants are stainless steel, CoCr alloys and titanium and its alloys. Stainless steel is known because of its excellent strength and corrosion resistance. Its usage becomes spread out due to strong, excellent fabrication properties, good biocompatibility, economical and easy to get. The type 302 stainless steel which is stronger and has higher corrosion resistance than vanadium steel was the first stainless steel for implants [1-4]. Then, its corrosion resistance has enhanced by additional of molybdenum which recognized as type 316 stainless steel [1-4]. By inclusion of Mo, the pitting corrosion resistance in salt water is raised. Afterwards, the type 316L stainless steel which has higher corrosion resistance in chloride solution was introduced by reducing the carbon content from 0.08 to 0.03 mass% [1-4]. However, it can corrode in the body with specific conditions such as in oxygen diminished and highly stressed region (e.g. the contacts under the screws of the bone fracture plate) [1-4]. Therefore, this type is only acceptable to apply for temporary implant devices, such as screws, hip nails and fracture plates.

Table 1.1 Implants devices and type of metals used [1- 4]

Fields	Implants devices	Metal used
Dentistry	Filling	Au, AgSn(Cu) amalgam
	Orthodontic wire	TiMo; TiNi; CoCrMo; 316L stainless steel
Craniofacial	Plate and screw	Ti6Al4V; Ti; CoCrMo; 316L stainless steel
Cardiovascular	Artificial valve	Ti6Al4V
	Stent	Ti ; CoCrMo; 316L stainless steel
Otorhinology	Artificial eardrum	316L stainless steel
Orthopedic	Artificial joints)	Ti6Al4V; Ti6Al7Nb; CoCrMo
	Bone fixation (plate, screw, pin	Ti6Al4V; Ti ;316L stainless steel

The application of Ti and its alloys in medical and dental fields become more popular because of their excellent mechanical properties, good biocompatibility, and excellent corrosion resistance. Titanium is also known as light metal with density is only 4.5 gcm^{-3} compared to 316 stainless steel with 7.9 gcm^{-3} and cast CoCrMo alloys with 8.3 gcm^{-3} of weights [1-5]. Ti6Al4V alloy is known because of its great pitting corrosion resistance and tensile strength [1-4]. TiNi (nitinol) becomes more suitable in various applications such as dental restoration wiring because of their shape memory effect [1-4]. On the other hand, CoCrMo alloys have been widely used in artificial joints because of their excellent wear resistance properties, especially for the wrought CoNiCrMo alloy that use for heavily loaded joints such as ankle implants [1-4].

Recently, other metals which are getting attention for implant applications are valve metals (Ti, zirconium (Zr), niobium (Nb), and tantalum (Ta)) and their alloys because of their excellent anti-corroded properties. Zirconium alloys are known because their chemical properties similar to Ti alloys. Ordinary Zr metal shows good mechanical strength and biocompatibility, therefore, this metal gets attention in surgical implants [1-6][1-7]. Compared with stainless steel, CoCr alloys and Ti, Zr has lower magnetic susceptibility and is more suitable for surgery performed in a magnetic resonance imaging (MRI) circumstance than the others [1-8]. Niobium is non-toxic and highly corrosion resistant element, also becomes a strengthener to Zr. Tantalum has higher corrosion resistance compared than Nb due to its higher stability of oxide [1-9]. However, Ta exhibits a limited mechanical resistance and high density [1-9]. Generally, valve metals are known as high density and non-magnetic metals. These properties are required for the implants used for MRI and X-ray imaging.

1.2 Structure and properties of metallic biomaterials

1.2.1 Microstructure

Metals are rarely used as single crystals but more commonly are polycrystalline and also mixtures of two or more different phases. Each phase is physically and chemically recognizable from the next and has a different crystal structure or a different composition. The microstructure of metals will influence their physical, chemical and mechanical properties. By modifying microstructure, we can optimize the mechanical properties of biomaterial.

Every pure metal is considered as a phase, in every solid solution and intermetallic compound. The pure metals should be alloyed with other metals to improve their properties. Mainly, alloys contain of two or more solid phases depend on their composition and temperature. One of the important methods to strengthen the metal alloys and enhance their properties is by alloying a metal with finely dissolved particles.

There are many ways to manipulate microstructures and mechanical properties of metallic biomaterials. The following sample just a few of more commonly methods that apply to the most commonly metallic biomaterials. Titanium and its alloys have getting more attention in the medical implant fields because of their remarkable mechanical properties, high corrosion resistance and do not cause an allergic problem. Ti has a high melting point (1668 $^{\circ}$C) with a hexagonal closely packed structure α until a temperature of 882.5 $^{\circ}$C. After that, it transforms into a body centered cubic structure β. The phase and microstructure stabilized in room temperature are two important factors which determine the classification of Ti alloys. Titanium alloys are categorized as α, near-α, α+β, metastable β, or stable β [1-10]. Commercially pure Ti implants are made of single-phase α Ti, which is typically mild. O, Al, C, and N, are alloying elements classified as α-stabilizers; meanwhile Mo, V, Nb, Ta, Fe, W, Cr, Si, Co, Mn and H are known as β-stabilizers. In addition, neutral alloy elements such Zr, α and near-α alloys show excellent corrosion resistance. However, they were found rarely as biomaterials due to their low strength in room temperature. Ti6Al4V and Ti6Al7Nb which have α+β phase exhibit higher strength. Meanwhile, the β alloys are well known because of their low Young modulus and superior corrosion resistance [1-11].

1.2.2 Chemical properties

A chemical stability is one of the most important factors which influence the durability of a material. For biomaterial applications, one of the important considerations of metals and alloys as implant materials is the failure by corrosion and it has remained as one of the challenging clinical problems. Corrosion appeared as a result of chemical and electrochemical reactions with its surrounding environment. Singh, *et al.* observed that corrosion resistance becomes a crucial factor in metallic biomaterials selection because it occurs due to the unavoidable corrosive body fluid [1-12]. When installed in human body, severe corrosion environment which includes blood and other components of the body fluid such as water, chlorine, sodium, plasma, proteins, and amino acids along with mucin in saliva was occurred on metallic implants [1-13]. Various anions such as phosphate, chloride, and bicarbonate ions, cations like, Ca^{2+}, K^+ Na^+, Mg^{2+} etc., organic matters of low-molecular-weight species as well as dissolved oxygen and relatively high molecular-weight polymeric elements are mostly components in aqueous medium of the human body [1-14][1-15].

During corrosion, the implants release unwanted metal ions which are non-biocompatible. Brittleness and fracture of the implant which is caused by erosion of the dissolution of metal will be occurred since a material starts to corrode. The corrosion will accelerate because of reduction of oxide layer and enlarge of unprotected surface area. If the metal fractures are not surgically repaired, more seriously problem may occur and will generate inflammation of the surrounding tissues. Okazaki, *et al.*

also indicated the fact that dissolved metal ions can increase in tissues, near the implant or may be moved to other area in the body [1-16]. As a consequence, the life of implant device is decreased and lead to revision surgery. The human life will be reduced by the corrosion problem. **Table 1.2** lists the effect of corrosion due to the usage of various implant biomaterials in human body. The selection of metals carefully is one of the easiest ways to prevent the corrosion. Generally, stainless steel, nonferrous metals and non-metallic materials are appointed because of their high corrosion resistance.

Table 1.2 Effects of corrosion in human body due to the usage of various implant biomaterials [1-17].

Implant biomaterials	Effect of corrosion
Aluminum	Epileptic effects and Alzheimer`s disease *
Vanadium	Toxic in the basic circumstances
Nickel	Dermatitis (skin effect)
Chromium	Disturbances of ulcers and central nervous system
Cobalt	Anemia B inhibiting iron from being adsorbed into the blood stream

* It was proved that Al was not a causative agent today.

In general, valve metals (Ti, Zr, Nb, and Ta) show good corrosion resistance due to they are covered by passivate thin oxide layer which creates a gap between the metal and the environment. The application of valve metals and their alloys also have been expanded for biomedical because they have high corrosion resistance in an oxidizing nature media or containing chloride ions [1-18]. Therefore, fast improvement has been found in the surgical implants area and medical devices area [1-19].

1.2.3 Mechanical properties

The compatibility of materials with the body is one of the most important properties required for biomaterials. The biomaterials used should not generate inappropriate deterioration of their structures and properties. They are also not produce any clinically crucial disadvantages for biological tissues. Mechanical stability and durability are most important factors required for biomaterials because they are related to clinical performance and reliability [1-20]. The biomaterials have to keep their behavior during application. For the long-term guarantee of the performance of implant materials, their stability of mechanical properties during usage is very crucial. It is important to create devices with minimum risk as long as the safety of human life is decided by artificial organs. Because of that, the accurate and statistical data of fatigue strength of biomaterials are necessary.

It is well known that when the materials are used for structural applications, the mechanical properties are required to be optimized. The important mechanical properties of biomaterial are modulus of elasticity, tensile strength, ductility, toughness, strain hardening capability and hardness. In applications, metallic biomaterials are usually used for load bearing devices (*e.g.* fracture fixation wires, screws, pins, and plates, hip and knee prostheses). They must have sufficient fatigue strength to endure the rigors of daily activity such as walking, chewing etc. Nowadays, one of the significant problems of metallic implant materials which getting more attention is stress shielding [1-21]. Stress shielding occurs because of different stress between the implant and bone. The much higher Young's modulus of metallic implants than that of bone causes the stress is transferred through the implant. It creates detached of the implant or refracture of the bone after removed due to bone absorption. Consequently, it is required metallic biomaterials with a similar modulus with bone (low modulus metallic biomaterials). Valve metals show the acceptable mechanical properties, thereby, they are become materials of interest for surgical implants.

1.3 Biocompatibility and osteoconductivity

Biocompatibility has been pointed to long-term implantable devices, which biologically and chemically inert. They do not generate dangerous effect to the human body. By developing of biotechnology, some level of biological activity in certain research fields, such as tissue engineering, gene and drug delivery systems are required. One of the latest definition of biocompatibility is "the ability of a biomaterial to conduct its appropriate role in a medical therapy, without lead to any terrible effects in the recipient, however producing the most advantages for tissue response in specific situation, and optimizing the clinically related capability of that therapy" [1-22].

For metallic biomaterials, biocompatibility includes the compliance of an artificial implant by the body as a whole and by the surrounding tissues. They do not produce irritation on the surrounding structures, do not encourage immoderate inflammatory response, do not activate immunologic and allergic reactions, and do not originate cancer. Since metals can corrode in an *in vivo* environment, metal biocompatibility is one of considerable concern for metallic devices as structural implants. The corrosion of metallic biomaterials creates disadvantageous effects to the implant itself and to the surrounding tissues. The chemical matters that dangerous for body parts and failures the mechanical properties of the implant are created by corrosion. For that reason, corrosion resistance of metallic biomaterials becomes most important factor for their biocompatibility. Valve metals and their alloys were chosen as biomaterials because of their good biocompatibility and high corrosion resistance.

Osseointegration is currently defined as the direct contact between bone and implant surface, and the bone response in rate, quantity and quality, are mostly affected by characteristics of implant material [1-23]. The characteristics of the implant surface become crucial in the process of osseointegration with early loading, as a biological mechanism on the interface of bone-implant influence the implant`s providence. During insertion, untimely loading can bother the healing process and may create implant failure. In order to improve the biocompatibility and osseointegration of metallic biomaterials, several surface treatments have been developed. Although valve metals and their alloys have excellent mechanical properties and biocompatibility, however, they have low osseointegration (poor bone-forming properties) due to lack of bioactivity. Thus, surface modifications become crucial to improve the osteoconductivity of valve metals and their alloys. In this dissertation, various surface modifications which applied in metallic biomaterials especially for valve metals and their alloys will be explained in Chapter 2.

1.4 Objectives

The previous literatures reviewed that the use of anti-corroded valve metals, such as Ti, Nb, Ta and Zr as implant materials become more popular recently. However, they do not have high osteoconductivity as untreated surfaces. It is well known that surface characteristics of metals implants such as surface roughness and wettability have a significant function in controlling the biological response of the host bone. Various surface modifications have applied for enhancing the osteoconductivity of implants materials. In this dissertation, I focus on creating the hydrophilic surface of valve metals and their alloys in order to improve their osteoconductivity by applying several surface modifications, such as anodizing, plasma irradiation, UV light irradiation and hydrothermal treatment.

1.5 Main contents of this research

This dissertation consists of eight chapters

Chapter 1 is general introduction for metals that used to implant materials and their characteristics.

Chapter 2 presents various surface treatments which applied in order to improve the osteoconductivity of metallic biomaterials and evaluation method of their osteoconductivities by *in vitro* and *in vivo* studies.

Chapter 3 explains the high osteoconductivity of pure Ti that produced by hydrothermal treatment. The results become crucial and used as reference for the following chapters.

Chapter 4 introduces the application of one-step hydrothermal treatment of valve metals in enhancing of osteoconductivity, based on results of chapter 3.

Chapter 5 exhibits the improvement of osteoconductivity of titanium alloys which provided by hydrothermal treatment.

Chapter 6 shows the effect of hydrothermal treatment on osteoconductivity of Zr-9Nb-3Sn alloy. Alloy elements, such as pure Zr and Nb were used as comparison.

Chapter 7 describes the future works that may be done in order to improve the osteoconductivity of metallic biomaterials.

Chapter 8 summarizes the results of this work and makes general conclusions of them.

Reference

[1-1] Lane WA. Some remarks on the treatment of fractures. Brith Med J 1895;1:861.

[1-2] Lambotte A. Technique et indication des prothèses dans le traitement des fractures. Presse Med 1909;17:321.

[1-3] Sherman WO. Vanadium steel bone plates and screws. Surg Gynecol Obstet 1912;14:629.

[1-4] Park J and Lakes RS. An introduction. Biomater 3rd ed 2007.

[1-5] Brandes EA and Brook GB. Smithells Metals Ref Book. 7th ed. Oxford, Butterworth-Heinemann 1992.

[1-6] Thompsen P, Larsson C, Ericson LE, Sennerby L, Lausmaa J, Kasemo B. Structure of the interface between rabbit cortical bone and implants of gold, zirconium and titanium. J Mater Sci: Mater Med. 1997;8:653.

[1-7] Sherepo KM, Red`ko IA. Use of xirconium for implants in traumatology and orthopedics. Med Tekh. 2004;2:22.

[1-8] Suyalatu, Nomura N, Oya K, Tanaka Y, Kondo R, Doi H, Tsutsumi Y, and Hanawa T. Microstructure and magnetic susceptibility of as-cast Zr-Mo alloys. Acta Biomater 2010;6:1033-1038.

[1-9] Gerardi S. ASTM Handbook, vol. 2. Metals Park: ASM International; 1993

[1-10] Polmear IJ. Titanium alloys, in Honeycombey R and Hancock P (Eds.), Light alloys 1981:248-315.

[1-11] Bania PJ. Beta titanium alloys and their role in the titanium industry, in Eylon D, Boyer RR, and Koss DA (Eds), Titanium alloys in the 1990s. Metal Mater Soc 1993:3-14

[1-12] Singh, R. and N.B. Dahotre, Corrosion degradation and prevention by surface modification of biometallic materials. J Mater Sci: Mater in Med 2007;18:725-751.

[1-13] Lawrence SK, Gertrude M. Shults. Studies on the relationship of the chemical onstituents of blood and cerebrospinal fluid. J Exp Med 1925; 42: 565-591.

[1-14] Scales JT, Winter GD, Shirley HT. Corrosion of orthopaedic implants, screws, plates, and femoral nail-plates. J Bone Joint Surg 1959; 41B: 810-820.

[1-15] Williams DF. Review-tissue-biomaterial interactions. J Mater Sci 1987;22: 3421-3445.

[1-16] Okazaki, Y. and E. Gotoh, Comparison of metal release from various metallic biomaterials in vitro. Biomater 2005;26:11-21.

[1-17] Aksakal B, Yildirim ÖS, Gul H. Metallurgical failure analysis of various implant materials used in orthopedic applications. J Fail Anal Prev 2004; 4:17-23.

[1-18] Pourbaix M. Electrochemical corrosion of metallic biomaterials. Biomater 1984;5:122.

[1-19] Biehl V, Breme J. Metallic biomaterials. Mat-wis u Werkstoff tech. 2001; 32:137.

[1-20] Hayashi K. Mechanical properties of biomaterials: relationship to clinical applications. In Contemp Biomater, J. W. Boretos and M. Eden, Eds., Noyes Pub., Park Ridge, N.J., 46-65 (1984).

[1-21] Niinomi M, Hattori T, Morikawa K, Kasuga T, Suzuki A, Fukui H, et al. Development of low rigidity β-type titanium alloy for biomedical applications. Mater Trans 2002;43:2970-2977

[1-22] Williams DF. On the mechanisms of biocompatibility. Biomater 2008;29:2941.

[1-23] Albrektsson T, Wennerberg A. Oral implant surfaces: Part 1- review focusing on topographic and chemical properties of different surfaces and in vivo responses to them. Int J Prosthodont 2004;17:536-543.

CHAPTER 2

Surface Treatment and Biological Evaluation Methods of Metallic Biomaterials

2.1 Surface treatment for enhancement of osseointegration

Surface treatment is one major concern on recent developments in metallic biomaterials [2-1]. Several surface treatments have been implemented to enhance the surface properties, that support the osseointegration mechanism [2-2][2-3]. Aim of these methods is to promote the osseointegration mechanism with stronger and faster bone formation which present preferable stability at the healing process [2-4][2-5]. In the latest decades, many surface treatments have concerned on the influence of implant surface properties on the protein subject to mechanisms of proliferation, cell adhesion, bone matrix deposition and differentiation, purposing on the origination of new biomaterial surfaces.

The surface characteristics are well known as important factors in the protein adsorption [2-6]. The protein over layer is considered to be unstatic and changes by time. The surface is a firstly area reached and adsorbed by protein with higher concentration in the biological liquid. Then, the protein is taken place by another one which more affinity to the surface, while it has low concentration. Thus, a varying layer formed of dissimilar protein is appeared and kept on the implant surface. Attaching of functionary cells during the healing process is the main function of this layer. Some proteins such as fibronectin, laminin, vitronectin, serum albumin and collagen accelerate osteogenic cells attached on Ti surfaces [2-7][2-8]. Consequently, the implant surface capacity to bind protein is believed as a significant aspect to make the osseointegration mechanism become successful as well as surface characteristics, such as surface morphology [2-9], physicochemical composition [2-7] and surface free energy [2-10] which influence the protein adsorption expansion. Ideally, cells of osteogenic adhere to the particular protein containing arginine-glycine-aspartic acid (RGD) sequences. This pattern is obtained in any different ECM proteins, such as fibronectin, laminin, osteopontin, and vitronectin [2-11]. The membrane receptors, named as integrins are used by osteogenic cells to attach to these binding patterns. As said before, the RGD-pattern is a motif of cell adhesive protein (*e.g.* fibronectin, vitronectin and various collagen types) for binding with integrins [2-11]. However, other areas enclosed by these proteins which also have binding ability with integrins and induce integrin-mediated cellular signaling cascades are found. In brief, some intracellular conditions as well as protein kinase C and Na^+/H^+ antiporter, intracellular proteins and tyrosine phosphorylation of membrane phosphoinositide hydrolysis are originated by integrin-mediated cell attachment to ECM [2-12]. As results, the communication of intracellular is created together with the transmission of extracellular signal to the nucleus. A response to this signal is produced by the osteogenic cell through proliferation, migration, and differentiation [2-13]. This response is very necessary to optimize the osseointegration by the actuation of osteoblastic pathways through integrin-mediated signaling. Therefore, the improvement of an implant surface by observing an osteogenic protein becomes a crucial field in implant surface science.

Several surface modifications have been applied for improving osseointegration of metallic biomaterials. The surface morphology modification or the Ti coating by bioactive materials has been getting more attention for many scientists [2-14]. Sand-blasting [2-15], acid-etching [2-16], alkali-etching [2-17], plasma spraying [2-18], electro polishing [2-19], anodic oxidation [2-20], hydroxyapatite (HA) [2-21] and calcium phosphate (CaP) [2-22] are commonly techniques used to modify the surface properties of Ti. Generally, the surface modifications generate any alteration in surface characteristics, such as surface energy, morphology, and physicochemical composition. Surface morphology (surface roughness) and wettability are considered as the most important factors which influence the tissue response to the implant. This is because they closely connected to the protein adsorption and cell attachment at early period [2-23[2-24]. Hence, this chapter more concentrates to these two categories.

2.1.1 Surface roughness

As mentioned before, surface roughness has a significant function in biological responses of biomaterial surfaces. It influences the cell attachment, spreading and subsequent proliferation as well as wettability. Attaching, spreading and proliferating of osteoblasts cells on smooth surfaces are faster than on rough surfaces, as reported by several *in vitro* studies [2-25]. In addition, some *in vivo* studies have detected that the osteoconductivity of TiO_2 coatings was improved by a smooth surface with $Ra/\mu m < 0.1$ and also hydrophilic surface. They also found that surface roughness, and hydrophilicity can differ base on the anodizing conditions and resulting in different osteoconductivities [2-26][2-27]. The common processes which have been reported in modifying implant roughness are hydroxyapatite (HAp) coating, sandblasting, acid etching, electrochemical anodization, and Ti plasma spraying.

2.1.2 Surface wettability (hydrophilicity)

Similar to the surface roughness, surface wettability is also known as a crucial factor in adsorption of protein containing RGD, such as fibronectin [2-28][2-29] onto the surface. It improves the adhesion and spreading of osteoblast precursors on implant surface. Cell adhesion is generally better on hydrophilic surfaces. According to some literatures, highly hydrophilic surfaces are suggested to be more beneficial than hydrophobic surfaces [2-30][2-31]. Some *in vitro* studies indicated that the cell differentiation and growth factor production of Ti were affected positively by the hydrophilic surfaces [2-32][2-33]. Besides, animal studies (*in vivo*) also revealed that osseointegration can be initiated on hydrophilic surface soon after implantation [2-34][2-35][2-36].

There are many researches on the hydrophilicity of organic biomaterials. However, it is only few literatures that investigated effect of the surface hydrophilicity on metallic biomaterial. Thus, some efforts have been done to obtain an optimum surface wettability of metallic biomaterials in performing more appropriate bone response. Anodic oxidation (or electrochemical anodization) is a versatile method to form oxidic films on top of metal foils. Metallic biomaterials develop a stable, passivating oxide layer when contacting air and not only involve the metals aluminum and Ti (most important), but also a variety of other metals as vanadium, Zr, hafnium, Nb, Ta, tungsten and others. Compared to other oxidation methods, anodic oxidation creates bioactive oxide film on surface of Ti and its alloy. Additionally, this process also can improve the corrosion resistance of Ti by increasing the thickness of oxide layer to diminish ion release. However, another researcher found that despite the hydrophilicity of anodized TiO_2 also was improved by anodizing process, its water contact angle (WCA) did not decrease lower than 20 degree. Therefore, an additional surface treatment is required to produce a more hydrophilic surface [2-37]. Besides, anodizing process was not effective to enhance the wettability of some titanium alloys such as TNTZ and TCFA [2-38].

Thereafter, other surface treatments such as ultraviolet (UV) irradiation [2-39], plasma irradiation [2-40], and hydrothermal treatment [2-41] have been applied and detected as processes which can produce high hydrophilicity on sol-gel or sputtered TiO_2 coatings on Ti substrate (display on **Table 2.1)**. Ultraviolet irradiation and plasma irradiation were chosen in creating hydrophilic surface due to they do not cause dynamic changes in the films, such as the surface morphology or crystal structure, but these processes are difficult to apply for complex-shaped substrates of metallic biomaterials with complex topographies, which typify many implants, because of the line-of-sight nature of the methods. In contrast, hydrothermal treatment can be applied regardless of the shape of implant, although this process may influence the film properties because of heat application. Hydrothermal treatment is one of the processes which can produce hydrophilic surface by cleaning the surface from contamination. This process is also known as a useful surface treatment method for producing Ti and its alloys with bioactive surface composition.

From above explanations, surface hydrophilicity gives more significant influence in improving osteoconductivity of metallic biomaterials compare than the other properties (such as film thickness, surface chemistry etc.) provided by coating process. Therefore, in this dissertation, I focus in creating hydrophilic surface on valve metals and their alloys by several surface modifications without coating process.

Table 2.1 Overview of surface modification methods in creating hydrophilic surface for metallic biomaterials

Surface modifications methods	Process	Advantages (+) / Disadvantages (−)
Anodizing	producing oxide film by coating process	(+) can form bioactive oxide film on surface of Ti and its alloy.
		(−) not effective to improve the wettability of several Ti alloys, such as TNTZ and TCFA
Hydrothermal treatment	cleaning the surface from contaminations	(+) can be applied to create hydrophilic surface on valve metals and their alloys
		(−) it takes time; (−) heat application influences the film properties
UV light irradiation	creating surface oxygen vacancies at bridging site, and this leads to conversion of relevant Ti^{4+} to Ti^{3+} sites	(+) simple, easy way and no longer time process; (+) do not cause dynamic changes in the films, such as the surface morphology or crystal structure
		(−) difficult to apply for complex-shaped substrates with complex topographies because of the line-of-sight nature of the methods; (−) only can be applied to titanium surface
Plasma irradiation	enhancing heat transfer of liquid-vapor phase change	(+) no longer time process; (+) do not cause dynamic changes in the films, such as the surface morphology or crystal structure
		(−) difficult to apply for complex-shaped substrates with complex topographies

2.2 Biological evaluation methods of metallic biomaterials

Because of the great numbers of obtainable implant categories, various surface and other characteristics should be handled carefully [2-42]. By understanding that surface modification methods may promote varying surface properties and affect the host-to-implant response, it necessary to perform some tests on implant surfaces as new biomaterials [2-43]. The evaluation must be treated with both *in vitro* and *in vivo* studies for their applications.

2.2.1 *In vitro* studies

In vitro refers to the technique of performing a given procedure in a controlled environment outside of a living organism. Many experiments in cellular biology are conducted outside of organisms or cells. Cell cultures are generally employed for investigating the osteoblastic cells response during contacting with various materials in bone tissue. They concentrated on the growth capacity, morphological aspect, and the condition of cells differentiation on substrate with different topography, chemical, and composition [2-44].

In vitro studies provide a description of the material behavior in certain condition and are usually carried out in simulated body fluid. The primary disadvantage of *in vitro* experimental studies is the difficulties in getting better and accurate results because each researcher used different cell cultures and also different methods. Besides, the behavior of early biological of novel implant materials achieved by *in vitro* studies is not be able to connect to *in vivo* behavior. The dynamic environment that requires for *in vivo* bone/implant interaction cannot reproduce by cell cultures. This outcome only can be confirmed by *in vivo* studies using animal models [2-45]. Therefore, *in vitro* study cannot be applied as the final test to propose the new implant materials.

2.2.2 *In vivo* studies

The evaluation method by *in vivo* study is applied as a consequence of the disadvantages of *in vitro* study (as explanation above). The *in vivo* studies are needed to get an approval from FDA (Food and Drug Administration, USA). Rabbits and dogs are the most customarily animal models used for dental bone-implant interface analysis. However, there are some disadvantages in using rabbit equated to bigger animals, for example the dimension since an amount of experimental and control implants are suggested *in each* animal. In addition, tibia and femur of rabbits have notably dissimilar bone structure (*e.g.* the amount of trabecular bone) contrasted to human. Differently the intraoral of canine surroundings produce a microstructure of bone with a trabecular/cortical ratio like which obtained in the mandible of human, also similar microflora and saliva.

Regardless of the various animals model (rabbits, rat, dogs, sheep, pigs or nonhuman primates), useful information can be retrieved from properly designed animal studies. In *in vivo* evaluation, biological or mechanical technique was used. The bone implant contact ratio ($R_{B\text{-}I}$) that often used as evaluated parameters in *in vivo* studies, amount and cellular content type and bone density are pointed to static parameters. Meanwhile, the biomechanical test, (torque, push-out, pull-out) usually is performed to determine the number of force needed by a torque to break down the interface of bone-implant which surround the varying implant surfaces. Unfortunately, obtained *in vivo* results by using different evaluation methods were not compared, and also the global standard of the evaluation method has not determined yet.

2.2.3 Evaluation method in this study

In this study, I used rat tibia to investigate effect of surface hydrophilicity of valve metals and their alloys by *in vivo* tests (as shown in **Figure 2.1**). Six rat tibias were used for each *in vivo* test. The experimental procedure for my *in vivo* study was similar to that described in a previous report [2-46]. Prior to surgery, all implants (metals rods) were cleaned ultrasonically in distilled water, sterilized in a steam autoclave, and immersed in a chlorhexidine gluconate solution. The 340–400 g 10-week-old male Sprague Dawley rats (Charles River Japan, Inc.) were used in this experimental. The animals were kept in quarantine for 7 days. The rats were anesthetized using the pentobarbital (25–30 mg kg^{-1}) and the incision sites (right and left) and surrounding area were shaved and cleaned with povidine iodine solution and 70% ethanol. Slightly oversized holes, which did not pass completely through the bone, were created with a low speed rotary drill under profuse saline irrigation, and the bone debris was washed away with saline. Eventually, implants were inserted in these holes, and Penicillin G was administered for the prevention of infection. The subcutaneous tissue and skin were then closed and sterilized with povidine iodine solution. After surgery, rats were housed

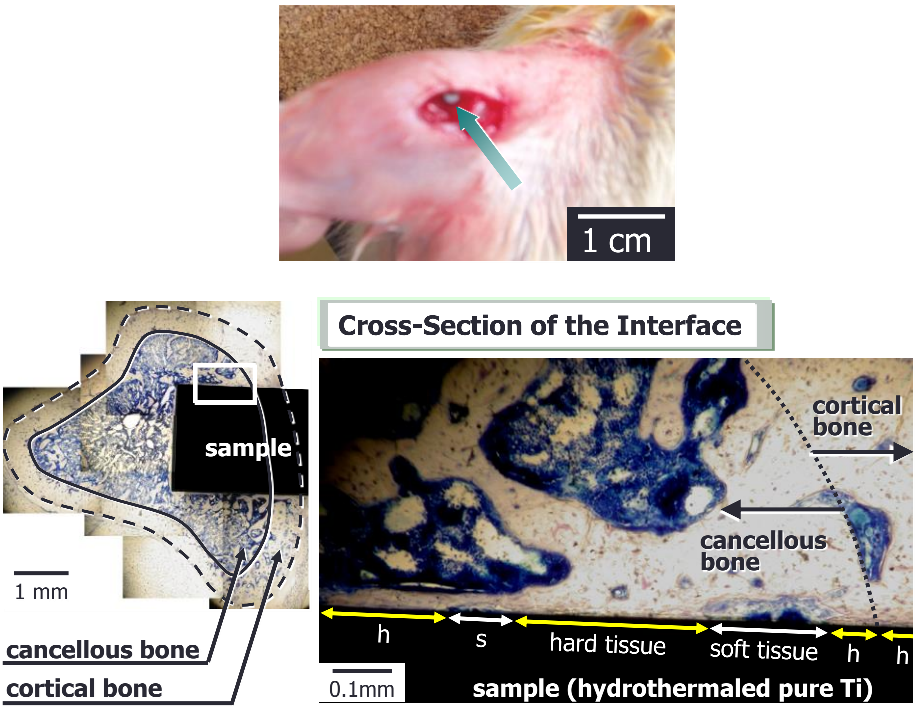

Figure 2.1 *In vivo* test method using rat tibia and calculation of bone implant contact ratio that represent the osteoconductivity of implant materials.

individually in polycarbonate cages, and monitored daily for any complications. Rats were sacrificed after 14 days, and the implants with their surrounding tissue were retrieved. The specimens were fixed in 10% neutral buffered formalin solution, rinsed in distilled water, dehydrated in a graded series of ethanol, and embedded in methyl methacrylate. Following polymerization, each implant block was sectioned to 20 μm thick slices. The final sections were stained with toluidine blue. The cancellous bone and the interface between the implant and the cortical bone were observed by optical microscopy. The total length of hard tissue formed at the interface was measured in the cortical bone and cancellous bone regions, respectively. The osteoconductivity was determined using following equation (2.1). $R_{B\text{-}I}$ represent as bone-implant contact ratio.

$$R_{\mathrm{B-I}}(\%) = \frac{\text{Total length of hard tissue formation on the implant}}{\text{Total length of implant}} \times 100 \qquad \textbf{(2.1)}$$

Significant differences in the $R_{B\text{-}I}$ were examined statistically using Tukey-Kramer method. Differences were considered statistically significant at the $p < 0.05$ level.

Reference

[2-1] Kohn DH. Metals in medical applications, Curr Op Solid State Mater Sci 1998;3:309.

[2-2] Wong M, Eulenberger J, Schenk R, Hunziker E. Effect of surface toplogy on the osseointegration of implant materials in trabecular bone. J Biomed Mater Res 1995;29:1567-1575.

[2-3] Wennerberg A, Alberktsson T. On implant surfaces: a review of current knowledge and oponions. In J Oral Maxillofac Implants 2010;25:63-74.

[2-4] Wennerberg A, Alberktsson T. Effects of titanium surface topography on bone integration: a systematic review. Clin Oral Implants Res 2009;20:172-184.

[2-5] Beutner R, Michael J, Schwenzer B, Scharnweber D. Biological nano-functionalization of titanium-based material surfaces: a flexible toolbox. J R Soc Interface 2010;7S93-S105.

[2-6] Puleo DA and Nanci A. Understanding and controlling the bone-implant interface. Biomater 1999;20:2311-2321.

[2-7] Park BS, Heo SJ, Kim CS, Oh JE, Kim JM, Lee G, Park WH, Chung CP, and Min BM. Effects of adhesion molecules on the behavior of osteoblast-like cells and normal human fibroblasts on different titanium surfaces. Biomed Mater Res A 2005;74:640-651.

[2-8] Yang Y, Cavin R, and Ong JL. Protein adsorption on titanium surfaces and their effect on osteoblast attachment. Biomed Mater Res A 2003;67:344-349.

[2-9] Lee MH, Oh N, Lee SW, Leesungbok R, Kim SE, Yun YP, and Kang JH. Factors influencing osteoblast maturation on microgrooved titanium substrata. Biomater 2010;31:3804-3815.

[2-10] MacDonald DE, Deo N, Markovic B, Stranick M and Somasundaran P. Thermal and chemical modification of titanium-aluminum-vanadium implant materials: effects on surface properties, glycoprotein adsorption, and MG63 cell attachment. Biomater 2004;25:3135-3146.

[2-11] Ruoslahti E. Rgd and other recognition sequences for integrins. Ann Rev Cell Develop Bio 1996;12:697-715.

[2-12] Plopper GE, McNamee HP, Dike LE, Bojanowski K and Ingber DE. Convergence of integrin and growth factor receptor signalling patways within

focal adhesion complex. Molec Bio Cell 1995;6:1349-1365.

[2-13] Sawyer AA, Hennessy KM, and Bellis SL. Regulation of mesenchymal stem cell attachment and spreading on hydroxyapatite by RGD peptides and adsorbed serum proteins. Biomater 2005;26:1467-1475.

[2-14] Oshida Y. Bioscience and Bioengineering of Titanium Materials, Elsevier 2007.

[2-15] Rosa AL, and Beloti MM. Rat bone marrow cell response to titanium and titanium alloy with different surface roughness. Clin Oral Impl Res 2003;14:43-48.

[2-16] Juodzbalys G, Sapragoniene M, Wennerberg A, and Baltrukonis T. Titanium dental implant surface micromorphology optimization. Oral Impl 2007;33:177-185.

[2-17] Kim HM, Kokubo T, Fujibayashi S, Nishiguchi S, and Nakamura T. Bioactive macroporous titanium surface layer on titanium substrate. Biomed Mater Res 2000;52:553-557.

[2-18] Vercaigne S, Wolke JG, Naert I, and Jansen JA Bone healing capacity of titanium plasma-sprayed and hydroxylapatite-coated oral implants. Clin Oral Impl Res 1998;9:261-271.

[2-19] Harris LG, Meredith DO, Eschbach L, and Richards RG. Staphylococcus aureus adhesion to standard micro-rough and electropolished implant materials. Mater Sci. Mater in Med 2007;18:1151-1156.

[2-20] Yamagami A, Yoshihara Y, and Suwa F. Mechanical and histologic examination of titanium alloy material treated by sandblasting and anodic oxidization. The Oral Max Impl 2005;20:48-53.

[2-21] Dalton JE, and Cook SD. In vivo mechanical and histological characteristics of HAcoated implants vary with coating vendor. Biomed Mater Res 1995;29:239-245.

[2-22] Liu Y, de Groot K, and Hunziker EB. Osteoinductive implants: the mise-en-scene for drug-bearing biomimetic coatings. Annals Biomed Eng 2004;32;398-406,0090-6964.

[2-23] Lincks J, Boyan BD, Blanchard CR, Lohmann CH, Liu Y, Cochran DL, Dean DD, Schartz Z. Response of MG63 osteoblast-like cells to titanium and titanium alloy is dependent on surface roughness and composition. Biomater

1998;19:2219-2232.

[2-24] Arimata Y and Iwata H. Effect of wettability and surface functional groups on protein adsorption and cell adhesion using well-defined mixed self-assembled monolayers. Biomater 2007;28:3074-3082.

[2-25] Anselme K, Bigerelle M. Topography effects of pure titanium substrates on human osteoblast long-term adhesion. Acta Biomater 2005;1:211-222.

[2-26] Yamamoto D, Kawai I, Kuroda K, Ichino R, Okido M, and Seki A. Osteoconductivity of anodized titanium with controlled micron-level surface roughness. Mater Trans 2011;52:1650-1654.

[2-27] Yamamoto D, Iida T, Arii K, Kuroda K, Ichino R, Okido M, and Seki A. Surface hydrophylicity and osteoconductivity of anodized Ti in aqueous solution with various solute ions. Mater Trans 2012;53:1956-1961.

[2-28] Kilpadi Kl, Chang PL, Bellis Sl. Hydroxyapatite binds more serum proteins, purified integrins, and osteoblast precursor cells than titanium or steel. J Biomed Mater Res 2001;57:258-267.

[2-29] Schneider G, Burridge K. Formation of focal adhesions by osteoblasts adhering to different substrata. Exp Cell Res 1994;214:264-269.

[2-30] Junker R, Dimakis A, Thoneick M, and Jansen JA. Effects of implant surface coatings and composition on bone integration: a systematic review. Clin Oral Implants Res 2009;20:185-206.

[2-31] Schwarz F, Wieland M, Schwartz Z, Zhao G, Rupp F, Geis-Gerstorfer J, Schedle A, Broggini N, Bornstein MM, Buser D, Ferguson SJ, Becker J, Boyan BD, and Cochran DL. Potential of chemically modified hydrophilic surface characteristics to support tissue integration of titanium dental implants. J Biomed Mater Res B App Biomater 2009;88:544-557.

[2-32] Rausch-fan X, Qu Z, Wieland M, Matejka M, and Schedle A. Differentiation and cytokine synthesis of human alveolar osteoblasts compared to osteoblast-like cells (MG63) in response to titanium surfaces. Dent Mater 2008;24:102-110.

[2-33] Zhao G, Raines AL, Wieland M, Schwartz Z and Boyan BD. Requirement for both micron- and submicron scale structure for synergistic responses of osteoblasts to substrate surface energy and topography. Biomaterials 2007;28: 2821-2829.

[2-34] Bornstein MM, Valderrama P, Jones AA, Wilson TG, Seibl R, and Cochran DL. Bone apposition around two different sandblasted and acid-etched titanium implant surfaces: a histomorphometric study in canine mandibles. Clin Oral Imp Res 2008;19:233-241.

[2-35] Buser D, Broggini N, Wieland M, Schenk RK, Denzer AJ, Cochran DL, Hoffmann B, Lussi A and Steinemann SG. Enhanced bone apposition to a chemically modified SLA titanium surface. J Dent Res 2004;83:529-533.

[2-36] Schwarz F, Ferrari D, Herten M, Mihatovic I, Wieland M, Sager M, and Becker J. Effects of surface hydrophilicity and microtopography on early stages of soft and hard tissue integration at nonsubmerged titanium implants: an immunohistochemical study in dogs. J Periodontology 2007;78:2171-2184.

[2-37] Yamamoto D, Arii K, Kuroda K, Ichino R, Okido M, and Seki A. Osteoconductivity of superhydropilic anodized TiO_2 coatings on Ti treated with hydrothermal processes. J Biomater Nanobiotech 2013;4:45-52.

[2-38] Yamamoto D, Kuroda K, Ichino R, and Okido M. Anodic oxide coatings on Ti alloys and their osteoconductivity. Mater Sci Forum 2012;706-709:612-616.

[2-39] Simonsen ME, Li Z, and Sogaard EG. Influence of the OH groups on the photocatalytic activity and photoinduced hydrophilicity of microwave assisted sol-gel TiO_2 film. App Surf Sci 2009;255:8054-8062.

[2-40] Zhang KX, Wang W, Hou JL, Zhao JH, Zhang Y, and Fang YC. Oxygen plasma induced hydrophilicity of TiO_2 thin films. Vacuum 2011;85:990-993.

[2-41] Takebe J, Ito S, Miura S, Miyata K, and Ishibashi K. Physicochemical state of the nanotopographic surface of commercially pure titanium following anodization hydrothermal treatment reveals significantly improved hydophilicity and surface energy profiles. Mater Sci and Eng C 2012;32:55-60.

[2-42] Binon PP. Implants and components: entering the new millennium. Int J Oral Maxillofac Implants 2000;15:76-94.

[2-43] Coelho PG, Granjeiro JM, Romanos GE, Suzuki M, Silva NR, Cardaropoli G, ThompsonVP, and Lemons JE. Basic research methods and current trendsof dental implant surfaces. J Biomed Mater Res B Appl Biomater 2009;88:579-596.

[2-44] Anselme K. Osteoblast adhesion on biomaterials. Biomaterials 2000;21:667-681.

[2-45] Lemons JE. Biomaterials, biomechanics, tissue healing, and immediate-function

dental implants. J Oral Implantol 2004;30:318-324.

[2-46] Kuroda K, Nakamoto S, Miyashita Y, Ichino R, and Okido M. Osteoconductivity of HAp films with different surface morphologies coated by the thermal substrate method in aqueous solutions. Mater Trans 2006;47:1391-1394.

CHAPTER 3

High Osteoconductive Surface of Pure Titanium by Hydrothermal Treatment

3.1 Introduction

Titanium has been one successful metals for implant applications especially in dental and orthopedic fields because of its superior properties such as good biocompatibility, good ductility, high fatigue and tensile strengths, lower allergenicity and high corrosion resistance arising from the formation of a self-healing passive oxide layer on their surface. However, pure Ti has low osseointegration due to it cannot bond with bone directly and contribute new bone formation on its surface at the early stage after implantation due to insufficient bioactivity [3-1]. Therefore, surface modification is become more essential to enhance its osteoconductivy.

In the previous chapter, it already explained that surface roughness and hydrophilicity are important factors of biomaterials which related with protein adsorption and cell adhesion after implantation into the body [2-23][2-24]. Surface treatments such as anodizing, hydrothermal treatment, UV light irradiation and plasma irradiation processes have been widely used in creating hydrophilic surfaces on implant materials. In this chapter, these various processes were applied to Ti samples which purpose to produce hydrophilic surfaces in improving their osteoconductivities. Then, I compared their osteoconductivities with as-polished and those of Ti implant produced by any other surface modifications. The osteoconductivity was evaluated in *in vivo* test. Finally, the effective surface modifications will become a fundamental and implemented to improve the osteoconductivity of other metallic biomaterials that will be described on the following chapters.

3.2 Materials and methods

3.2.1 Pre-treatment of Ti substrates

Commercially pure Ti (cp-Ti) plates with 1.1 cm^2 in surface area and cp-Ti rods with 2 mm in diameter and 5 mm in length were used as substrates to evaluate surface properties and *in vivo* testing of as-polished, as-anodizing, as-hydrothermal and anodizing + hydrothermal. The substrates were ground with emery papers up to #2000, and followed by polishing using Al_2O_3 particles with 0.05 µm in size. Then, they were cleaned and degreased with ethanol in an ultrasonic cleaner for 5 min, and finally dried in air. After this pre-treatment, following treatments were carried out.

3.2.2 Hydrothermal process

The hydrothermal process was applied to pure Ti after polishing. The samples were immersed in a beaker of 300 ml distilled water and put in an autoclaving unit. The temperature of hydrothermal vessel was set at 453 K and kept at this condition for 180 min. Regarding to a previous research, the hydrothermal treatment performed at a temperature of 453 K for 180 min was sufficient to produce a small and stable WCA [2-27]. After hydrothermal treatment, the beaker was directly taken out from autoclave unit and the samples were cooled naturally to the room temperature in the baker.

3.2.3 Anodizing process

The Ti substrate after polishing was used for anodizing process. A Ti substrate and a Pt coil were used as the anode and cathode electrodes, respectively. The electrolyte for anodizing treatment was 0.1 M H_2SO_4 aqueous solution with pH around 1.0. The anodizing process was performed by applied voltage up to 100 V at 0.1 Vs^{-1} at room temperature as reported by Yamamoto *et al.* [2-26]. This processing could produce a TiO_2 coating film with Ra (arithmetical means of roughness) < 0.1 µm.

3.2.4 Anodizing + hydrothermal process

The anodized Ti plate was hydrothermally treated at 453 K for 180 min in 300 ml distilled water using an autoclaving unit.

3.2.5 UV light irradiation process

UV light irradiation process used Ti substrates after polished. The wave length of UV light used about 250 nm.

3.2.6 Plasma irradiation process

The as-polished Ti substrate was treated by plasma irradiation process using nitrogen (N_2) gas with a flow rate of 20 L/min. The distance between samples and plasma head was 10 mm. Output of this process was 500 W.

3.2.7 Samples storage

After any treatments, the samples were sterilized at temperature 394 K for 20 min. Then, the surface treated samples were stored in the following three conditions at room temperature in air, in distilled water and in five times concentrated phosphate buffered saline (×5 PBS(−), pH 7.5). The composition of 5PBS(−) was 685 NaCl, 13.5 KCl, 50 Na_2HPO_4, and 8.8 KH_2PO_4 in mM.

3.2.8 Surface characterization

The surface morphologies of the all samples after various processes were observed using SEM. The coated films were determined by XPS and XRD. The surface roughness was measured using means a confocal laser scanning microscope with 150 µm × 112 µm in an area of measurement and was expressed as the arithmetical means of the surface roughness (Ra). The WCA was estimated using a 2 µL droplet of distilled water

3.2.9 *In vivo* test

All rod samples after various processes and keeping in different storages media were subjected to *in vivo* testing. In this research, the method of *in vivo* test is refer previous research [2-46] which already explained in Chapter 2.

3.3 Results and Discussion

3.3.1 Surface characterization of investigated samples

The SEM, optical micrograph and surface roughness (Ra) of samples are displayed in **Figure 3.1**. Compared to as-polished samples (a), the sample surfaces of as-anodizing (b), as-hydrothermal (c) and anodized + hydrothermal (d) show a change of colour become yellow as an interference colour, which indicates that an oxidation was occurred during process. This phenomenon was proved by XRD analysis where there was found only a peak of TiO_2 (anatase) in all treated samples except as-polished sample as shown in **Figure 3.2**. However, the intensity of the anatase peak of hydrothermal samples (c) becomes weak and it cannot be detected more clearly compared with the other samples. It is probably because the oxide layer formed by only hydrothermal treatment was too thin. Besides, all the sample surfaces were non porous and only some fine particles attached to the hydrothermal surfaces. Although three types

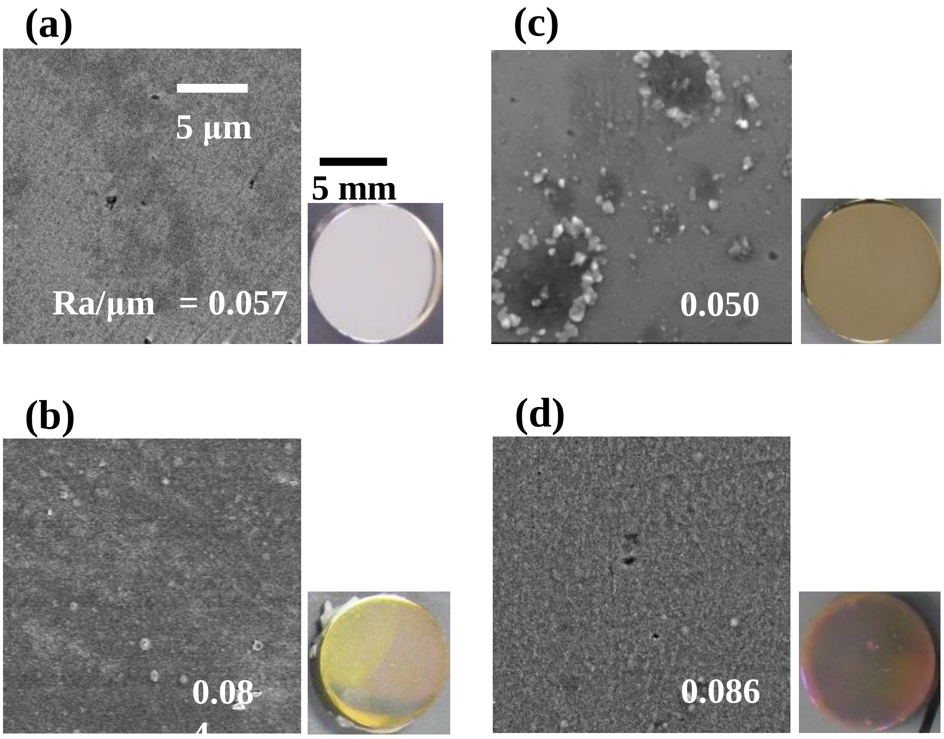

Figure 3.1 Surface morphology, optical micrograph and Ra of Ti samples with various surface modifications: (a) as-polished; (b) as-anodized; (c) as-hydrothermal and (d) anodized + hydrothermal.

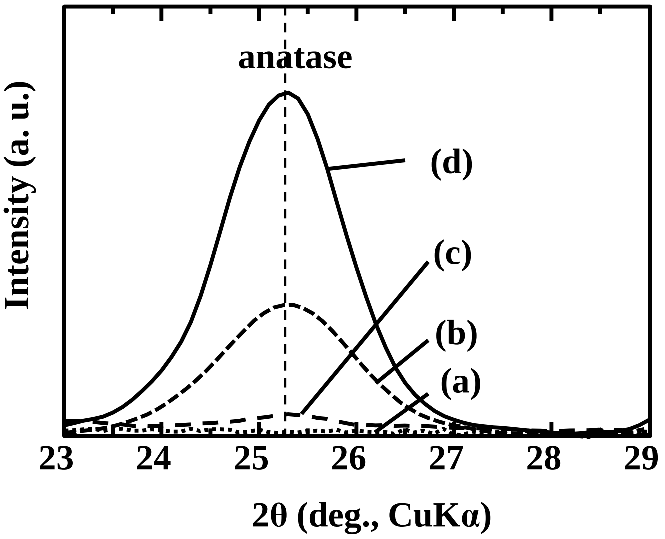

Figure 3.2 XRD patterns of Ti samples with various surface modifications: (a) as-polished; (b) as-anodized; (c) as-hydrothermal; and (d) anodized + hydrothermal.

of treated samples (as-anodized, anodized + hydrothermal and as-hydrothermal samples) contribute to slight increase in surface roughness compared to as-polished, the smooth surfaces still kept maintain with Ra/µm < 0.1. It can be said that the anodizing and hydrothermal processes give no significant effect on the surface roughness, so we can ignore Ra effect on *in vivo* testing.

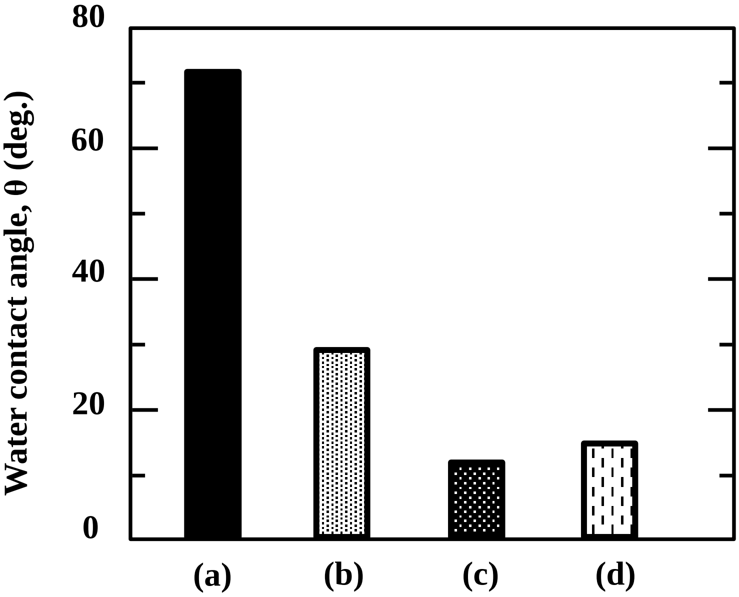

Figure 3.3 The WCA of Ti samples with various surface modifications: (a) as-polished; (b) as-anodized; (c) as-hydrothermal, and (d) anodized + hydrothermal.

On the other hand, a contrast phenomenon is shown in wettability (**Figure 3.3**) where the WCA of the as-polished sample decreased from 71 (deg.) to 28 (deg.) after anodizing. Then, the sample surfaces of hydrothermal with and without anodizing become more hydrophilic when the WCA continued to decrease until 13 and 10 (deg.). This figure proves that anodizing and hydrothermal treatments were effective to create hydrophilic surfaces on pure Ti. As additional data, we also investigated and compared both anodizing and hydrothermal treatment with two more surface treatments (UV light irradiation and plasma irradiation) as display in **Figure 3.4**. We found that all surfaces samples showed the similar tendency where the WCA after processes decreased significantly compared to as-polished Ti and became more hydrophobic when all samples stored in air for longer time. However, even though UV light irradiation and plasma irradiation processes can create the hydrophilic surfaces, they are difficult to apply for complex-shaped substrates of implant materials with complex topographies, because of the line-of-sight nature of the methods. Therefore, in this research I only applied anodizing and hydrothermal treatment processes to improve the osteoconductivity of pure Ti and other valve metals such Nb, Ta, Zr and their alloys.

Regarding to previous research, the high WCA can be caused by adsorption of hydrocarbon which come from air atmosphere [3-2]. In a fact, determination of the influence of adsorbed oxygen (O) and carbon (C) on the treated samples were analyzed using XPS.

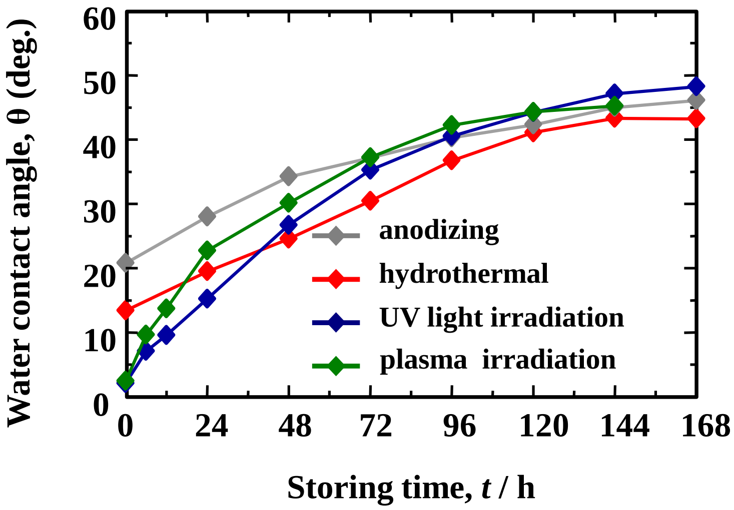

Figure 3.4 The WCA of Ti samples with various surface modifications such as anodizing, hydrothermal treatment, UV light irradiation, and plasma irradiation.

From Figure 3.5, it shows that all of the investigated samples mainly composed by Ti, O and C that was detected as surface contaminant in the XPS data. The O_{1S} XPS spectrum deconvoluted into three peaks (530.1, 531.5, and 532.5 eV) originated from anhydrous oxide (O^{2-}), hydroxyl group (OH^{-}) and hydrate and/or adsorbed water (H_2O), in the same way as in a previous report [3-3]. Meanwhile, the C_{1S} spectrum also contained three peaks originating from C−H, C−O, and C = O. The ratios of the proportion of OH^{-} to that of TiO_2, [OH^{-}]/ [TiO_2] and C−H to that of TiO_2, [C−H]/[TiO_2] of the surface modified titanium in different processes are shown in Figure 3.5. The [OH^{-}]/[TiO_2] values in the surface layers modified with anodizing process in H_2SO_4 solution are smaller than those without the treatment (as-polished). Then, this value decreased slightly after additional hydrothermal treatment. These results show that the amount of OH^{-} group on anodized sample was not change and affected by hydrothermal process.

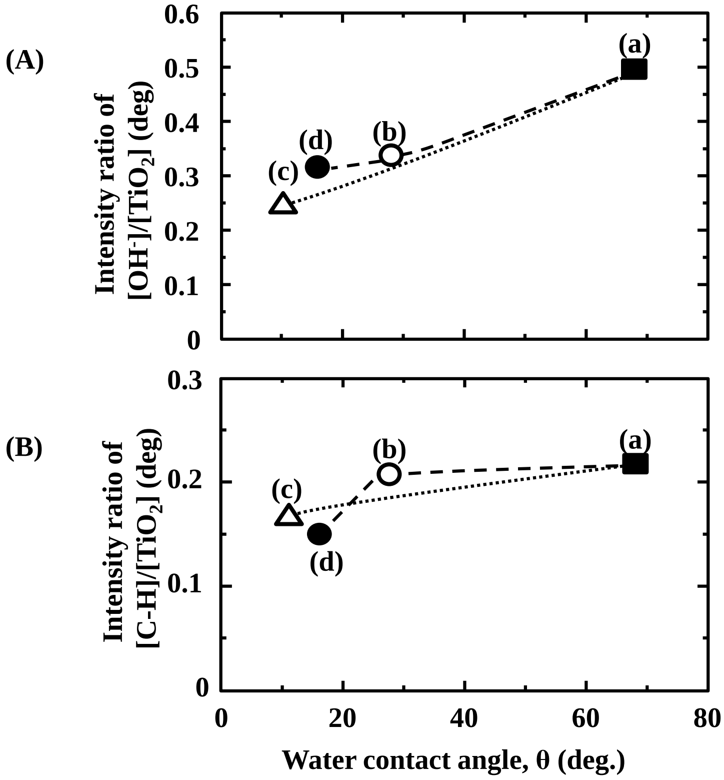

Figure 3.5 The correlation between WCA and the amount (A) OH^{-} group and (B) the adsorbed C−H of Ti samples with various surface modifications: (a) as-polished; (b) as-anodized; (c) as-hydrothermal; and (d) anodized + hydrothermal.

In contrast, the [C−H]/ [TiO_2] values of as-polished and as-anodized samples are almost similar and then continue to reduce after additional hydrothermal treatment on anodized titanium samples. This trend also can be found in Ti samples after applying only hydrothermal process without anodizing. It suggested that the reason why the Ti surface becomes more hydrophilic when hydrothermal process was applied to the sample was because of the reduction in adsorbed hydrocarbon.

Storing samples in different environments will influence their WCA. It can be seen in **Figure 3.6**, when the all treated samples stored in the air (A), the WCA increased by increasing of the storage time. This fact is also shown to the samples stored in the distilled water (B). However, this tendency became reverse for all investigated samples when they stored in the PBS(−) especially in a higher concentration of PBS(−) solution, ×5 PBS(−) (C).

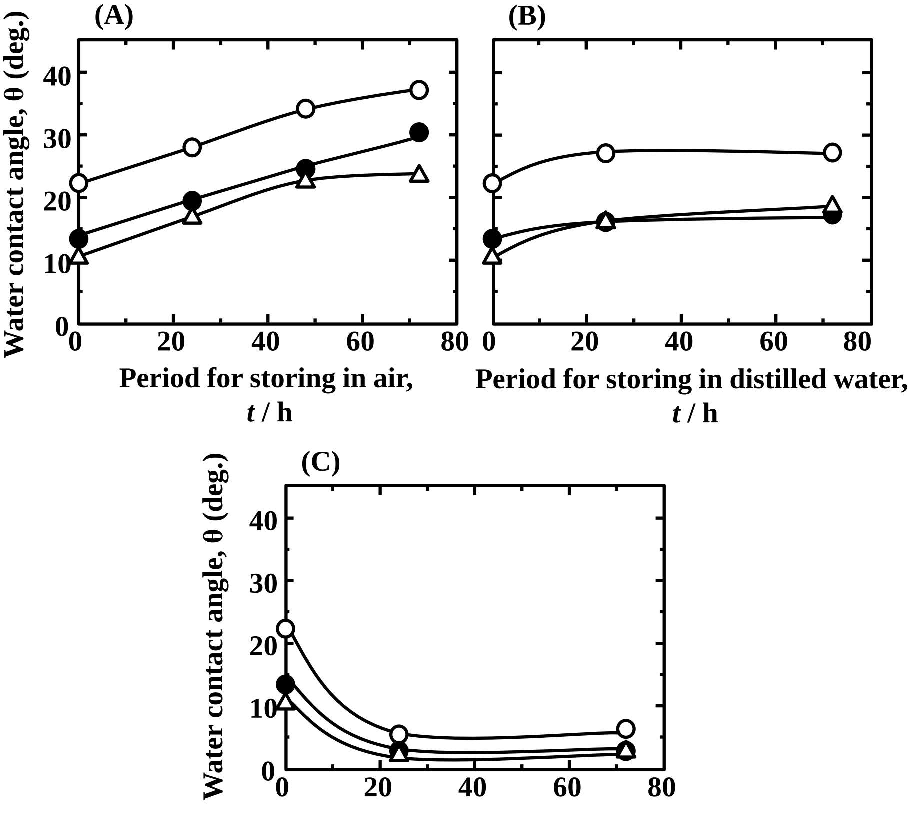

Figure 3.6 Changes in WCA under different storing conditions: in air, distilled water, and ×5 PBS(−) on Ti samples processed with various surface modifications (○: as-anodized; ●: anodized + hydrothermal; and △ : as-hydrothermal.

Their WCA became decreased by increasing of the storage time. The difference of WCA with time in the various storage environments were effected by chemical species on the surface [3-4].From the same figure, we also can see that the WCA of hydrothermal sample is lower than that of as-anodized and anodized + hydrothermal samples after keeping in the air, distilled water and ×5 PBS(−). The contamination was removed by hydrothermal treatment at high temperature, high pressure and made a clean surface, so that, the hydrophilicity was improved likely. A drastically lessening of WCA of as-hydrothermal samples to become less than 10 (deg.) was occurred when the samples immersed into the ×5 PBS(−). It can be indicated that by immersing each substrate in ×5 PBS(−), the sample surface became super-hydrophilic. Various inorganic solute ions such as Na^+ and Cl^- in high concentration ×5 PBS(−) were absorbed on the hydrothermaled clean surface, and has improved more hydrophilic, as likely [3-4].

3.3.2 *In vivo* test

Yamamoto *et al.* explained that the $R_{B\text{-}I}$ of the cortical and cancellous bone parts of the anodized samples in various aqueous solutions had same trend, but the metabolism in cancellous bone was faster than the cortical bone and it took a long time to find the bone reaction to anodized specimens (14 d) [2-27]. Therefore, in this analysis, we focus on the $R_{B\text{-}I}$ values from the cortical bone part. Titanium substrates after various processes and different storage condition were subjected to *in vivo* testing and their osteoconductivity were observed. **Figure 3.7** shows the effect of the WCA on the osteoconductivity ($R_{B\text{-}I}$ in the cortical bone) of pure titanium in different processes. As a comparison, we used a previous *in vivo* data for TiO_2-coated samples after anodizing in several electrolyte solutions with Ra/μm < 0.1 [2-26][3-5][2-27]. This figure explains that as-polished sample which hydrophobic surface has low osteoconductivity (12%). By decreasing of WCA, the osteoconductivity of as-polished titanium was increased after applying anodizing (43%) and additional hydrothermal process on anodized sample (49%). The notable improvement of osteoconductivity was detected when the hydrothermal sample immersed into the ×5 PBS(−) and become a super-hydrophilic surface. Even though there was a small difference of the $R_{B\text{-}I}$ between as-anodized + hydrothermal (58%) and as-hydrothermal samples (50%) after storing them into ×5 PBS(−), it can be said that a better osteoconductivity can be achieved by applying hydrothermal process even though without anodizing.

Factors with essential influence in the osteoconductivity are surface roughness, contact angles or wettability. However, surface roughness was nearly constant in this experiment. On the other hand, a high contact angle with a hydrophobic surface generates poor cell attachment and as a contrary, a low contact angle contributes to high surface energy, which is another factor that provides to better cell attachment. Hydrophilicity is a crucial property of implants which enhances the adhesion, spreading, and proliferation of cells on their surfaces. Cell adhesion proteins adsorption on the implant surfaces is notably affected by surface wettability. As a consequence, it strengthens the adhesion and spreading of osteoblast precursors on implant surfaces [2-28][2-29].

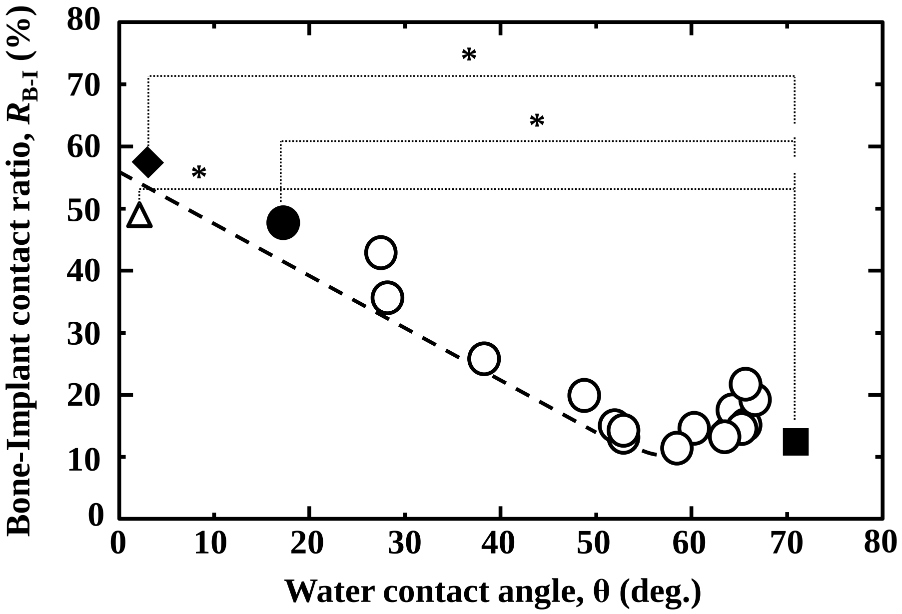

Figure 3.7 Relationship between $R_{B\text{-}I}$ value (in cortical bone) and WCA of Ti samples with various surface modifications and subsequent storage methods: ■ as-polished; ○ anodized in different aqueous solutions (Ra/μm < 0.1); ● anodized + hydrothermal, then stored in distilled water; ◆ anodized + hydrothermal, then stored in ×5 PBS(−) solution; and △ as- hydrothermal, then stored in ×5 PBS(−) solution.

The surface with high wettability can improve the healing process of bone at early period on the interface of cell-biomaterial by reducing the RGD adsorption and enhancing cell performance on the implant surfaces [3-6][3-7][3-8]. Furthermore, hydrophilic surfaces can encourage the biomineralization process. In this study, hydrothermal process without anodizing which applied in to Ti substrates contributed the hydrophilic surface with lower WCA as shown in **Figure 3.3**. It showed the enhanced of osteoconductivity compared to as-polished Ti. Furthermore, by storing into ×5 PBS(−), the hydrothermal sample surface become super-hydrophilic and it indicated a high osteoconductivity .

3.4 Conclusions

In this chapter, it was observed that hydrothermal process without coating (anodizing) which followed by immersion into ×5 PBS(−) produced a super-hydrophilic surface (WCA < 10 deg.) and improved the osteoconductivity of pure Ti significantly about 50% higher than as-polished samples and also higher than that of anodized TiO_2 samples in H_2SO_4. It can be concluded that combination process between hydrothermal treatment and storing in ×5 PBS(−) was effective to enhance the osteoconductivity of pure Ti. This process is used as a reference to the next chapters.

REFERENCES

[3-1] Garcia-Alonso MC, Saldana L, Valles G, Gonzalez JL-Carrasco Gonzalez, Cabrero J, Martinez ME, Gil-Garay E and Munuera, L. *In Vitro* corrosion behavior and osteoblast response of thermally oxidized Ti6Al4V alloy. Biomater 2003;24:19-26.

[3-2] Att W, Hori N, Takeuchi M, Ouyang J, Yang Y, Anpo M, and Ogawa T. Time-dependent degradation of titanium osteoconductivity: an implication of biological aging of implant materials. Biomater 2009;30:5352-5363.

[3-3] Tanaka Y, Nakai M, Akahori T, Niinomi M, Tsutsumi Y, Doi H, and Hanawa T. Characterization of air-formed surface oxide film on Ti-29Nb-13Ta-4.6Zr alloy surface using XPS and AES. Corr Sci 2008;50:2111-2116.

[3-4] Yamamoto D, Arii K, Kuroda K, Ichino R, Okido M, and Seki A. Osteoconductivity of superhydropilic anodized TiO_2 coatings on Ti treated with hydrothermal processes. J Biomater Nanobiotech 2013;4:45-52.

[3-5] Yamamoto D, Kawai I, Kuroda K, Ichino R, Okido M, and Seki A.

Osteoconductivity and hydrophilicity of TiO_2 coatings on Ti substrates prepared by different oxidizing processes. Bioinorg Chem App 2012;2012, Article ID: 495218.

[3-6] Rupp F, Scheideler L, Olshanska N, De Wild M, Wieland M, and Geis J-Gerstorfer. Enhancing surface free energy and hydrophilicity through chemical modification of microstructured titanium implant surfaces. J Biomed Mater Res A 2006;76A:323-334.

[3-7] Yamamoto H, Shibata Y, and Miyazaki T. Anode glow discharge plasma treatment of titanium plates facilitates adsorption of extracellular matrix proteins to the plates. J Dent Res 2005;84:668-671.

[3-8] Shibata Y, Hosaka M, Kawai H, and Miyazaki T. Glow discharge plasma treatment to titanium plates enhances adhesion of osteoblast-like cells to the plates through the integrin-mediated mechanism. J Oral Max Impl 2002;17:771-777.

CHAPTER 4

Enhancement of Valve Metals Osteoconductivity by One-Step Hydrothermal Treatment

4.1 Introduction

Ti, Zr, Nb and Ta, which are well-known, anti-corrosive valve metals, are widely used as biomedical metallic materials. The stable and protective oxide film that can form on the surface (mainly consisting of TiO_2, ZrO_2, Nb_2O_5 and Ta_2O_5, respectively) exhibits excellent corrosion resistance, chemical inertness and low solubility, especially in oxidizing media or even in the media containing chloride ions [4-1][4-2]. These metals are also recognized as nontoxic elements and biologically inert. However, due to insufficient bioactivity, many researchers have tried to focus on surface-modification methods in order to improve better osseointegration properties [4-3].

As concluded in Chapter 3, hydrothermal treatment was effective to improve the osteoconductivity of pure Ti. In this chapter, this process was intended to apply in order to improve the osteoconductivity of other valve metals such as Zr, Nb, and Ta. Then, these results were compared with that of as-polished, as anodized and both anodized + hydrothermally treated samples. Their osteconductivities were then evaluated using *in vivo* tests.

4.2 Materials and methods

4.2.1 Sample preparation

Two different sample shapes, made from pure valve metals (Ti, Ta, Zr and Nb), were used to characterize the surface and to evaluate osteoconductivity in the *in vivo* test. Plates (12 mm × 4 mm) were used for surface characterization and rods (Ø2 mm ×

5 mm) were used for the *in vivo* test. The samples were abraded with emery papers up to #2000, buffed with Al_2O_3 particles of 0.05 µm size, degreased with ethanol for 5 min in an ultrasonic cleaner, and finally dried at room temperature spontaneously. They are designated "as-polished" samples in following sections.

4.2.2 Anodic oxidation

The valve-metal samples and a Pt coil were set as the anode and cathode, respectively, without a reference electrode being employed. The electrolyte solution used was 0.1 M H_2SO_4, and it was stirred by a magnetic stirrer at a constant temperature (298 K) in a water bath during anodizing. The anodizing voltage was increased from 0 V to the final voltage of 150 V at a rate of 0.1 Vs^{-1}. The samples were then sterilized in an autoclave at 121 °C for a period of 20 min. They are designated the "as-anodized" samples in the following explanation.

4.2.3 Hydrothermal process

The one-step hydrothermal process was applied to as-polished samples and designated "hydrothermally treated" samples in the next description. They were immersed in beaker containing 300 cm^3 distilled water and put into an autoclave. The temperature of the hydrothermal vessel was set at 453 K and was maintained for 180 min. After the hydrothermal treatment, the beaker was taken directly out of the autoclave and cooled naturally to room temperature.

4.2.4 Anodizing + hydrothermal process

The as-anodized samples were hydrothermally treated at 453 K for 180 min in 300 cm^3 distilled water using an autoclave. The samples that were both anodized and subjected to the hydrothermal treatment are simply designated "anodized + hydrothermally treated" samples in the following sections.

4.2.5 Samples storage

The samples were stored under three storage media (at room temperature): in air, in distilled water and in five times concentrated phosphate buffered saline (×5 PBS(–)). The storing period of samples in the different media was carried out up to 168 h. For *in vivo* test, we used untreated and hydrothermally treated samples after 24 h immersed in ×5 PBS(–).

4.2.6 Surface characterization

The surface morphologies of the treated and untreated samples were observed by SEM. The crystalline structures and chemical compositions of the valve metals were determined by XPS and XRD. The surface roughness was measured using a confocal laser scanning microscope with 150 µm × 112 µm in an area of measurement. The arithmetical means of the Ra were used as the surface roughness. The WCA) was measured at three points on each sample surface using a 2 µL droplet of distilled water after 24 h in air and the average value was used as the WCA value.

4.2.7 *In vivo* test

The hydrothermaled samples which stored in ×5 PBS(–) were used for *in vivo* tests. The experimental procedure for our *in vivo* study was similar to that described in a previous report [2-46] as explained in Chapter 2.

4.3 Results

4.3.1 Surface characteristics

As shown in **Figure 4.1**, XRD analysis detected the presence of tiny TiO_2 (anatase) and ZrO_2 peaks on the surface of the Ti and Zr samples after anodizing and hydrothermal treatments. However, the peak intensities became relatively weak on hydrothermally treated Ti and Zr surface samples. This was probably because their oxide layers were thin. In contrast, no oxide peaks for Nb and Ta samples were revealed by XRD for all treated samples. It was considered to be an amorphous oxide [4-4]. **Figure 4.2** shows the representative surface morphologies of the treated and untreated sample surfaces with various surface roughnesses. A small amount of pores with fine white particles attached to the surface was observed for all treated samples.

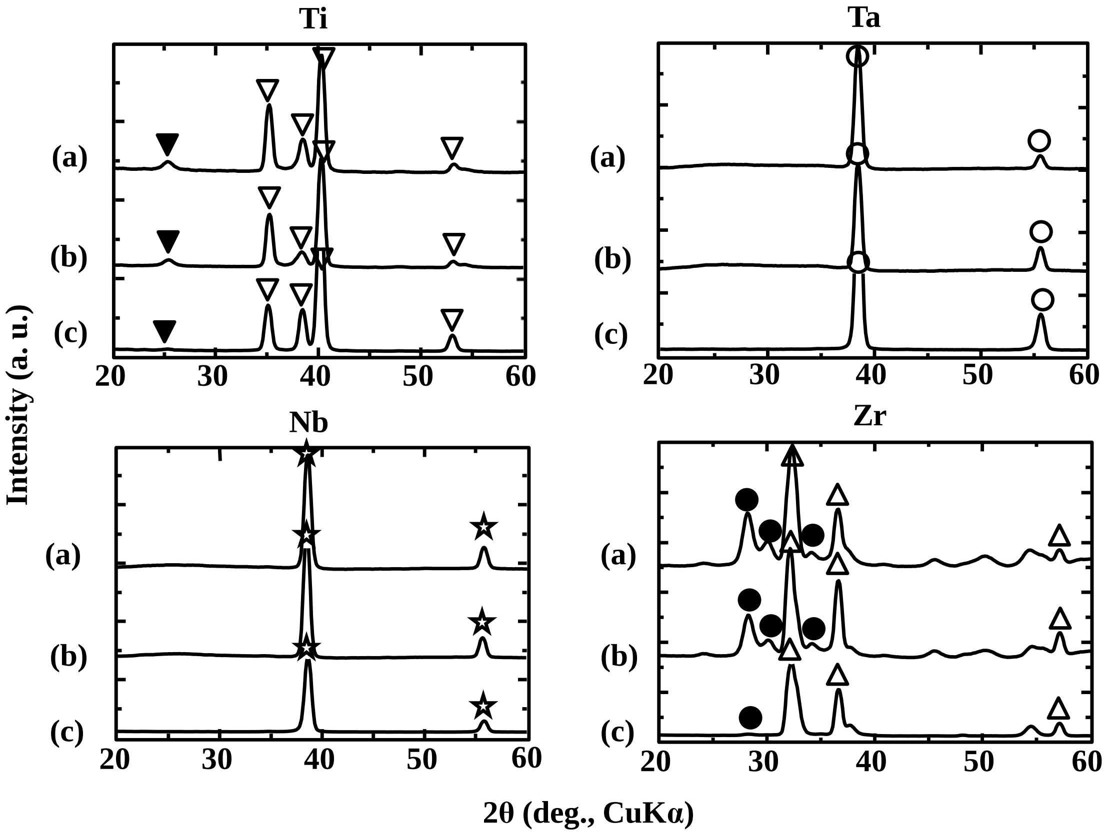

Figure 4.1 XRD patterns of Ti, Nb, Ta, Zr at different surface treatments: (a) as-anodized; (b) anodized+hydrothermally treated; and (c) hydrothermally treated. Symbols: ▼= TiO_2 (anatase), ▽= α-Ti, ○= Ta, ☆= Nb, ●=ZrO_2, and △= Zr.

In comparison, the as-polished samples exhibited a smooth surface. No significant difference in surface roughness was observed between treated and untreated surfaces. The average Ra of the treated samples remained similar to the initial Ra of untreated samples (Ra/µm < 0.1). This Ra value was associated with higher levels of osteoconductivity compared with samples that had a high Ra value [3-5]. The WCA measurements showed that the values of all contact angles of as-polished valve metals were in the range of 60–80 (deg.); namely, all the surfaces were hydrophobic surfaces (**Figure 4.3**). Contamination by a large amount of hydrocarbon on the surface, which comes from atmospheric air can produce high WCA. An implant with a hydrophobic surface exhibits a low level of biocompatibility because of its poor cell attachment and protein adsorption. Furthermore, the WCA of Ti, Nb, Ta and Zr samples were decreased by applying an anodizing process. However, their surfaces were still hydrophobic with WCA higher than 60 (deg.).

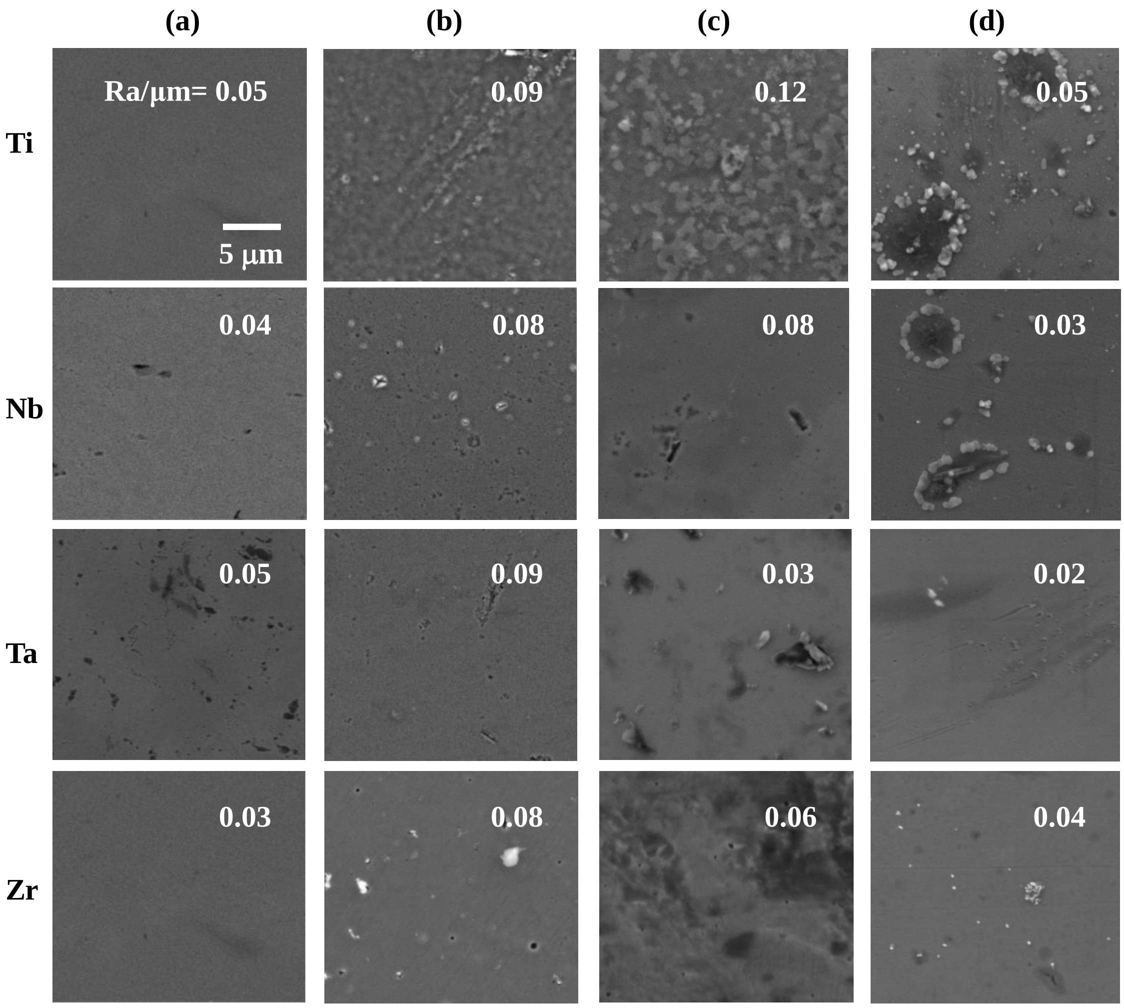

Figure 4.2 Surface morphology, optical micrograph and surface roughness (Ra) of Ti, Nb, Ta and Zr, each of which was (a) as-polished, (b) as-anodized, (c) anodized + hydrothermally treated and (d) hydrothermally treated.

A drastic reduction can be noticed with the as-anodized Ti sample, where its WCA decreases to 16 (deg.). This implies that the anodizing process only had a positive effect on pure Ti by producing a hydrophilic surface. On the other hand, when the hydrothermal process was implemented on all anodized samples, their WCA decreased. A significant decrement can be observed on the surfaces of anodized + hydrothermally treated Ti sample, where its WCA was more hydrophilic (16 (deg.)) than that of Nb, Ta and Zr (35, 30 and 30 (deg.), respectively). This similar trend was also detected on the WCA of samples processed by the one-step hydrothermal treatment, although their values were a bit higher than those of the anodized + hydrothermally treated samples.

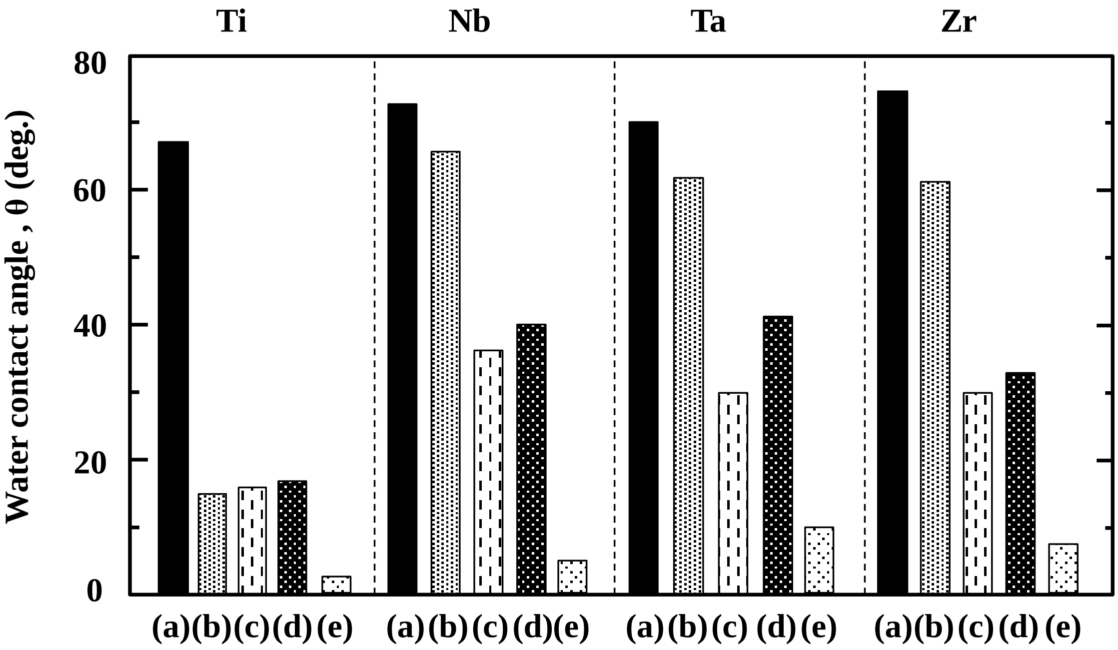

Figure 4.3 The WCA of Ti, Nb, Ta and Zr samples with various surface modifications: (a) as-polished; (b) as-anodized; (c) anodized + hydrothermally treated; (d) hydrothermally treated and (e) hydrothermally treated after 24 h immersed in ×5 PBS(−).

Therefore, hydrophilic surfaces of valve metals can be produced by one-step hydrothermal treatment. Moreover, the surfaces of hydrothermally treated samples immersed in ×5 PBS(–) became super-hydrophilic, with WCA < 10 (deg.). This might be due to an adsorption of inorganic solute ions such as Na^+, Cl^- etc. onto the surface [3-4]. The storage media used also influences the surface hydrophilicity (WCA) of treated samples. As displayed in **Figure 4.4**, when the hydrothermally treated samples were stored in air, the WCA tended to increase significantly with increasing storage time, meanwhile storage in distilled water caused the WCA to only slightly increase. However, this trend was reversed when the hydrothermaled samples were stored in ×5 PBS(–). The WCA continuously decreased with an increase in storage time. A comparable trend was also detected in the as-polished and anodized samples after storage in ×5 PBS(–). Among these samples, the lowest WCA were obtained for hydrothermaled samples stored in ×5 PBS(–).

The presence of OH^- groups and adsorbed hydrocarbons (C–H) are two components that must influence the surface hydrophilicity.

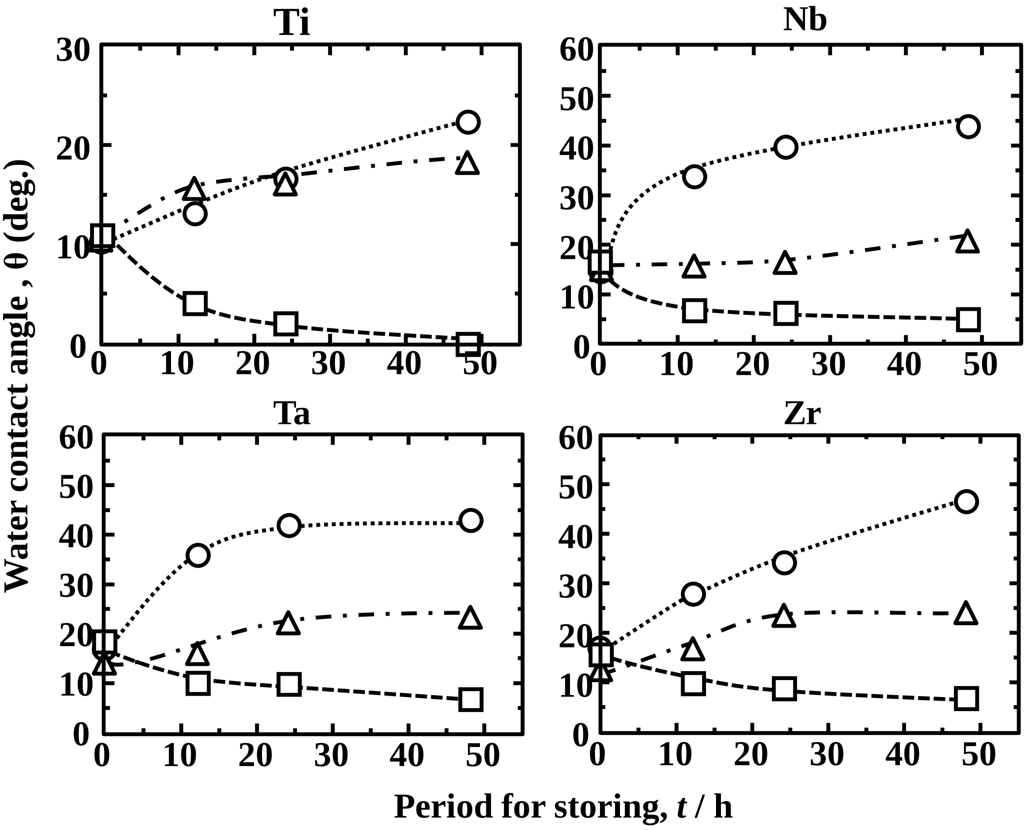

Figure 4.4 Relationships between WCA and periods of immersed of hydrothermally treated valve metals under different storage media: ○ in air; △ in distilled water; and ☐ in ×5 PBS solution(−).

Each component has the opposite effect on WCA [4-5]. Adsorption of hydrocarbons, presenting as surface contamination, can be produced on titanium during finishing processes such as machining, and surface treatments such as anodizing and sterilization. **Figure 4.5** shows the XPS survey results of untreated and treated valve metals. The method of analysis for the spectrum was as well as previous report [2-13]. This figure shows that the amount of adsorbed C–H in the untreated samples was higher than that of the treated samples. By applying a hydrothermal treatment with and without anodizing, the amount of adsorbed C–H in the samples reduced remarkably. In contrast, the amount of OH^- groups did not increase and appears to be unaffected by hydrothermal processing. It is concluded that the reduction in adsorbed hydrocarbons is one of the reasons why the surface of the valve metals becomes more hydrophilic after hydrothermal processing.

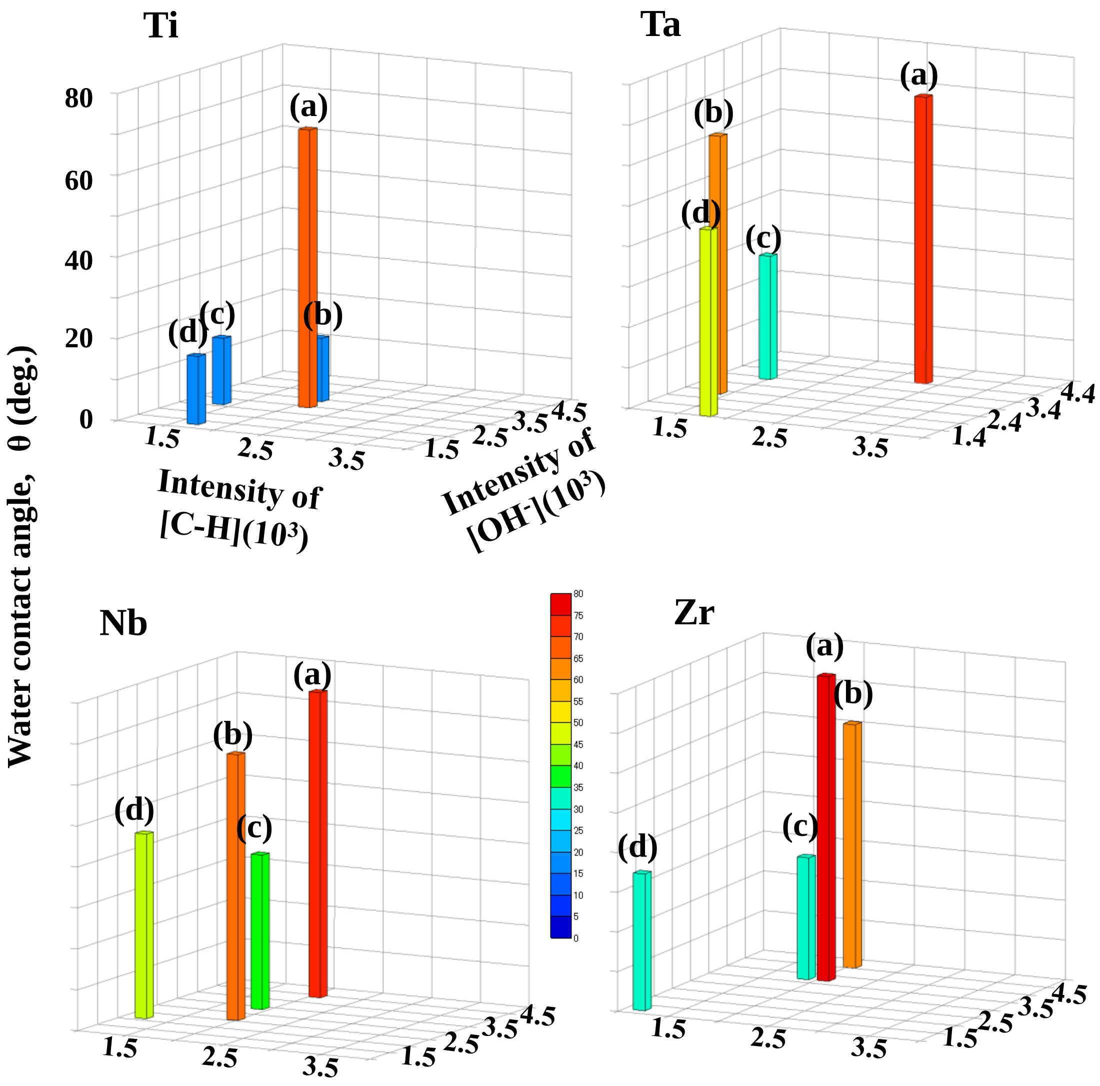

Figure 4.5 Quantitative relationship among surface OH^- group, adsorbed C–H, and WCA for single metals of Ti, Nb, Ta, and Zr under different conditions: (a) as-polished, (b) as-anodized, (c) anodized + hydrothermally treated and (d) hydrothermally treated . The amounts of OH^- and C–H were calculated from O_{1S} and C_{1S} spectrum from XPS analysis. The color scale in the spectrum represents the range of WCA.

4.3.2 *In vivo* test

To produce super-hydrophilic surfaces, the hydrothermally treated samples were stored in ×5 PBS(–) and their osteoconductivity in the cortical bone region was observed by *in vivo* testing. The previous results relating to anodized Ti samples in

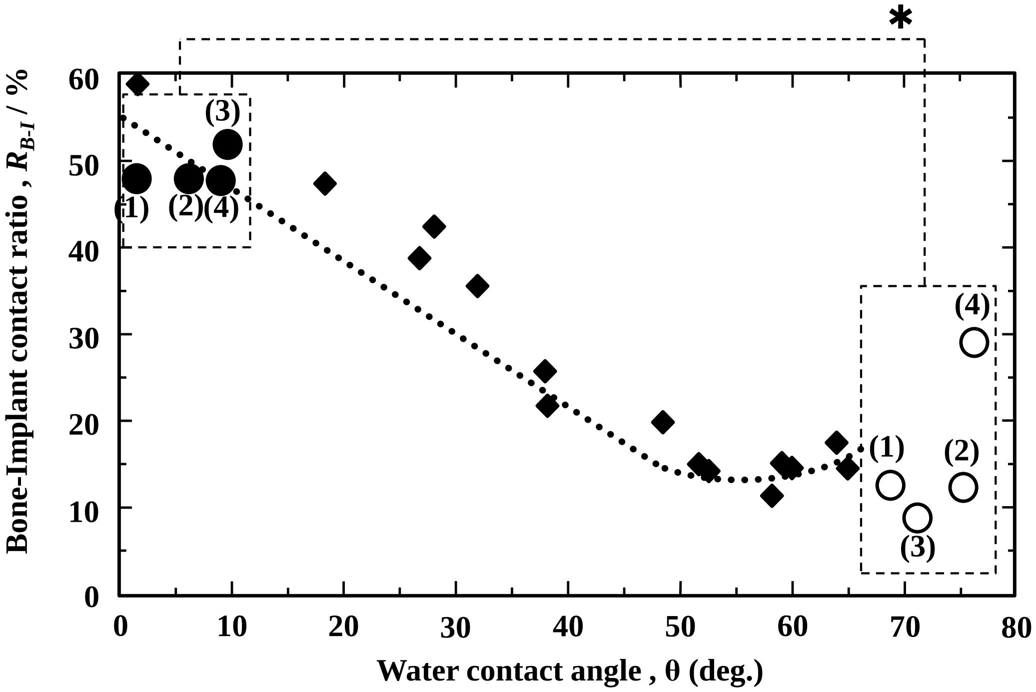

Figure 4.6 Relationship between R_{B-I} value (in cortical bone) and WCA of valve metals samples with various surface modifications and subsequent storage methods: ○ as-polished, ● hydrothermally treated after immersed in ×5 PBS(-) solution of (1) Ti, (2) Nb, (3) Ta and (4) Zr samples and ◆ anodized Ti in different aqueous solutions (Ra/µm < 0.1); $*p < 0.05$.

various electrolyte solutions with Ra/µm < 0.1 are plotted in **Figure 4.6** as a comparison [2-12][2-13]. The as-polished samples with WCA > 60 (deg.) contributed to the R_{B-I} value in a range around 8–30%, respectively. It can state that all valve metals in an as-polished condition have low osteoconductivity. However, by decreasing the WCA and producing super-hydrophilic surfaces after immersion in ×5 PBS(–), the R_{B-I} of hydrothermally treated valve metals increases remarkably, resulting in a range of 49–55%. It was evidenced from i*n vivo* bone morphogenesis near Ti implant as shown in **Figure 4.7**. After hydrothermaled treated Ti sample was immersed in ×5 PBS(–), the hard tissue formation become increased remarkably compared to as-polished sample. These results indicate that the osteoconductivity of valve metals (Ti, Nb, Zr and Ta) was enhanced most notably by a combination of one-step hydrothermal treatment and immersion in ×5 PBS(–).

4.4 Discussion

The surface properties of an implant such as surface roughness and wettability play a critical role in the biological responses at the interface between the bone tissue and the implant [4-6]-[4-14]. From the present experimental data, surface roughnesses for all treated valve metals were nearly constant compared to untreated samples. It is mainly concluded that no influence of the anodizing and hydrothermal treatments on the roughness of the oxide, suggesting the correlation between surface roughness and osteoconductivity can be ignored in this research. This consideration was supported by other previous reports indicating no significant difference in the effect of surface roughness on osteoblast cell attachment and proliferation [4-15][4-16]. On the other hand, surface wettability influences the adsorption of cell-adhesive proteins, such as fibronectin, on the surface of implants, and enhances the adhesion and spreading of osteoblast precursors on implant surfaces [2-28][2-29][4-17]. By increasing wettability, the attachment of cells and interaction between implant surfaces and the biological environment also become improve. In the present study, all the hydrothermaled valve metals surfaces were hydrophilic compared with the untreated and anodized surfaces. They continued to become super-hydrophilic after immersed in ×5 PBS(–). Consequently, the osteoconductivity of valve metals improved significantly after combination processes of hydrothermal and immersion in ×5 PBS(–) . It was confirmed by increasing of hard tissue formation of hydrothermaled samples in cortical bone after 14 days implantation compared with untreated valve metals. The higher $R_{B\text{-}I}$ values on hydrophilic surface in the present study corresponds to the previous *in vivo* report by Park *et al.*, which found that hydrophilic surface revealed higher $R_{B\text{-}I}$ than hydrophobic surfaces [4-18]. Considering that hard tissue formation is affected by adsorption of fibronectin, these results correspond with other study that investigated the adsorption of fibronectin on the surface with varied WCA [4-19]. Results of our study also match to other reports which demonstrated that osteoblast cell adhesion, differentiation, and bone formation were significantly enhanced on hydrophilic Ti surfaces compared to hydrophobic surfaces *in vitro* and *in vivo* [2-36][4-20][3-7][3-8].

Since the cells can attach efficiently to hydrophilic surfaces compared with hydrophobic surfaces, it can be said that all the hydrothermaled surfaces in the present

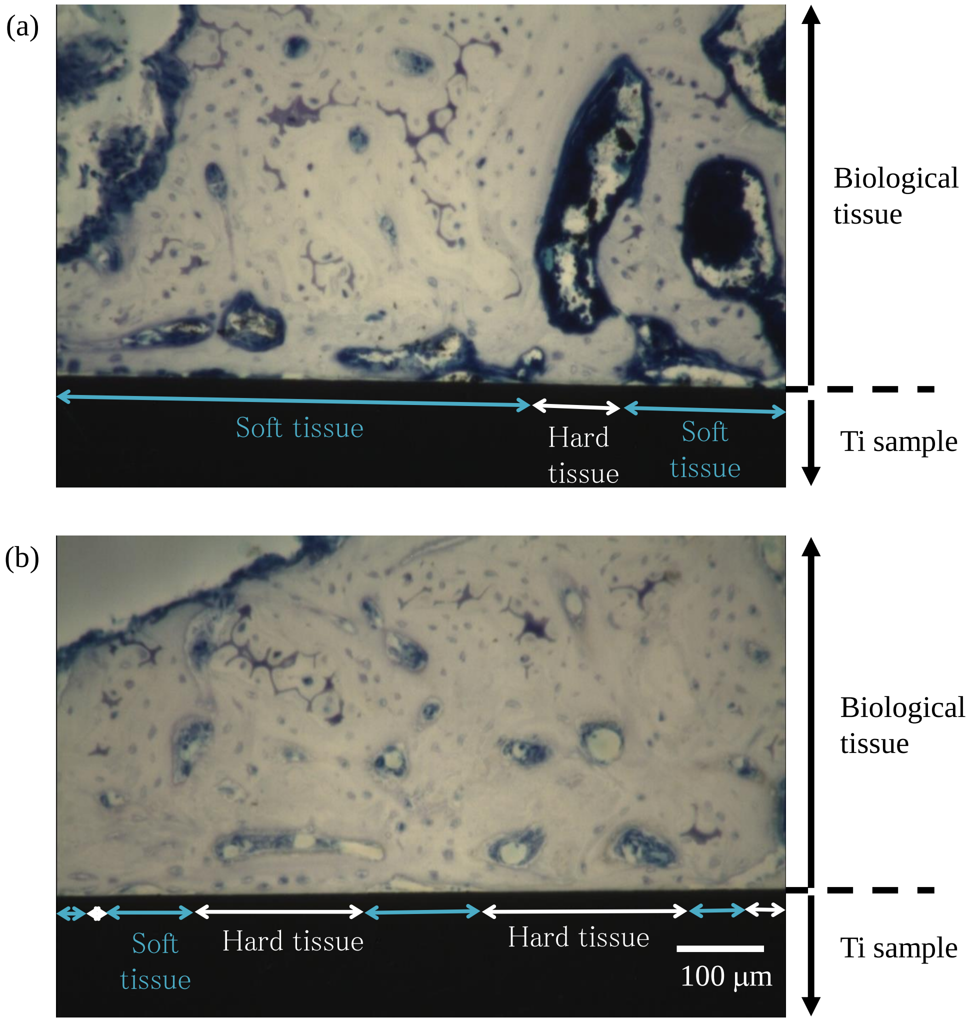

Figure 4.7 Optical micrographs of Ti sample surface and bones (cortical bone part) in: (a) as-polished and (b) hydrothermally treated after immersed in ×5 PBS(−) solution.

work are advantageous on cell attachment. However, further study is still needed to elucidate the relationship between hydrophilicity and osteoconductivity.

4.5 Conclusions

In this chapter, I can conclude that :

1. Anodizing only useful to improve the wettability of pure Ti, but it was not work to other valve metals (Zr, Nb, and Ta).
2. However, by applying hydrothermal processing, with or without anodizing, the sample surface of all valve metals becomes more hydrophilic (WCA $\leq$ 30 deg.).

Then, by storing in ×5 PBS(–), the hydrothermaled samples surfaces became more hydrophilic with WCA < 10 deg. The hard tissue formation ratio for the hydrothermally treated valve metals in the cortical bone region (the osteoconductivity) increased by up to 55% after immersed in ×5 PBS(–). It can be concluded that one-step hydrothermal treatment is not only effective to produce a highly osteoconductive on pure Ti, but also on other valve metals (Zr, Nb, and Ta).

References

[4-1] Guo WY, Sun J, and Wu JS. Electrochemical and XPS studies of corrosion behavior of Ti–23Nb–0.7Ta–2Zr–O alloy in Ringer's solution. Mater Chem Phys 2009;113:816.

[4-2] Huang P, Xu KW, and Han Y. Preparation and apatite layer formation of plasma electrolytic oxidation film on titanium for biomedical application. Mater Lett 2005;59:185.

[4-3] Le Guehennec L, Soueidan A, Layrolle P, and Amouriq Y. Surface treatments of titanium dental implants for rapid osseointegration, Dent Mater 2007;23:844-854.

[4-4] Habazaki H, Uozumi M, Konno H, Shimizu K, Skeldon P, and Thompson GE. Crystallization of anodic titania on titanium and its alloys. Corr Sci 2003;45:2063-2073.

[4-5] Yu J, Zhao X, Yu J, and Zhong G. The grain size and surface hydroxyl and content of super-hydrophilic TiO_2/SiO_2 composite nanometer thin films. J. Mater. Sci. Lett 2001;20:1745.

[4-6] Benoit DSW, Schwartz MP, Durney AR, and Anseth KS. Small functional groups for controlled differentiation of hydrogel-encapsulated human mesenchymal stem cells. Nature Mater 2008;7:816-823.

[4-7] Castner DG, and Ratner BD, Biomedical surface science: foundations to frontiers. Surf Sci 2002;500:28-60.

[4-8] Sul YT. The significance of the surface properties of oxidized titanium to the bone response: special emphasis on potential biochemical bonding of oxidized titanium implant. Biomater 2003;24:3893-3907.

[4-9] Baier RE, Meyer AE, Natiella JR, Natiella RR, and Carter JM. Surface

properties determine bioadhesive outcomes: methods and results. J Biomed Mater Res 1984;18:337-355.

[4-10] Takebe J, Itoh S, Okada J, and Ishibashi K. Anodic oxidation and hydrothermal treatment of titanium results in a surface that causes increased attachment and altered cytoskeletal morphology of rat bone marrow stromal cells in vitro. J Biomed Mater Res 2000;51:398-407.

[4-11] Nandakumar AK, Yang L, Habibovic P, and van Blitterswijk C. Calcium phosphate coated electrospun fiber matrices as scaffolds for bone tissue engineering. Langmuir 2010;26:7380-7387.

[4-12] Klenke FM, Liu Y, Yuan H, Hunziker EB, Siebenrock KA, and Hofstetter W. Impact of pore size on the vascularization and osseointegration of ceramic bone substitutes in vivo. J Biomed Mater Res A 2008;85A;777-786.

[4-13] Yuan H, Fernandes H, Habibovic P, de Boer J, Barradas AMC, de Ruiter A, Walsh WR, van Blitterswijk CA, and de Bruijn JD. Osteoinductive ceramics as a synthetic alternative to autologous bone grafting. Proc of Natl Acad Sci 2010;107:13614-13619.

[4-14] Ripamonti U, Crooks J, Khoali L, and Roden L. The induction of bone formation by coral-derived calcium carbonate/hydroxyapatite constructs. Biomater 2009;30:1428-1439.

[4-15] Cooper LF, Masuda T, Whitson SW, Yliheikkil P, and David A. Formation of mineralizing osteoblast cultures on machined, titanium oxide grit-blasted and plasma-sprayed titanium surfaces. J Oral Max Impl 1999;14:37-47.

[4-16] Anselme K, Linez P, Bigerelle M, Le Maguer D, Le Maguer A, Hardouin P, Hildebrand HF, Iost A, and Leroy JM. The relative influence of the topography and chemistry of TiAl6V4 surfaces on osteoblstic cell bahaviour. Biomater 2000;21:1567-77.

[4-17] Omori M, Tsuciya S, Hara K, Fujio M, Kuroda K, Yajima A, Okido M, Hibi H, and Ueda M. The application of atmospheric pressure plasma treatment for immobilizing stem cells from human exfoliated deciduous teeth-conditioned medium on titanium implants. Biomater, in submission.

[4-18] Park JW, Jang JH, Lee CS, and Hanawa T. Osteoconductivity of hydrophilic microstructured titanium implants with phosphate ion chemistry. Acta Biomater

2009;5:2311-2321.

[4-19] Wei J, Igarashi T, Okumori N, Maetani T, Liu B, and Yoshinari M. Influence of surface wettability on competitive protein adsorption and initial attachment of osteoblasts. Biomed Mater 2009;4:31-35.

[4-20] Eriksson C, Nygren H, and Ohlson K. Implantation of hydrophilic and hydrophobic titanium discs in rat tibia: cellular reactions on the surfaces during the first 3 weeks in bone. Biomater 2004;25:4759-4766.

CHAPTER 5

Hydrothermal Treatment of Titanium Alloys for the Enhancement of Osteoconductivity

5.1 Introduction

Approximately 70–80% of implants are manufactured from metallic biomaterials, such as stainless steels, CoCr alloys, and Ti and its alloys [5-1]. Among them, Ti and its alloys indicate the highest biocompatibility, corrosion resistance, and specific strength (ratio of tensile strength to density) compared with stainless steel and CoCr alloys. The Ti-6Al-4V (Ti64) alloy with the α+β phase type has been used widely as a high-strength biomedical alloy. However, the possible toxic effect by the released V ions on the human body has created some concern [5-2][5-3][5-4]. The V-free Ti alloys, such as Ti-6Al-7Nb (Ti67) (α+β type), were introduced into clinical use as a substitute for Ti64 because, as noted, the V ions in practical Ti64 were toxic [5-5].

It was pointed out that metallic implants which high modulus brought the resorption in the body because of the stress-shielding effect. For that reason, β-single phase Ti alloys, such as TCFA and TNTZ, have been introduced for implant biomaterials because the values of the Young's modulus for these Ti alloys are smaller than the Young's modulus for the α-phase Ti alloys [5-6][5-7][5-8]. However, Ti and Ti alloys are recognized as bioinert materials due to the lack of direct chemical bonding to the host bone tissues after implantation as mentioned before. Therefore, as a way to improve their osseointegration, surface modification has become essential [5-9]. In chapter 3, I applied a hydrothermal treatment for the surface modification of pure Ti, compared with anodized, ultraviolet irradiation and plasma irradiation samples. It was detected that the osteoconductivity of pure Ti improved markedly after hydrothermal treatment without anodizing [5-10]. So, by using this result as a reference, in this chapter, a single hydrothermal surface treatment was performed on Ti alloys to confirm whether the hydrothermal treatment was also effective in improving their osteoconductivities or not, and it was compared with anodized Ti alloys samples. The osteoconductivity was investigated using *in vivo* tests.

5.2 Materials and methods

5.2.1 Materials preparation

Two different sample shapes made from pure Ti, Ti64, Ti67, TNTZ, and TCFA were used to characterize the surface and to evaluate the osteoconductivity in the *in vivo* tests. Plates with the dimensions of 12 mm × 4 mm were used for surface characterization, and rods with a diameter of 2 mm and length of 5 mm were used for *in vivo* tests. The samples were classified into the following three groups: Group I, as-polished; Group II, as-anodized; and Group III, as-hydrothermally treated. All samples were abraded with emery papers up to # 2000, polished by Al_2O_3 particles 0.05 μm in size, degreased with ethanol for 5 min in an ultrasonic cleaner, and finally dried at room temperature. In Group II, anodizing was performed using a final voltage of 100 V (65 V for TCFA) at a rate of 0.1 Vs^{-1}. The electrolyte solution was 0.1 M H_3PO_4 and this was stirred using a magnetic stirrer at a constant temperature (25 °C) in a water bath during anodizing. After anodizing, the specimens were sterilized using an autoclave unit at 121 °C for 20 min. In Group III, hydrothermal treatment was carried out for the Group I as-polished specimens using 300 ml of distilled water in a hydrothermal unit at a temperature of 180 °C for 180 min.

It has been reported that storage environment has a notable effect on the surface hydrophilicity of the material [3-4]. Therefore, the specimens were stored under two storage environments (at room temperature), namely in air and in five times concentrated phosphate buffered saline (×5 PBS(–)).The storage period of the samples in the different media was up to 168 h. For the *in vivo* tests, we used untreated and hydrothermally treated samples that were stored for more than 24 h in ×5 PBS(–).

5.2.2 Surface characterization

The surface morphologies of three group specimens were observed using SEM. The crystal structure of the Ti alloys and their surface elements were determined using XRD and XPS. The anodized and hydrothermal films were etched using Argon ion bombardment (at 2 kV) and the element depth profiles were obtained using Auger electron spectroscopy. The surface roughness was measured using a confocal laser scanning microscope with 150 μm × 112 μm in an area of measurement. The Ra measurements were employed as the surface roughness. The WCA was estimated using a 2 μL droplet of distilled water.

5.2.3 *In vivo* test

In the *in vivo* tests, as-polished, anodized, and hydrothermaled samples after stored for 24 h in ×5 PBS(–) were used for *in vivo* tests. The experimental procedure for *in vivo* tests was similar to that described in previous chapter.

5.3 Results and discussion

5.3.1 Surface properties

Figure 5.1 shows the colors of the untreated and treated samples in this work. The gray metallic color was revealed in the as-polished samples. After anodizing, each specimen displayed a different color (yellow, rose, purple, green, and dark gold) in contrast to the uniform gold color appearance of the hydrothermaled samples. The change in color showed that an oxidation layer was formed during anodizing and hydrothermal treatment, which is confirmed by the XRD patterns obtained from the untreated and treated Ti and Ti alloy specimens, as shown in **Figure 5.2**. For all treated specimens, no other oxide peaks, except anatase (TiO_2), were detected on the Ti or the Ti alloys surfaces. However, the peak was considered as amorphous TiO_2 on anodized samples because the peak intensities were relatively weak and also become broaden on the anodized TNTZ and TCFA surfaces.

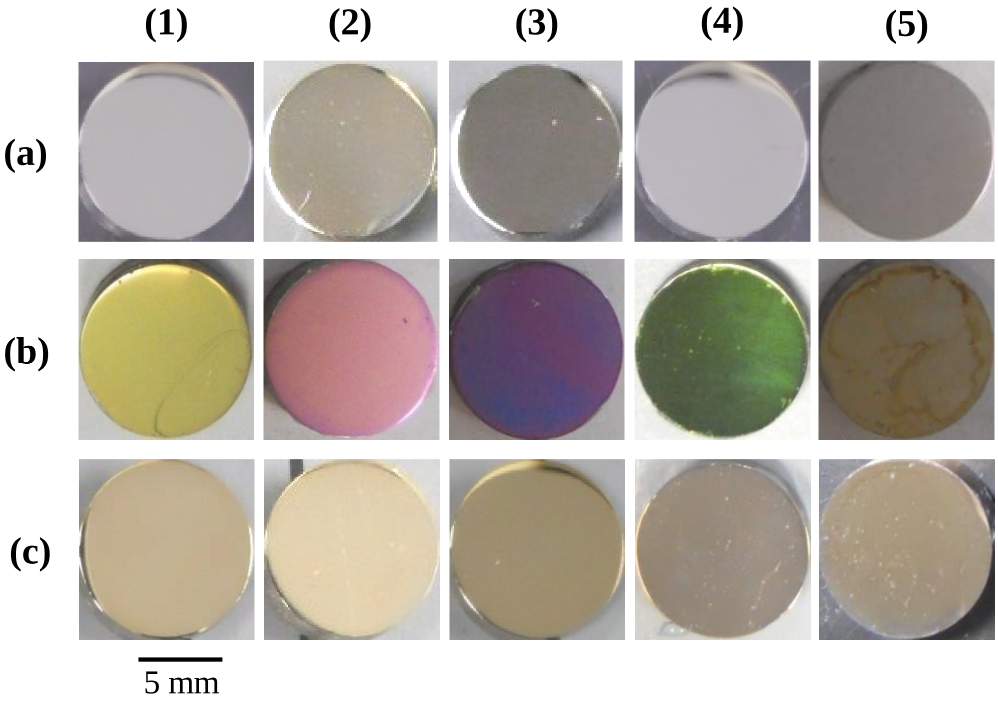

Figure 5.1 Photographs of samples of (1) Ti, (2) Ti64, (3) Ti67, (4) TNTZ, and (5) TCFA, each of which was (a) polished, (b) as-anodized in H_3PO_4 solution, and (c) as-hydrothermaled in distilled water at 180 °C for 180 min.

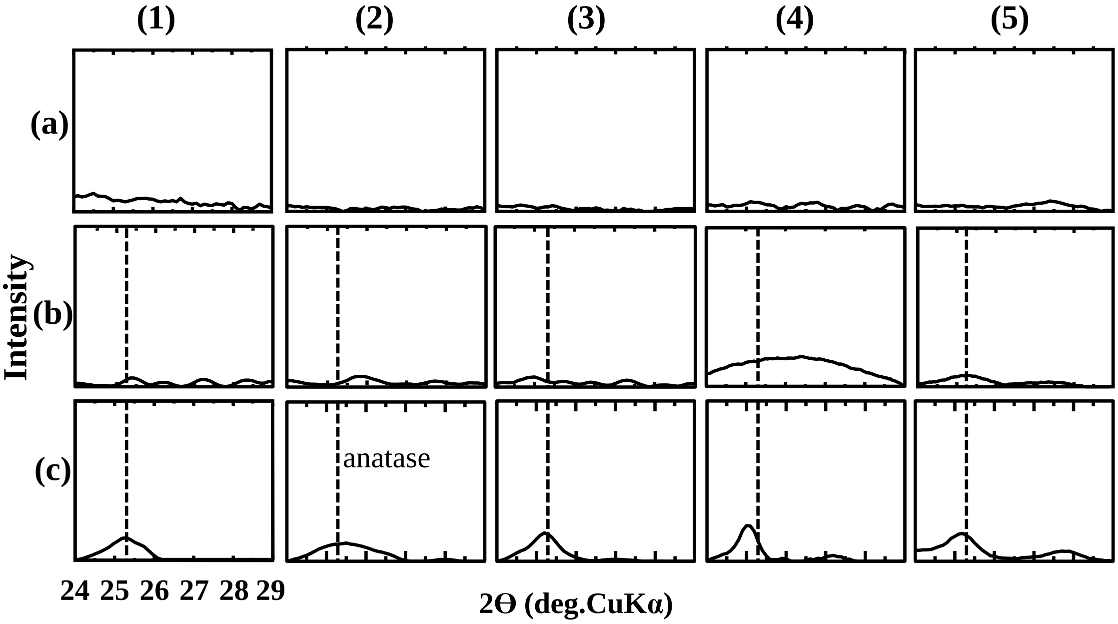

Figure 5.2 XRD patterns of (1) Ti, (2) Ti64, (3) Ti67, (4) TNTZ, and (5) TCFA using different treatments: (a) polished, (b) as-anodized in H_3PO_4 solution, and (c) as-hydrothermaled in distilled water at 180 °C for 180 min.

In addition, the results of the Auger spectroscopy also showed high amounts of alloy elements (Nb and Cr) and P, supplied from the H_3PO_4 solution, on the surfaces of anodized TNTZ and TCFA (**Figure 5.3**), respectively. On the other hand, alloy elements were hardly detected on the Ti64 and Ti67 film surfaces after anodizing. The anodized films of Ti64 and Ti67 had similar chemical profiles to the films of pure Ti. Meanwhile, the surfaces of all hydrothermaled Ti alloys were covered with oxide coatings consisting of anatase-type TiO_2 containing negligibly small amounts of alloy elements (**Figure 5. 4**).

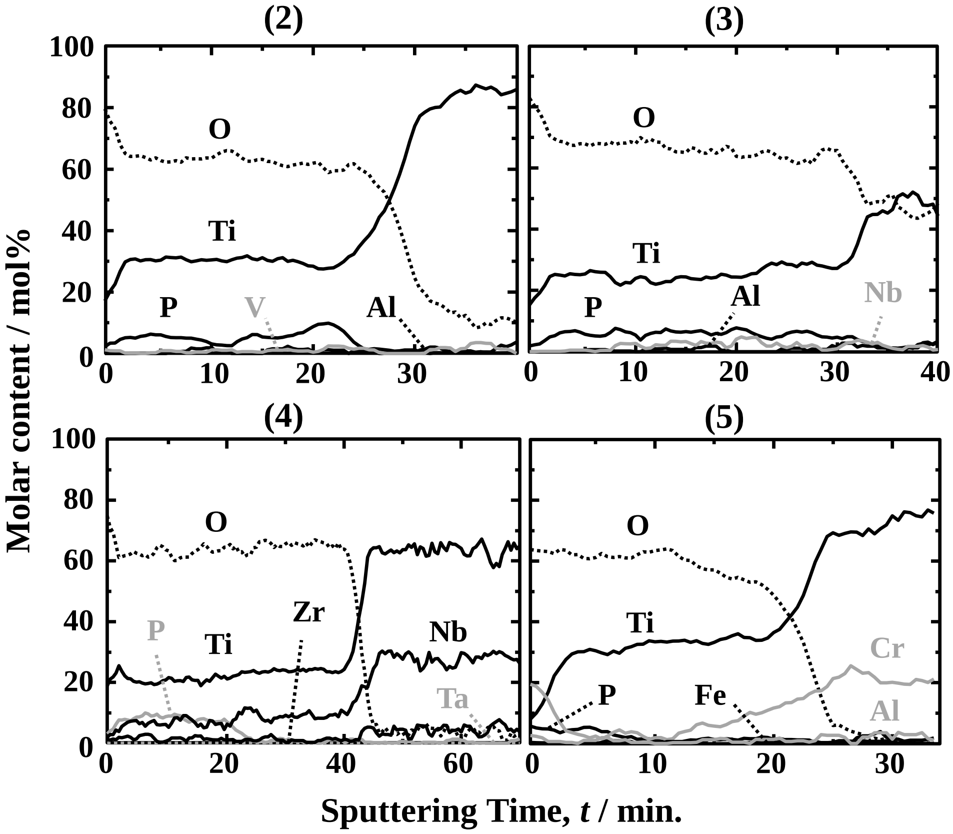

Figure 5.3 Depth profiles of as-anodized Ti alloys in the H_3PO_4 solution: (2) Ti64, (3) Ti67, (4) TNTZ, and (5) TCFA samples.

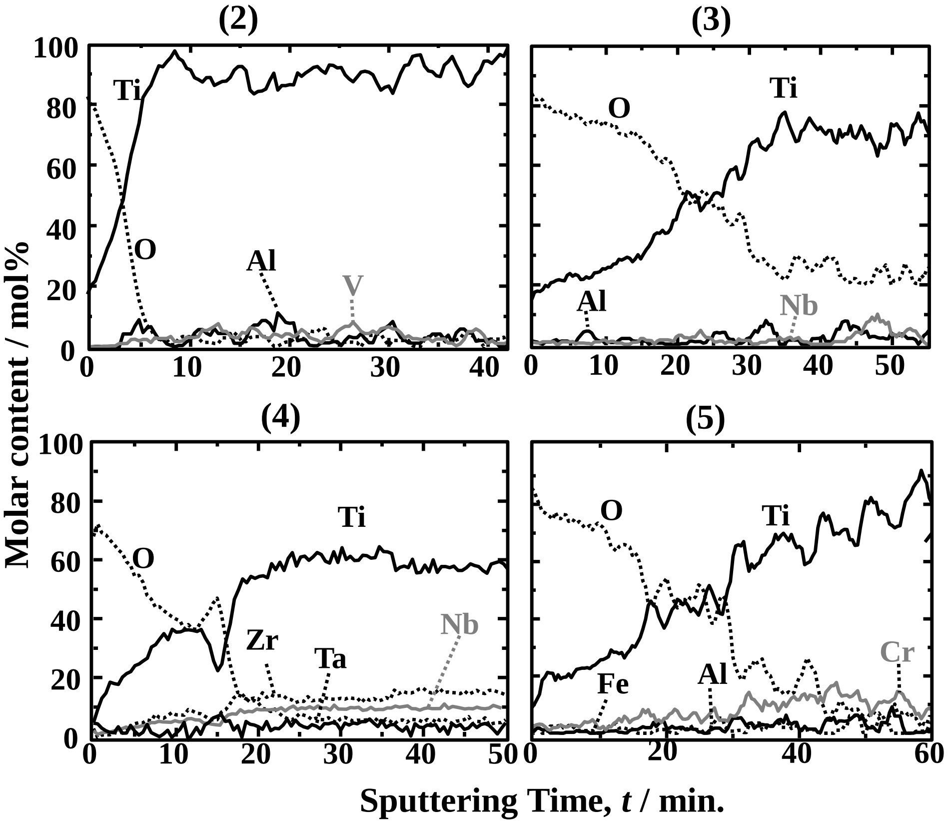

Figure 5.4 Depth profiles of as-hydrothermaled Ti alloys in distilled water at 180 °C for 180 min: (2) Ti64, (3) Ti67, (4) TNTZ, and (5) TCFA samples.

The morphology and surface roughness appeared to be comparable in all prepared samples (**Figure 5.5**). SEM observation showed almost identical surface morphologies in all investigated samples, with relatively smooth surface morphologies. Surface roughness has been shown to be a significant factor influencing the osteoconductivity of materials [2-26]. The treated samples exhibited similar average roughness values (Ra/μm < 0.01) compared with the as-polished samples.

The anodizing and hydrothermal treatments generated a change in the wettability of the pure Ti and the Ti alloys. The results from the contact angle measurements showed that all the as-polished samples (**Figure 5.6**, column a) had hydrophobic surfaces, with their WCA in the range of 60 degree (deg.) to 70 deg. (**Figure 5.6**). After anodizing, (1) Ti, (2), Ti64, and (3) Ti67 exhibited a hydrophilic surface.

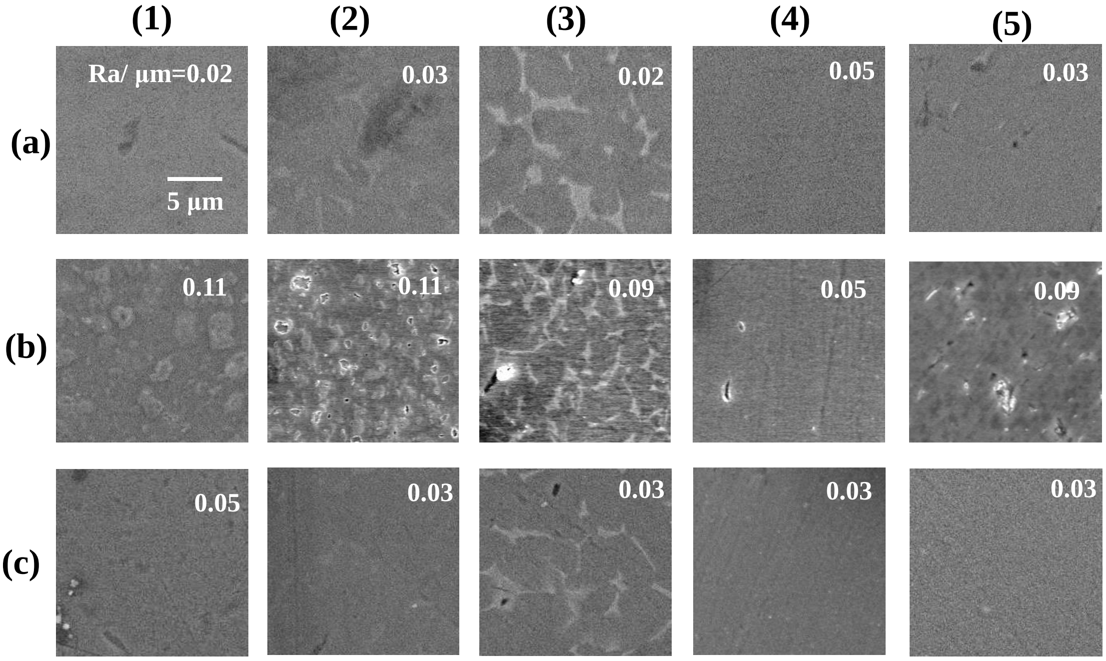

Figure 5.5 Surface morphology and surface roughness (Ra) of (1) Ti, (2) Ti64, (3) Ti67, (4) TNTZ, and (5) TCFA, each of which was (a) polished, (b) as-anodized in a H_3PO_4 solution, and (c) as-hydrothermaled in distilled water at 180 °C for 180 min.

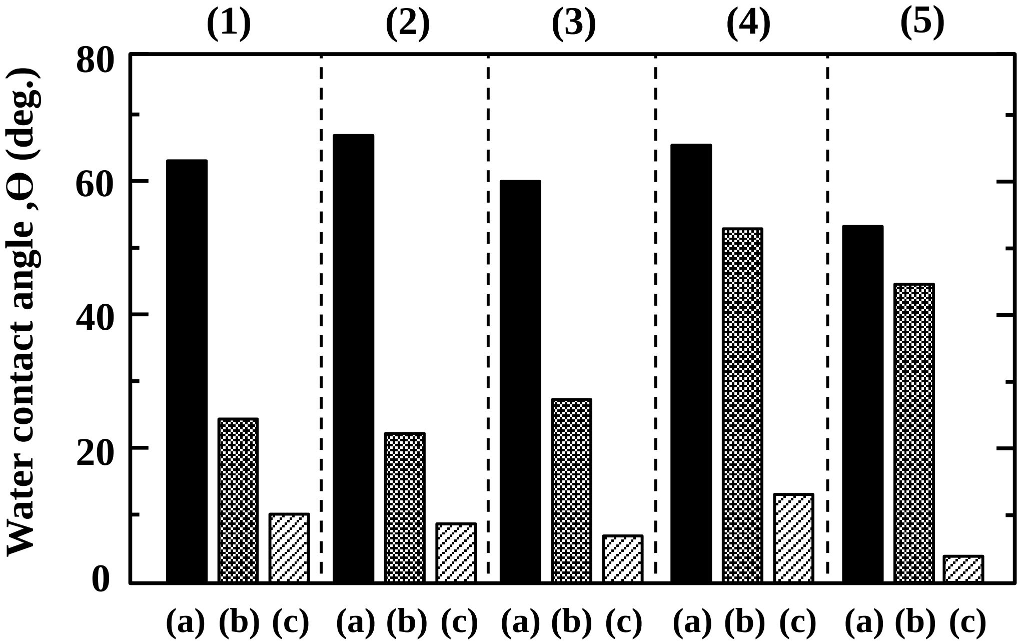

Figure 5.6 Water contact angle (WCA) of (1) Ti, (2) Ti64, (3) Ti67, (4) TNTZ, and (5) TCFA samples with various surface modifications: (a) polished, (b) as-anodized in H_3PO_4 solution, and (c) as-hydrothermaled in distilled water at 180 °C for 180 min.

The WCA of Ti, Ti64, and Ti67 decreased remarkably from 63 deg., 67 deg., and 60 deg. to 25 deg., 22 deg., and 28 deg., respectively. However, this trend was not observed on (4) TNTZ and (5) TCFA samples. Only a slight reduction at 65 deg. and 53 deg. to 53 deg. and 45 deg., respectively, was found. This fact identified that the anodizing treatment had a significant impact on the surface hydrophilicity of Ti, Ti64, and Ti67 samples, but that it did not influence the anodized TNTZ and TCFA samples. As is well known, a larger amount of C–H adsorption can generate a hydrophobic surface. In contrast, the surfaces of pure Ti and all types of Ti alloys, including TNTZ and TCFA, became more hydrophilic when a hydrothermal treatment was applied on their surfaces. This was shown by the significant reduction in their WCA to lower than 15 deg. The existence of alloy elements (Nb and Cr) and oxides on the surfaces of TNTZ and TCFA did not bring hydrophobicity directly but changed the surface properties of these Ti alloys. The amount or rate of adsorption of hydrocarbon (C–H) (shown in **Figure 5.8**) changed, probably due to the existence of those alloy elements.

As surface hydrophilicity can change with time [3-2], it is important to maintain hydrophilic surfaces until surgery. Optimum methods must be considered when preparing the hydrophilic surfaces and to maintain their hydrophilicity by selecting appropriate storage environments. **Figure 5.7** shows the effect of storage environments (air and ×5 PBS(–)) on the WCA of the untreated and treated Ti and Ti alloys. When samples were stored in air, the WCA of the treated samples tended to increase with increasing storage time. In contrast, a significant reduction in the WCA with an increase in storage time was observed on untreated and treated samples after storage in ×5 PBS(–). Furthermore, the low WCAs were maintained after 24 h in storage. Between the different storage media, the hydrothermaled samples stored in ×5 PBS(–) showed the lowest WCA value. The Ti and Ti alloy surfaces became super hydrophilic with a WCA lower than 5 deg. The adsorbed solute ions, such as Na^+ and Cl^- ions, strongly influenced the surface hydrophilicity of TiO_2 coatings [3-4]. It can be concluded that storing Ti alloys samples in a ×5 PBS(–) solution maintained the super hydrophilic surface effectively for longer time.

XPS analyses of the surface of the hydrothermaled treated alloys show that all the investigated samples consisted primarily of Ti and O (**Figure 5.8**).

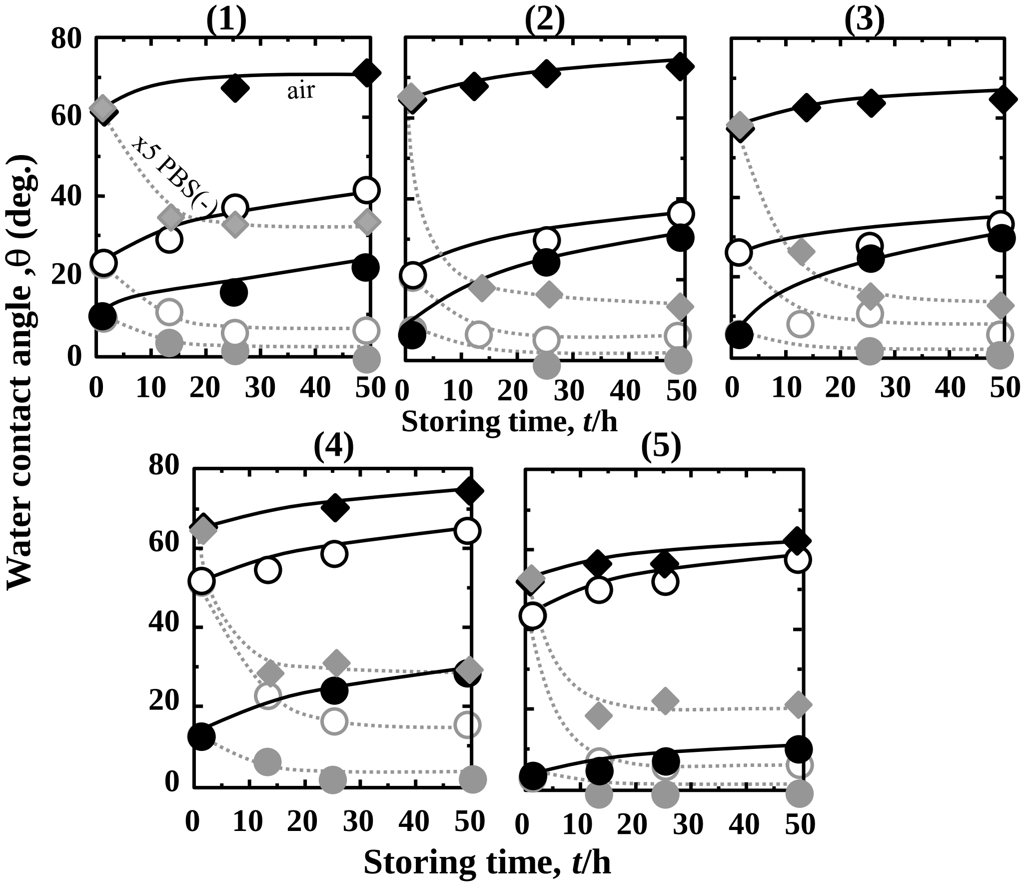

Figure 5.7 Relationship between the WCA and periods of hydrothermaled samples of (1) Ti, (2) Ti64, (3) Ti67, (4) TNTZ, and (5) TCFA samples stored in: ─ air and … ×5 PBS(–) with various surface modifications: ◆ as-polished, ○ as-anodized in H_3PO_4 solution, and ● as-hydrothermaled in distilled water at 180 °C for 180 min.

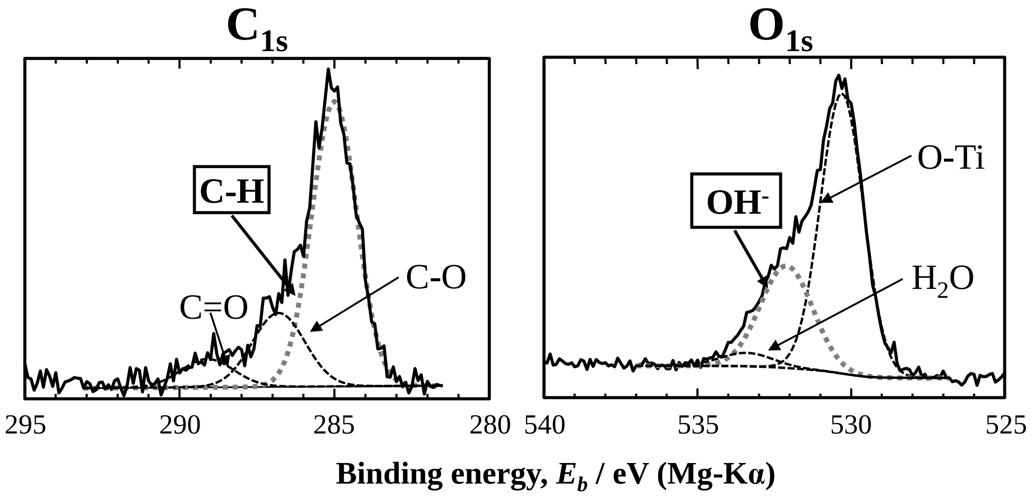

Figure 5.8 High-resolution XPS spectra of the C_{1S} and O_{1S} of Ti after hydrothermal treatment with distilled water.

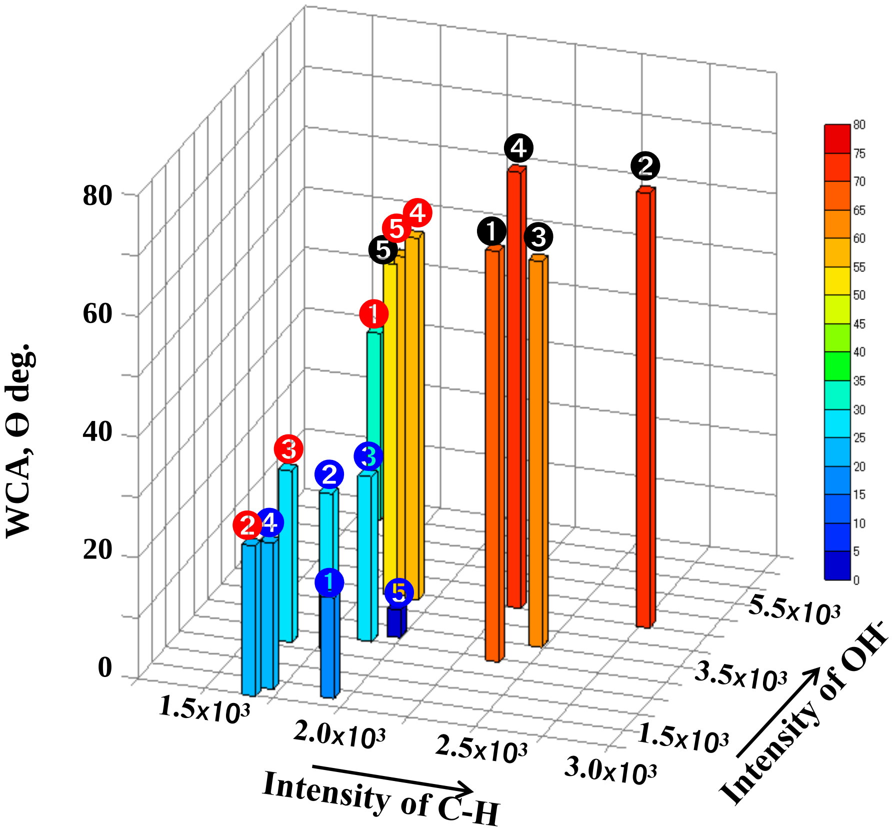

Figure 5.9 Quantitative relationship between surface OH^- group, adsorbed C–H, and WCA for (1) Ti, (2) Ti64, (3) Ti67, (4) TNTZ, and (5) TCFA with various surface modifications: ● as-polished, ● as-anodized in H_3PO_4 solution, and ● as-hydrothermaled in distilled water at 180 °C for 180 min. The amount of OH^- and C–H was calculated from the O_{1S} and C_{1S} spectrum from XPS analysis. The color scale in the spectrum represents the range of WCA.

The O_{1S} XPS spectrum deconvoluted into three peaks (531.8, 533.5, and 529.9 eV) originating from the hydroxyl group (OH^-), hydrate, and/or adsorbed water (H_2O), and from the Ti−O group from TiO_2, respectively, in the same way as previously [3-3]. Meanwhile, the C_{1S} spectrum also contained three peaks originating from the hydrocarbon (C−H), etheric (C−O), and carbonyl (C=O) groups on the surface at around 285, 286.8, and 289.1 eV, respectively. The OH group and adsorbed C–H can influence surface hydrophilicity. Adsorption of hydrocarbons as surface contamination can be produced during storage after processing, as well as from the surface treatment of the Ti, such as machining, anodizing, and sterilization. Surface contamination can increase the WCA, allowing the surface to become more hydrophobic [4-9]. In this experiment, the values of the adsorbed hydrocarbon (C−H) of the Ti and Ti alloys after hydrothermal treatment were smaller than those of the untreated samples (**Figure 5.9**). At the same time, hydrothermal treatment reduced the WCA of the Ti and the Ti alloys significantly. In contrast, although the WCA of the samples was low, amount of hydroxyl (OH^-) group content on the film surfaces decreased after hydrothermal treatment. This indicates that the reduction of adsorbed C–H after hydrothermal treatment (by surface cleaning) influences the surface hydrophilicity more than the reduction of OH^- groups.

5.3.2 *In vivo* test

In the *in vivo* tests, I used as-polished, anodized, and hydrothermaled samples after being stored for 24 h in ×5 PBS(–). The previous *in vivo* results for the TiO_2-coated samples with a ratio of Ra/μm less than 0.1 are also shown in **Figure 5.10** [2-26][2-27]. As shown in **Figure 5.10**, the as-polished samples with hydrophobic surfaces provided low osteoconductivity (ca. 10% of the $R_{B–I}$ value). This tendency also occurred on TNTZ and TCFA after anodizing, where their $R_{B–I}$ values and WCA were almost similar to those of the as-polished samples. However, a different condition was detected on the Ti, Ti64, and Ti67 samples after anodizing. The $R_{B–I}$ values of these three anodized samples with a WCA of approximately 30 deg. was about 40% more than the untreated samples. This confirmed that a single anodizing treatment could enhance the osteoconductivity of pure Ti, Ti64, and Ti67, but not the osteoconductivity of TNTZ and TCFA. However, the osteoconductivity of Ti and all types of Ti alloys increased significantly when a hydrothermal treatment followed by immersion in ×5 PBS(–) was implemented. With the super hydrophilic surfaces (WCA < 5 deg.), the $R_{B–I}$ value of the Ti and the Ti alloys rose by 40–50% more than for the as-polished specimens.

Surface hydrophilicity plays a critical role in the biological response of biomaterial surfaces. It can influence the cells attachment, as long as the phase of attachment has appeared through an early process involving physicochemical connections between cells and surfaces or via changes in the proteins adsorption [2-28][2-29][4-17]. High hydrophilicity enhances the interaction between the implant surface and the biological environment [4-9][5-11][5-12]. All hydrothermaled samples in this study were hydrophilic compared with the as-polished and as-anodized samples. Thus, from

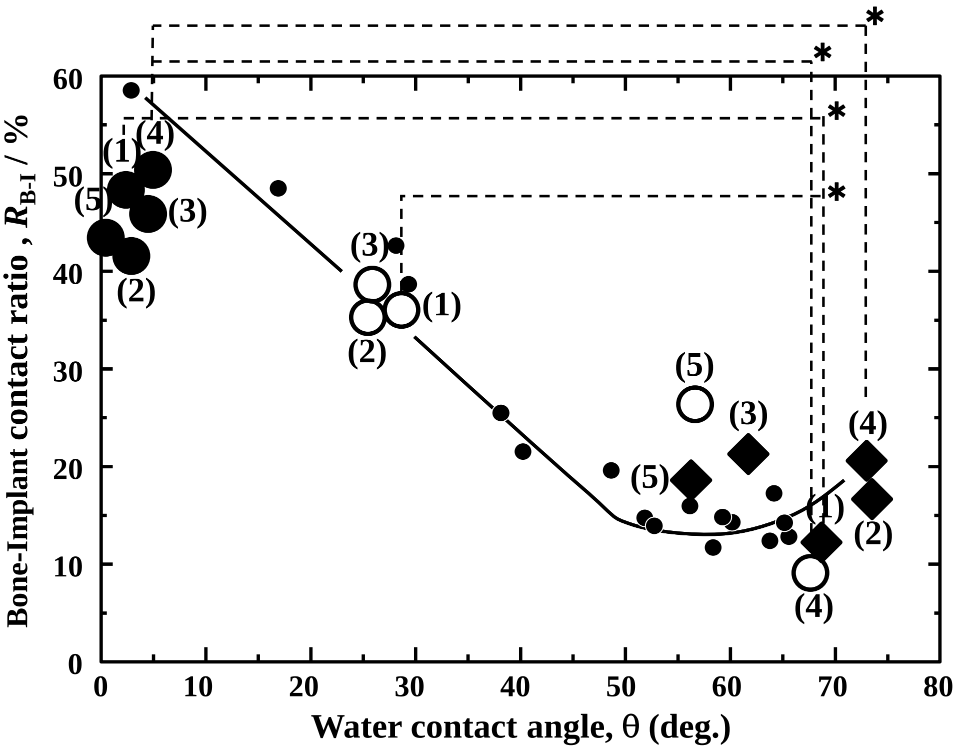

Figure 5.10 Correlation between the $R_{B–I}$ value and the WCA of Ti and Ti alloys samples with several surface modifications and different storage media: ◆ as-polished, ○ as-anodized in H_3PO_4 solution, ● as-hydrothermaled in distilled water at 180 °C for 180 min after stored in ×5 PBS(–) solution of (1) Ti, (2) Ti64, (3) Ti67, (4) TNTZ, and (5) TCFA; and • as-anodized Ti in different aqueous solutions (Ra/μm < 0.1), $^*p < 0.05$.

this result we may conclude that the surface hydrophilicity of Ti alloys was improved, showing a significant improvement in their osteoconductivity after an applied combination of hydrothermal treatment and immersion in a ×5 PBS(–) solution.

5.4 Conclusions

In this chapter, it showed that anodizing process only enhanced the osteoconductivity for pure Ti, Ti64, and Ti67 alloys, but not for other Ti alloys (TNTZ and TCFA. In contrast, hydrothermal treatment followed by stored in ×5 PBS(–) solution improved the osteoconductivity of Ti and Ti alloys almost 50% compared with that of the untreated samples. Thus, it may concluded that the combination of a one-step hydrothermal treatment and immersion in ×5 PBS(–) solution could be a simple and effective way of enhancing the osteoconductivity of Ti alloys.

References

[5-1] Niinomi M, Nakai M, and Hieda J. Development of new metallic alloys for biomedical applications Acta Biomater 2012;8:3888-3903.

[5-2] Thair L, Mudali UK, Rajagopalan S, Asokamani R, and Raj B. Nitrogen ion implantation and in vitro corrosion behavior of as-cast Ti–6Al–7Nb alloy. Corros Sci 2003;45:1951-1967.

[5-3] Lopez MF, Jimenez JA, and Gutiérrez A. Corrosion study of surface-modified vanadium-free titanium alloys. Electrochem. Acta Biomater 2003;48:1395-1401.

[5-4] Morant C, Lopez MF, Gutiérrez A, and Jimenez JA. AFM and SEM characterization of non-toxic vanadium-free Ti alloys used as biomaterials. Appl Surf Sci 2003;220:79-87.

[5-5] Semlitsch M. Classic and new titanium alloys for production artificial hip joints. Titan 1986;2:721-740.

[5-6] Akahori T, Niinomi M, Fukui H, and Suzuki A. Fatigue, fretting fatigue and corrosion characteristics of biocompatible beta type titanium alloy conducted with various thermo-mechanical treatments. Mater Trans 2004;45:1540-1548.

[5-7] Tanaka Y, Nakai M, Akahori T, Niinomi M, Tsutsumi Y, Doi H, and Hanawa T. Characterization of air-formed surface oxide film on Ti–29Nb–13Ta–4.6Zr alloy surface using XPS and AES. Corros Sci 2008;50:2111-2116.

[5-8] Chen HT, Hsiao CH, Long HY, Chung CJ, Tang CH, Chen KC, and He JL. Micro-arc oxidation of beta-titanium alloy: structural characterization and osteoblast compatibility. Surf Coat Tech 2009;204:1126-1131.

[5-9] de Jonge LT, Leeuwenburgh SCG, Wolke JGC, and Jansen JA. Organic–inorganic surface modifications for titanium implant surfaces. Pharma Res 2008;25:2357-2369.

[5-10] Zuldesmi M, Waki A, Kuroda K, and Okido M. High osteoconductive surface of pure titanium by hydrothermal treatment, J Biomat Nanobiotech 2013;4:284-290.

[5-11] Dexter SC. Influence of substratum critical surface tension on bacterial adhesion-in situ studies. J Coll Interf Sci 1979;70:346-354.

[5-12] Baier RE, Shafrin EG, and Zisman. Adhesion: mechanisms that assist or impede it. Sci 1968;162:1360-1368.

CHAPTER 6

Osteoconductivity of Hydrophilic Surface of Zr-9Nb-3Sn Alloy with Hydrothermal Treatment

6.1 Introduction

Nowadays, MRI has become a dominant diagnostic device in orthopedics and brain surgery [6-1]. However, when metallic orthopedic implants are inserted into a patient's body and MRI is performed; defects and distortions sometimes appear in the MRI images (artifacts) and they are caused by difference in the magnetic susceptibilities of the metals and living tissues [6-2][6-3]. To decrease the artifacts, medical devices with magnetic susceptibility close to living tissues are required. Zirconium becomes more suitable for surgery performed in an MRI because it has near magnetic susceptibility of living tissue compared with stainless steel, CrCo alloys and Ti.

Zirconium and its alloys have become candidate for surgical implants due to their acceptable mechanical strength (more rigidly than Ti and Ti alloys) and good biocompatibility as well as superior corrosion resistance [6-4][6-5]. However, Zr and its alloys promote low osseointegration with poor bone-forming properties and some cases, lower than the reported for Ti alloys [6-6][6-7]. Therefore, various surface modifications have been employed to improve the osteoconductivity of the Zr and its alloy as well as Ti and Ti alloys. Anodizing is a simple and economic process to obtain a uniform surface oxide film despite the geometry of the specimen. Many literatures obtained that anodizing has been extensively used with the aim of improving the osteoconductivity performance of Ti and Ti alloys [6-8][6-9][2-26][2-27][6-10][6-11]. However, based on to Chapter 4, this process was not effective to enhance the osteoconductivity of pure Zr due to it still produced a hydrophobic surface on anodized Zr and Nb. In contrast, hydrothermal treatment became a benefit surface modification that can improve osteoconductivity because it created the hydrophilic surface not only on pure Ti, but also on pure Zr and

Nb surfaces [5-10][6-12]. For that reason, in this chapter, I investigated whether a hydrothermal treatment without coating was also benefit or not to improve the osteoconductivity of Zr-9Nb-3Sn alloy. Its osteoconducticvity was evaluated by *in vivo* tests.

6.2 Materials and methods

6.2.1 Sample preparation

Two different sample shapes, made from Zr–9Nb–3Sn, pure Zr, and pure Nb were used to characterize the surface and to evaluate osteoconductivity in an in vivo test. Plates (12 mm × 4 mm) were used for surface characterization and rods (∅2 mm × 5 mm) for *in vivo* testing. The samples were abraded with emery paper up to #2000, polished by Al_2O_3 particles with a size of 0.05 μm, degreased with ethanol for 5 min in an ultrasonic cleaner, and finally dried at room temperature. After that, the following processes were applied to the samples.

6.2.2 Anodic oxidation

Zr–9Nb–3Sn, pure Zr, Nb samples, and a Pt coil were set as the anode and cathode, respectively. A reference electrode was not used. The electrolyte solution was 0.1 M H_2SO_4 and was stirred by a magnetic stirrer at a constant temperature (25 °C). The anodizing voltage was increased from 0 V to a final voltage of 150 V at a rate of 0.1 Vs^{-1}, which was chosen to prevent sparking. Sterilization of anodized samples was conducted in an autoclave unit at temperature of 121 °C for 20 min.

6.2.3 Hydrothermal process

The hydrothermal process was applied to Zr–9Nb–3Sn, pure Zr, and Nb samples after polishing. The samples were immersed in a beaker containing 300 mL distilled water and placed in a hydrothermal unit. The temperature of the hydrothermal vessel was set at 180 °C and maintained at this condition for 180 min. After hydrothermal treatment, the beaker was removed from the hydrothermal unit and cooled naturally to room temperature.

6.2.4 Surface characterization

The surface morphologies of the treated and untreated samples were observed by SEM. The crystal structure and chemical composition of Zr–9Nb–3Sn were determined by XRD and XPS, respectively. The surface roughness was obtained using a confocal laser scanning microscope with 150 μm × 112 μm in a measurement area. The Ra defined the surface roughness. WCA was estimated using a 2 μL droplet of distilled water.

6.2.5 In *vivo* test

In this study, all hydrothermaled samples which stored in to ×5 PBS(–) solution were subjected to *in vivo* testing. The experimental procedure for *in vivo* tests was similar to that described in previous chapter.

6.3 Results and discussion

6.3.1 Surface characteristics

Surface roughness has an important function in the biological response of implant surfaces. Using previous research as a reference, it was observed that the osteoconductivity of TiO_2 coatings was enhanced by a fine surface finish (Ra/μm < 0.1) [2-26]. SEM displayed identical surface morphologies in all treated samples, which had smooth surfaces with Ra/μm about 0.1 or less, similar to the as-polished samples (as shown in **Figure 6.1**).

Figure 6.2 shows the crystal structure of the surface-treated Zr–9Nb–3Sn, pure Zr, and Nb samples obtained by XRD analysis. For the surface-treated Zr–9Nb–9Sn alloy, the ZrO_2 oxide peak was distinctly visible on the surface. This peak was also seen on the pure Zr surface for several of the surface treatments applied. However, the peak intensity of Nb_2O_5 oxide became relatively weak and was almost undetectable for the Zr–9Nb–3Sn and the pure Nb metal surfaces. It was considered an amorphous oxide [4-4].

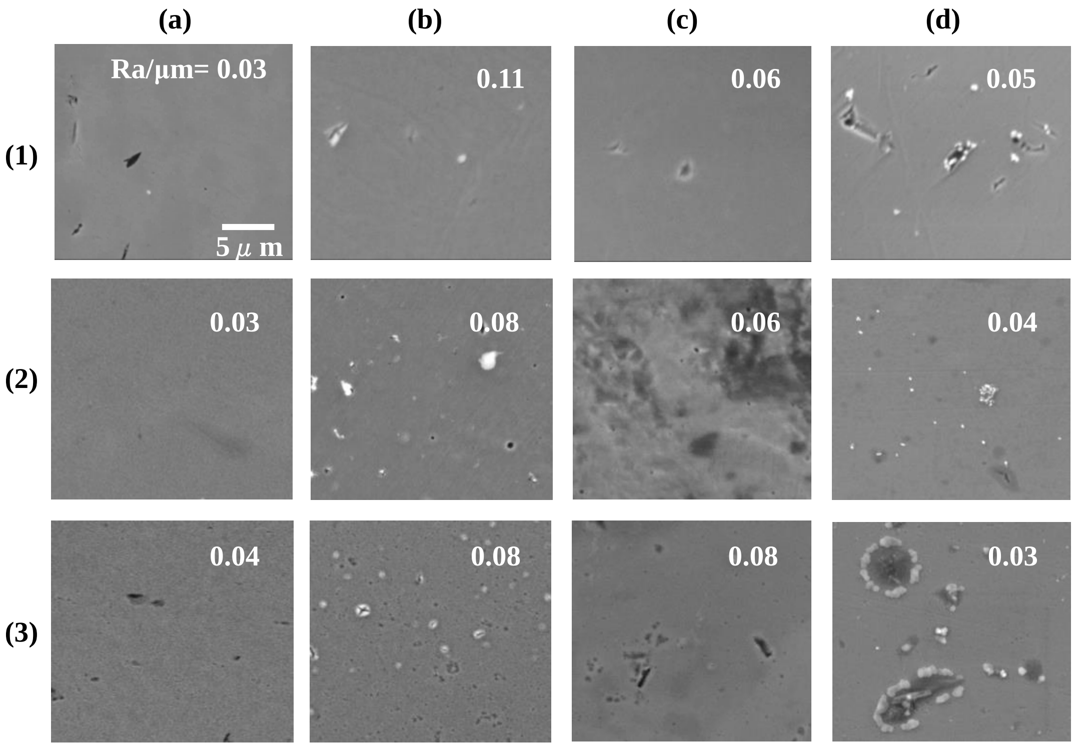

Figure 6.1 Surface morphology and surface roughness (Ra/μm) of (1) Zr-9Nb-3Sn, (2) Zr, and (3) Nb each of which was (a) as-polished, (b) as-anodized in a H_2SO_4 solution, (c) anodized + hydrothermaled and (d) as-hydrothermaled in distilled water at 180 °C for 180 min.

6.3.2 Surface hydrophilicity

Surface hydrophilicity has a remarkably function in the proteins adsorption on the surface as cell adhesion [4-4]. Generally, cell adhesion presents better behavior on hydrophilic surfaces [4-18]. In our results, a similar trend was observed for pure Zr and Nb. In addition, anodizing did not provide a significant enhancement of the hydrophilicity of Zr–9Nb–3Sn alloy. This can be seen in **Figure 6.3** where, despite the WCA of this Zr alloy decreasing after anodizing, it still produced a hydrophobic surface with WCA > 60°. The high WCAs of pure Zr and Nb after anodizing (higher than 60°) probably influenced the hydrophobicity of this Zr alloy. In contrary, the WCAs of Zr–9Nb–3Sn and its alloy elements (Zr and Nb) decreased significantly after hydrothermal treatment with and without anodizing, becoming lower than 30°. This means that the hydrothermal treatment produced hydrophilic surfaces for pure Zr and Nb, as well as for Zr–9Nb–3Sn alloy.

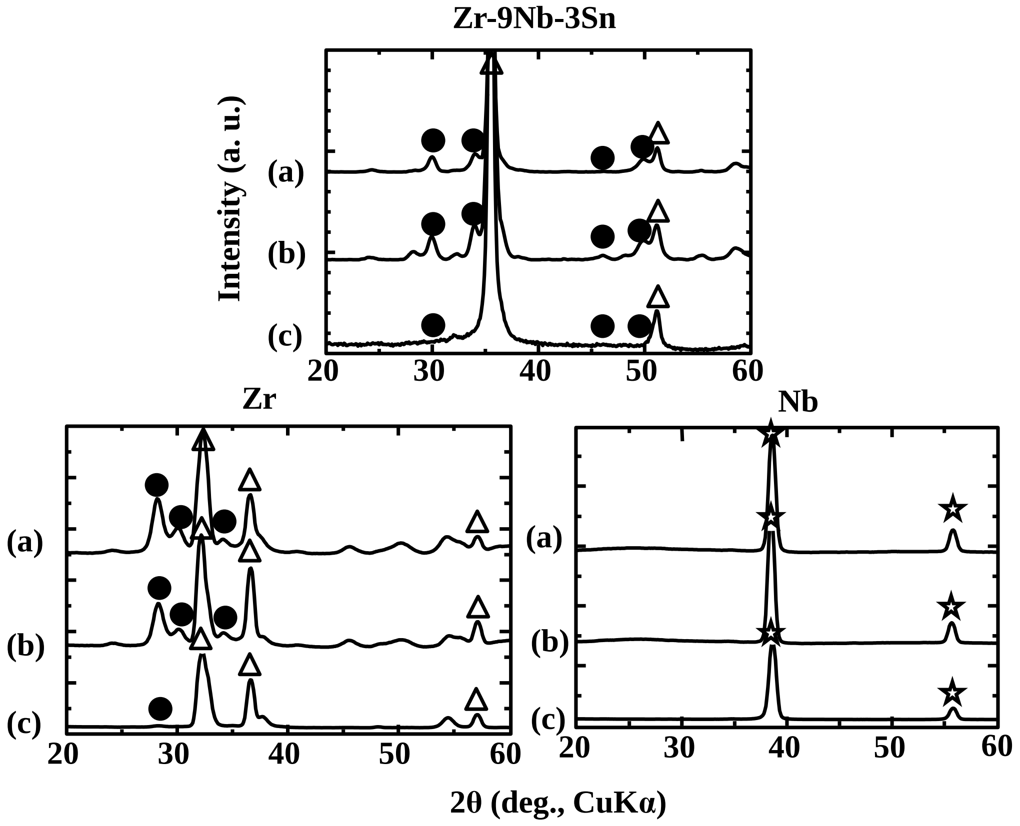

Figure 6.2 XRD patterns of Zr-9Nb-3Sn, Zr, and Nb using different treatments: (a) as-anodized in H_2SO_4 solution, (b) anodized+hydrothermaled and (c) as-hydrothermaled in distilled water at 180 °C for 180 min. Symbols: ☆= Nb, △= Zr, and ●=ZrO_2.

After obtaining the hydrophilic surface by hydrothermal treatment, it is important to maintain this condition before implantation because the hydrophilicity can change over time. When the hydrothermaled samples were stored in air and distilled water for longer time periods, their WCAs increased remarkably and their surfaces became hydrophobic. The adsorption of contaminants from the air or solution was probably one of the reasons for this increase in the WCAs (see **Figure 6.4**). In contrary, the surface hydrophilicity of Zr–9Nb–3Sn, pure Zr, and Nb was retained or slightly improved when stored in ×5 PBS(−) for 24 h. This is because of adsorbed solute ions in PBS(−), such as Na^+ and Cl^- [3-4]. This result shows that storing in ×5 PBS(−) is effective in maintaining the super hydrophilic surfaces on hydrothermaled Zr–9Nb–3Sn, pure Zr, and Nb samples, as well as on those of pure Ti [5-10].

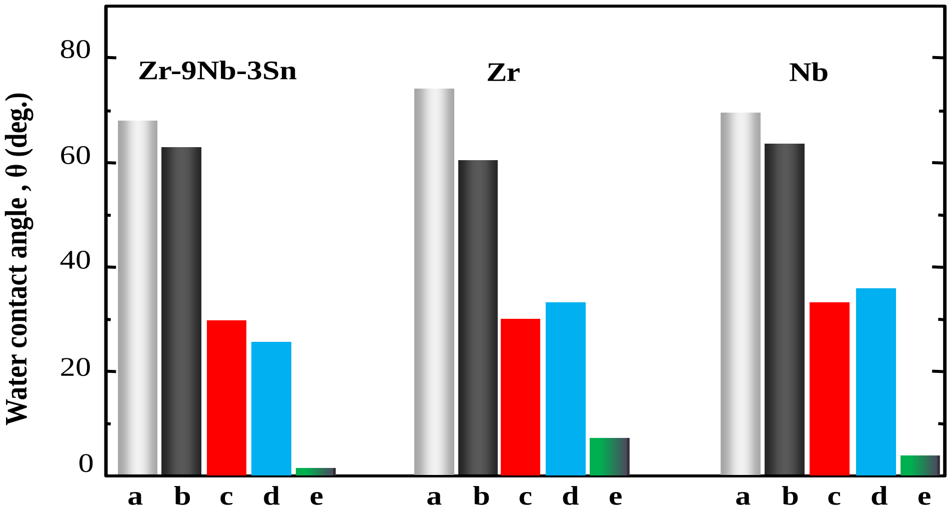

Figure 6.3 WCA of Zr-9Nb-3Sn, Zr, and Nb samples with various surface modifications: (a) as-polished, (b) as-anodized in a H_2SO_4 solution, (c) anodized + hydrothermaled, (d) as-hydrothermaled in distilled water at 180 °C for 180 min., and (e) as-hydrothermaled after stored in ×5 PBS(−).

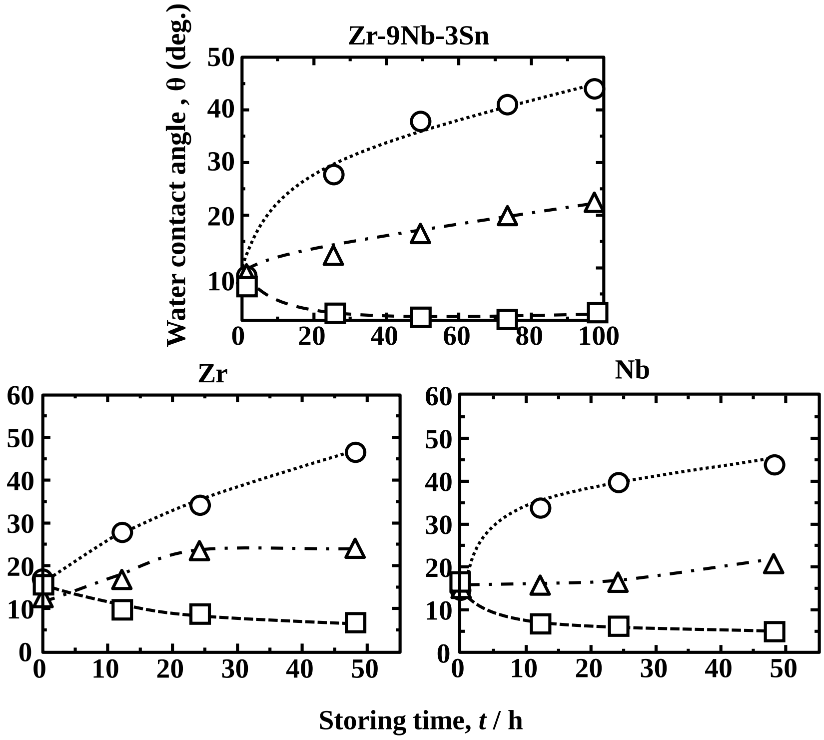

Figure 6.4 Relationship between the WCA and periods of hydrothermaled samples of Zr-9Nb-3Sn, Zr, and Nb stored in: ○ in air; △ in distilled water; and □ in ×5 PBS (−).

6.3.3 XPS analysis

Zirconium has a strongly bonding capacity to atmospheric elements, such as carbon, nitrogen, oxygen, and hydrogen. Adsorption of C–H is taken place in air or solution contained organic contaminants under normal conditions. C–H elements as a surface contaminant can adsorb during storage after surface treatment. The hydroxyl (OH^-) group is another factor that can influence the surface hydrophilicity, in a similar manner as adsorbed C–H. As shown in **Figure 6.5**, XPS analysis showed the change in adsorption of C–H and OH^- groups on the surface hydrophilicity (WCA) of samples after several different surface treatments. Spectrum analysis was performed as described in our previous report [6-13]. As for pure Zr and Nb, the adsorption of C–H for the Zr–9Nb–3Sn alloy decreased significantly after hydrothermal treatment, in the same manner as pure Ti [5-10]. By cleaning the surfaces to remove contamination, the hydrophilicity of pure Zr, Nb, and Zr–9Nb–3Sn improved notably. This result agrees with an earlier study, which reported that surface contamination increased the WCA and the surface became more hydrophobic [4-9]. *In vitro* studies have also indicated that surface contamination reduced cell attachment and spreading [6-14]. On the other hand, the binding of the OH^- group did not have a similar trend for each sample and seemed to be unaffected by hydrothermal treatment. Therefore, the reduction of the adsorbed C−H was the most important factor for improvement of the hydrophilicity of hydrothermaled Zr–9Nb–3Sn, pure Zr, and Nb samples.

6.3.4 *In vivo* test

As-polished and hydrothermaled samples of Zr–9Nb–3Sn and its alloy elements were used for *in vivo* testing after being stored for 24 h in ×5 PBS(–). As a reference, the previous *in vivo* results for TiO_2-coated samples with Ra/μm < 0.1 are also shown in **Figure 6.6** [2-26][2-27]. It can be seen that by decreasing the WCA, the osteoconductivity ($R_{B–I}$) of the hydrothermaled samples (Zr–9Nb–3Sn, pure Zr, and Nb) after stored in ×5 PBS(−) became higher by 40–45% compared with that of the as-polished samples.

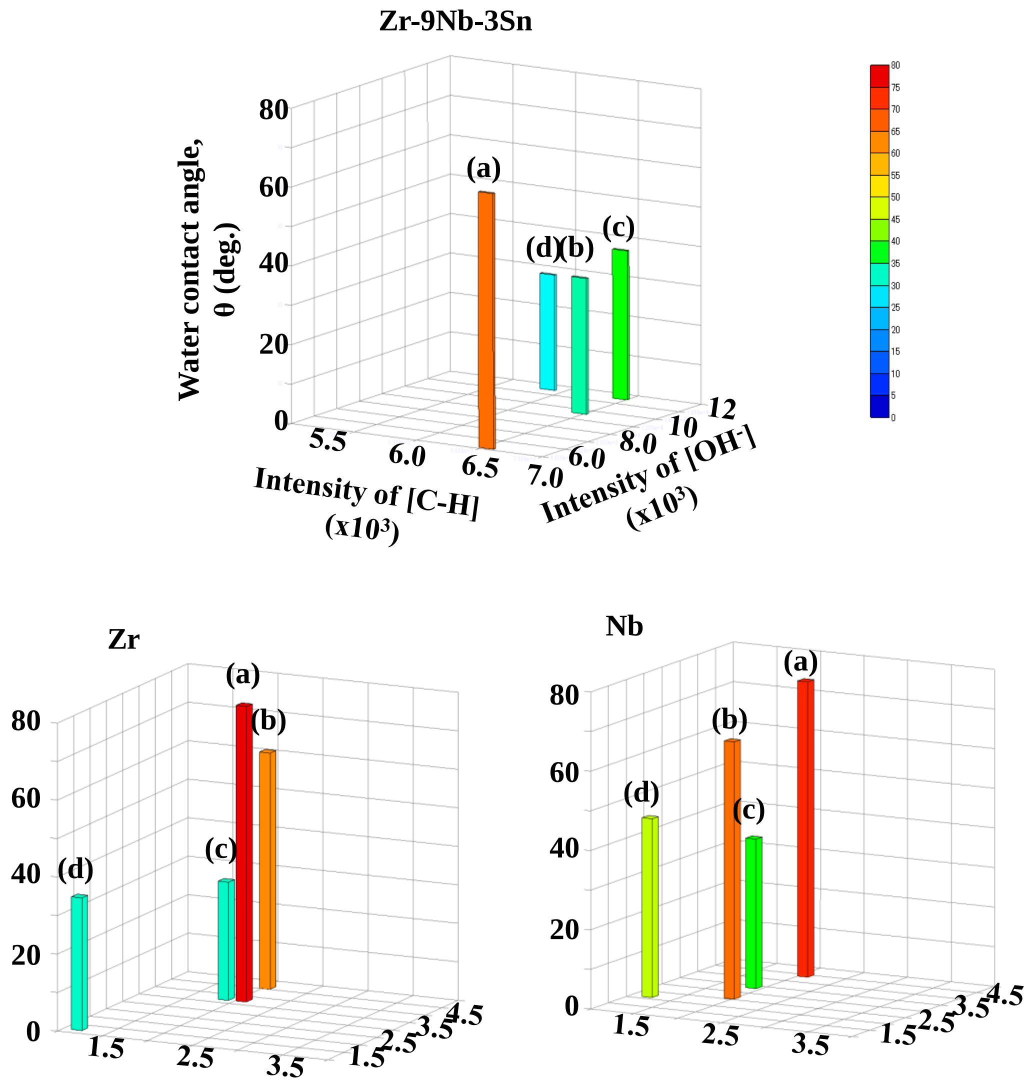

Figure 6.5 Quantitative relationship among surface OH⁻ group, adsorbed C–H, and WCA for single metals of Zr-9Nb-3Sn, Zr, and Nb under different conditions: (a) as-polished, (b) as-anodized, (c) anodized + hydrothermally treated and (d) hydrothermally treated . The amounts of OH⁻ and C–H were calculated from O_{1S} and C_{1S} spectrum from XPS analysis. The color scale in the spectrum represents the range of WCA.

According to previous reports, the adsorption of cell adhesive proteins containing RGD, such as fibronectin, was affected by surface hydrophilicity. As results, it enhanced the adhesion and spreading of osteoblast precursors on these surfaces [2-28][2-29].

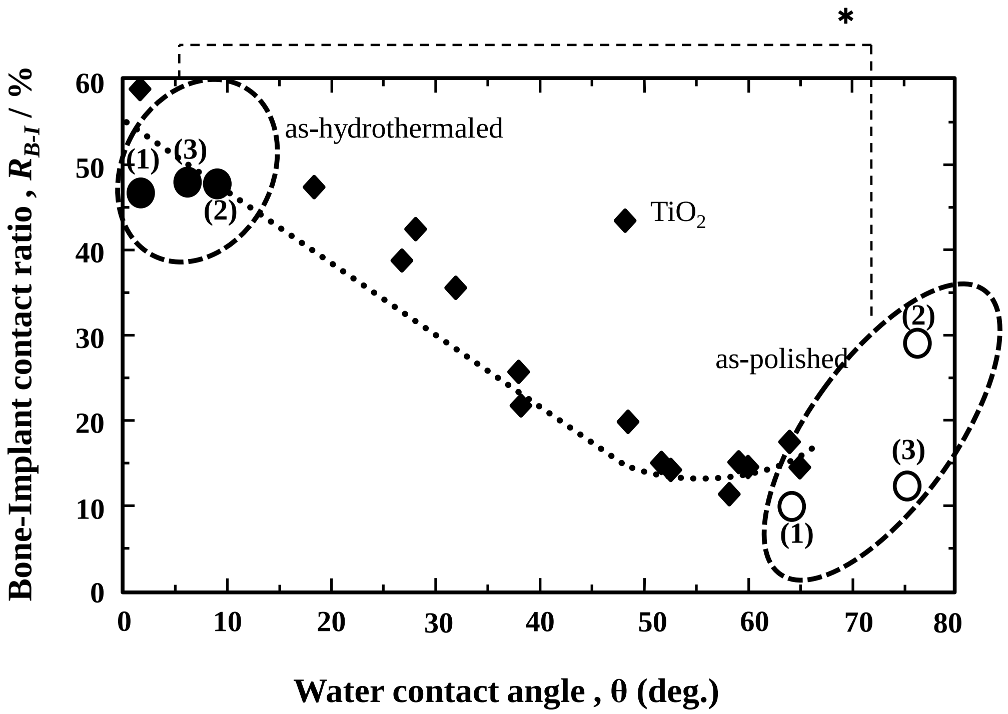

Figure 6.6 Relationship between $R_{B\text{-}I}$ value (in cortical bone) and WCA of (1) Zr-9Nb-3Sn, (2) Zr, and (3) Nb samples with various surface modifications and subsequent storage methods: ○ as-polished, ● as-hydrothermaled after immersed in ×5 PBS(−) solution and ◆ anodized Ti in different aqueous solutions (Ra/μm < 0.1); *$p < 0.05$.

My results correspond with those of previous *in vitro* and *in vivo* studies that detected that early bone formation, osteoblast cell adhesion, and differentiation of hydrophilic implant surfaces were notably improved compared than hydrophobic ones [2-35][4-20][6-15][3-7][3-8].

Therefore, I conclude that the combination of hydrothermal treatment and ×5 PBS(−) immersion is an effective method of enhancing the osteoconductivity of Zr–9Nb–3Sn, as well as pure alloy elements (pure Zr and Nb), by producing hydrophilic surfaces.

6.4 Conclusions

In this chapter, the hydrothermally treated surfaces for Zr–9Nb–3Sn and its pure alloy elements (Zr and Nb) after storage in ×5 PBS(−) were super hydrophilic with higher osteoconductivity (an increase of 40–45%) compared with untreated samples. This suggests that hydrothermal treatment may be a promising surface treatment for improving the osteoconductivity of implant materials such as Zr, Nb, and Zr–9Nb–3Sn alloy in future. However, further studies are needed to improve our understanding of the effect of surface treatments and hydrophilic surfaces on fibronectin adsorption.

References

[6-1] Hanawa T. Metals for medicine. Sendai, Japan Institute of Metals; 2010.

[6-2] Bui FM, Bott K, and Mintchev MP. A quantitative study of the pixel-shifting, blurring and nonlinear distorsions in MRIimages caused by the presence of metal implants. J Med Eng Tech 2000;24:20-27.

[6-3] Shafiei F, Honda E, Takahashi, and Sasaki T. Artifacts from dental casting alloys in magnetic resonance imaging. J Dent Res 2003;82:602-606.

[6-4] Thomsen P, Larsson C, Ericson LE, Sennerby L, Lausmaa J, and Kasemo B. J. Mater. Sci. Mater. Med. 8 (1997) 653–665.

[6-5] Sherepo KM, and Red'ko IA. Biomed Eng 2004;38:77-79.

[6-6] Hanawa T. Evaluation techniques of metallic biomaterials in vitro. Sci Tech Adv Mater 2002;3:289-295

[6-7] Hanawa T. Metal ion release from metal implants. Mater Sci Eng C 2002;24:745-752.

[6-8] Song HJ, Park SH, Jeong SH, and Park YJ. Surface characteristics and bioactivity of oxide films formed by anodic spark oxidation on titanium in different electrolytes. J Mater Proc Tech 2009; 209:864-870.

[6-9] Cui X, Kim HM, Kawashita M, Wang L, Xiong T, Kokubo T, and Nakamura T. Preparation of bioactive titania films on titanium metal via anodic oxidation. Dent Mater 2009;25:80-86.

[6-10] Yamamoto D, Kuroda K, Ichino R, and Okido M. Proc. 18th Int. Conf. on Proc and Fabri of Adv Mater 2009:1311-1319.

[6-11] Liu X, Chu PK, and Ding C. Surface modification of titanium, titanium alloys, and related materials for biomedical applications. Mater Sci Eng R 2004; 47:49-121.

[6-12] Le Guehennec L, Soueidan A, Layrolle P, and Amouriq Y. Surface treatments of

titanium dental implants for rapid osseointegration. Dent Mater 2007;23:844-854.

[6-13] Zuldesmi M, Waki A, Kuroda K, and Okido M. Enhancement of valve metal osteoconductivity by one-step hydrothermal treatment. Mater Sci Eng C 2014;42:405-411.

[6-14] Vezeau P, Keller J, and Wightman J. Reuse of healing abutments: an in vitro model of plasma cleaning and common sterilization techniques. Impl Dent 2000; 9:236-246.

[6-15] Zhao G, Schwartz Z, Wieland M, Rupp F, Geis-Gerstorfer J, Cochra DL, and Boyan BD. High surface energy enhances cell response to titanium substrate microstructure. Biomed Mater Res A 2005;74:49-58.

CHAPTER 7

Future Works

A current research area in biomaterials focuses on how materials properties, such as surface morphology (roughness, wettability etc), can influence the osseintegration of implant materials; indeed, this is a common theme for many papers. In my present work, various surface modifications were applied to create hydrophilic surface on metallic implant materials (valve metals and their alloys). The effect of surface hydrophilicity on their osteoconductivities was investigated. It was found that hydrophilic surface which produced by hydrothermal treatment can improve the osteoconductivity of valve metals and their alloys. However, several important questions still remain. Do the surface modifications improve the adsorption of cell-adhesive protein? Which kind of protein that most important for cell attachment? Is that protein easy to adsorb on the hydrophilic surfaces? What happen on the attached cell after protein adsorption? So, the further investigations in a short time will help to get the answers.

I predict that metallic biomaterials still have been proposed to apply in a number of future medical applications. However, the current generation of implants are still with problems (such as, corrosion), and long-term performance is still an issue particularly in younger high-demand patients. It is hoped that continuing development in implant technology research will improve the condition of implants in the future. The use of wide methods in the manipulation of surface morphology of implants and incorporating the use of various inorganic and organic components would directly influence the response in the local bone-implant interface and the apposition of new bone. With the development of new techniques and strategies on metallic surfaces to better mimic the human bone structure, this would result in a new generation of orthopedic implants with improved implant integration and bone healing.

CHAPTER 8

General Conclusions

1. In chapter 1, several properties of metallic biomaterials (such as microstructure, chemical properties, and mechanical properties) including biocompatibility and osteoconductivity were represented clearly.
2. In chapter 2, various surface treatment to improve the osteoconductivity of metallic biomaterials were explained in detail. In addition, two biological evaluation methods (*in vitro* and *in vivo* studies) were informed.
3. In chapter 3, various processes (such as anodizing, hydrothermal treatment, UV light irradiation, and plasma irradiation) were applied to Ti samples which purpose to produce hydrophilic surfaces in improving its osteoconductivity. Although UV light irradiation and plasma irradiation processes created the hydrophilic surfaces on pure Ti, they are difficult to apply for complex-shaped substrates of implant materials with complex topographies, because of the line-of-sight nature of the methods. Super-hydrophilic surface (WCA<10 deg.) with osteoconductivity higher about 50% than as-polished Ti was provided on hydrothermaled pure Ti after stored in x5 PBS(−).
4. In chapter 4, based on results from chapter 3, hydrothermaled treatment was carried out for valve metals (Ti, Zr, Nb, and Ta). Anodizing process was done to the valve metals as a comparison. The surface hydrophilicity of pure Ti showed the enhancement after anodizing, however, it was not work to anodized Zr, Nb, and Ta samples. Their surface were still in hydrophobic conditions. In contrast, the improvement of surface hydrophilicity was detected after the hydrothermal treatment was implemented to all valve metals. This condition became more valuable when the hydrothermaled valve metals stored in x5 PBS(−) where their osteoconductivity increased up to 55 % compared than those of as-polished samples. It means that, hydrothermaled treatment that continue by storing in x5 PBS(−) is not only effective to improve the osteoconductivity of pure Ti, but also effective for

other valve metals (Zr, Nb, and Ta).

5. In chapter 5, hydrothermaled treatment was conducted to Ti and Ti alloys (such as Ti64, Ti67, TNTZ, and TCFA) in order to improve their osteoconductivities. Anodizing data were used for comparison. It was found that anodizing only succesful to produce hydrophlic surfaces on Ti, Ti64, and Ti67 and improved their osteoconductivities, but it was not useful for TNTZ and TCFA alloys. The hydrophobic surfaces were still formed on the surface of these two alloys. Consequently, the osteoconductivities of anodized TNTZ and TCFA were low and almost similar to those of as-polished conditions. On the other hand, all the Ti alloys surface became hydropilic after hydrothermaled treatment. When the hydrothermaled Ti and Ti alloys were continue stored in x5 PBS(−), the superhydrophilic surface were formed on all Ti alloys and improved their osteoconductivities significantly. It can be said that a combination process between hydrothermaled treatment and storing in x5 PBS(−) became a simple and effective way of enhancing the osteoconductivity of not only for pure Ti, but also forTi alloys.
6. In chapter 6, the effect of hydrothermal treatment on osteoconductivity of Zr-9Nb-3Sn and its alloy was examined. Anodizing data also used as comparison. Similar to its pure metal alloys (Zr and Nb), anodizing did not provide a significant enhancement of the hydrophilicity of Zr-9Nb-3Sn alloy. However, this condition became different when hydrothermal treatment was applied to Zr-9Nb-3Sn alloy. Its surface became hydrophilic after hydrothermal treatment. In addition, higher osteoconductivity was produced on hydrothermaled Zr-9Nb-3Sn alloy after stored in x5 PBS(−). This suggests that hydrothermal treatment become one of the promising surface treatments which improve the osteoconductivity of implant materials such as Zr, Nb, and Zr-9Nb-3Sn alloy in future.
7. In chapter 7, several recommendations were explained for improving of this dissertion in the next future

List of publications

Journal papers:　published

1. Zuldesmi M, Waki A, Kuroda K, and Okido M. High osteoconductive surface of pure titanium by hydrothermal treatment, J Biomat Nanobiotech 2013;4:284-290.
2. Kuroda K, Zuldesmi M, and Okido M. Osteoconductivity of hydrothermal-treated valve metals. Mater Sci Forum 2014;783-786:1298-1302.
3. Zuldesmi M, Waki A, Kuroda K, and Okido M. Enhancement of valve metal osteoconductivity by one-step hydrothermal treatment. Mater Sci Eng C 2014;42:405-411.

Journal papers:　submitted

1. Zuldesmi M, Waki A, Kuroda K, and Okido M. Hydrothermal treatment of titanium alloys for the enhancement of osteoconductivity.Mater Sci Eng C 2015 (accepted).
2. Zuldesmi M, Kuroda K, Okido M, Ueda M, and Ikeda M. Osteoconductivity of hydrophilic surfaces of Zr-9Nb-3Sn alloy with hydothermal treatment. Mater Sci Eng C 2014 (submitted)

List of international conferences

1. Waki A, Zuldesmi M, Kuroda K, and Okido M, and Niinomi M. High osteoconductivity surface of valve metal by hydrothermal treatment. The 5th International Symposium on Designing, Processing and Properties of Advanced Engineering Materials (ISAEM-2012), November 5-8, 2012, Toyohashi, Japan.
2. Zuldesmi M, Waki A, Kuroda K, and Okido M. Improvement of Osteoconductivity of Pure Titanium by Hydrothermal Treatment. International Symposium on Eco Topia Science 13 (ISETS 13), December 13-15, 2013, Nagoya University, Nagoya, Japan.
3. Waki A, Zuldesmi M, Kuroda K, and Okido M, Ueda M, Ikeda M, and Niinomi M. Highly Osteoconductive Surface of Ti Alloys using Hydrothermal Treatment. International Symposium on Eco Topia Science 13 (ISETS 13), December 13-15, 2013, Nagoya University, Nagoya, Japan.
4. Kuroda K, Zuldesmi M, and Okido M. Osteoconductivity of hydrothermal-treated valve metals. International Conference on Processing & Manufacturing of Advanced Materials, Processing, Fabrication, Properties, Applications, Thermec` 2013, Dec.2-6 , 2013, Las Vegas, USA.
5. Zuldesmi M, Kuroda K, and Okido M. Hydrothermal surface modification of titanium alloys for enhancement of osteoconductivity. The 15th IUMRS-International Conference in Asia (IUMRS-ICA 2014), August 24-30, 2014, Fukuoka University, Fukuoka, Japan.
6. Zuldesmi M, Kuroda K, and Okido M. Osteoconductivity of Hydrothermaled Zr-9Nb-3Sn Alloy for Biomedical Application. The 15th International Symposium on Advanced Materials Development and Integration Novel Structured Metallic and Inorganic Materials (AMDI 5), November 19, 2014, Tokyo Medical and Dental University, Tokyo, Japan.

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Zuldesmi