# Modifying Ti6Al4V Implant Surfaces:

# **Cell Responses and Corrosion Resistance of Annealed Titania Nanotubes**

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# THESIS

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# Dedication

I would like to dedicate this work to my family and friends, who have been unconditionally supportive of my endeavors throughout my life, and continue to support me in my pursuit of a career in medicine. I would particularly like to dedicate my work to my parents, my sister, and Bethany Otto. Their emotional and financial support made this work and my pursuits possible. I am forever grateful to them.

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# List of Abbreviations

Ad	Anodized
Ad+TO	Anodized and thermally oxidized
BET	Brunauer-Emmett-Teller surface area
С	Capacitor
CoCrMo	Cobalt Chromium Molybdenum alloy
СРЕ	Constant phase element
Cp-Ti	Commercially pure titanium
E <sub>corr</sub>	Corrosion potential
EIS	Electrochemical impedance spectroscopy
FESEM	Field emission scanning electron microscopy
FTIR	Fourier transform infrared spectroscopy
I <sub>corr</sub>	Corrosion current density
I <sub>pass</sub>	Passivation current density
ОСР	Open circuit potential
R	Resistor
Ti-6Al-4V	Titanium alloy grade V
ТО	Thermal oxidation
TNT	Titania nanotube
W	Warburg diffusion element
WCA	Water contact angle

#### **Summary**

The following body of work aimed to investigate the surface modifications of electrochemical anodization, to fabricate titania nanotubes, and thermal oxidation on Ti-6Al-4V alloy for biomedical application as it relates to surface wettability and resistance to corrosion. Treatment groups of interest were smooth, thermally oxidized at 600° for 3 hours, anodized at 60 V for 2 hours, and a combination of anodization followed by thermal oxidation under the same respective conditions. The specific focus was to evaluate the ways in which these modifications may enhance surface properties relating to osseointegration and corrosion resistance of orthopedic and dental implants in bone. Wettability, surface characterizations of Fourier transform infrared spectroscopy, field emission scanning electron microscopy, Brunauer-Emmett-Teller surface area analysis and standard corrosion testing were performed, as well as a proposed cell culture study.

Results showed that thermally oxidized nanotubes had the most hydrophilic surface, and that the surface retained its hydrophilic behavior over 25 days of aging while other surface treatments did not. The anodization process proved to yield predominantly amorphous  $TiO_2$  with little anatase, while the thermal oxidation process proved to yield predominantly rutile. Nanotube dimensions were determined to be 100 nm inner diameter, 170 nm outer diameter, and 5 µm length. The nanotube surface area was determined to be 0.33 m<sup>2</sup>/g, and the region exposed to the corrosion electrolyte was determined to be  $\sim$ 300 cm<sup>2</sup>. Regarding corrosion testing, the annealed nanotubes showed shifts in the noble (positive) direction regarding open circuit potential and corrosion potential, while also showing the

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# **Summary (continued)**

lowest corrosion current density. However, the amorphous nanotubes showed the better passivation behavior than the annealed nanotubes, particularly in anodic regions slightly higher than the corrosion potential. This behavior was attributed to the presence of rutile crystalline structure in the nanotube layer and compact oxide layer acting as a semiconductor in electron transfer and metal ion evolution, while inhibiting the movement of anionic oxygen species to the metal-oxide interface. The annealed nanotubes offered the overall greatest wettability and corrosion resistance, however, it was determined that future work can be done to optimize both titania nanotube dimensions for cellular responses, as well as rutile/anatase content for corrosion resistance and passivation behavior

# Chapter 1

#### Introduction

#### 1.1 Biomaterials and Metallic Implants

Biomaterials have long been incorporated into clinical treatment of various physiological disorders, giving rise to a unique subset of research in materials science. Examples of such materials can be found in artificial heart valves, blood vessel stents and orthopedic and dental implants [1]. Biomedical implants face an array of challenges and must equilibrate both function and integration. Deviations from this crucial balance can lead to implant failure. Specific aspects of importance to the evaluation and longevity of an implant are its mechanical, chemical and tribological properties, its biocompatibility, and the overall health of the implant recipient patient [2].

Certain pure metals and their alloys, such as Ti and CoCrMo, have been used in biomedical orthopedic and dental implants (Figure 1.1) due to their mechanical strength and relative biocompatibility regarding adverse immune responses [3]. However, many of these metallic implants fail within 12-15 years due to mechanical, chemical, tribological, surgical, manufacturing and biocompatibility problems [2]. Additionally, the clinical issues surrounding implant corrosion are of significant importance. As the population continues to age, demands for longer-lasting implants are increasing. It is estimated that by the end of 2030, total hip replacements and knee arthroplasties will rise by 174% and 673% respectively, in addition to an increase in implant revision surgeries [1]. The current clinical problems and expected increases in demand for bone replacement implants have made the study of corrosion and wear resistance, in conjunction with osseointegration, an imperative endeavor.



**Figure 1.1** Representation and radiograph of materials and used in orthopedic hip implants and dental implants [4, 5].

Current materials used for biomedical implants in bone include 316L stainless steel, cobalt chromium alloys and titanium and its alloys [1, 6]. Of particular interest to this study is the selection of titanium-based materials for implantation. Titanium shows high strength, low density, low modulus, high corrosion resistance, and enhanced biocompatibility and osseointegration. Yet, titanium also has poor shear strength and undergoes severe mechanical wear as well as being bioenert, thus producing a poor osseointegration response [1]. Mechanical wear and corrosion leads to metal ion release, and can induce electric fields and voltages at the implant surface, leading to localized cellular death, inflammation, joint loosening and the need for revision surgeries [7-9]. While the nature of corrosion and the deleterious effects of ion release, both local and systemic, have been studied extensively, spontaneous potentials that induce

currents at the implant surface as a result of corrosion and mechanical wear, and its effect on integrated tissue, have undergone limited examination with implant surface modifications. Because titanium is bioenert, it has become imperative to investigate treatments to modify the implant surface in order to improve osseointegration. In particular, as new surface treatments of metallic implants are evaluated with respect to the osteogenic cellular response, it is necessary to gain an understanding of their performance with regard to corrosion and protection from potential differences at the implant surface. Current surface treatments of interest include electrochemical anodization, in order to develop nano-scale surface topography for cellular growth, and thermal oxidation to tailor a protective layer to corrosion on the surface [10-12]. Furthermore, the ability to combine both processes with fabricating titania nanotubes followed by thermal oxidation are examined as ell. The nature of these particular surface modifications and the evidence of increased wettability of thermally oxidized titania nanotubes, extrapolation to osteogenic cell culture response, and increased resistance to corrosion of both as-received and thermally oxidized titania nanotubes will be further discussed.

# 1.2 Surface Treatments of Titanium

## 1.2.1 Thermal Oxidation

Thermal oxidation of titanium and titanium alloys has two results, 1) a compact oxide layer is formed, and 2) the structure of  $TiO_2$  changes from amorphous to crystalline, depending on the annealing temperature. The formation of the oxide layer can be described in a three-step process (Figure 1.2). First,

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oxidants from the local atmosphere of the reservoir, in the case of this study ambient air, transport to the outer surface of the metal substrate (Ti-6Al-4V). As the redox reaction takes place at the bulk metal interface, the oxide layer grows, creating a diffusion zone. Second, oxidants (primarily oxygen gas) transport through the oxide layer by diffusion. Finally, oxidants arrive at the bulk metal-oxide layer interface where they continue to oxidize the bulk metal, growing the oxide layer [13].



Figure 1.2 Schematic of thermal oxidation process.

The crystalline structure of titanium exists in two forms,  $\alpha$  and  $\beta$ . The  $\alpha$  form occurs at low temperatures and is a closely packed hexagonal crystal structure. The  $\alpha$  form is also stabilized by alloying elements. The  $\beta$  form exists above temperatures of 883° C and has a body-centered cubic structure. Thermal treatment of titanium and its alloys below the  $\beta$  transus temperature yields a mixture of the structures. For example, Ti-6Al-4V is an  $\alpha$ + $\beta$  alloy normally used in biomedical implants in the annealed condition [1]. Similarly, TiO<sub>2</sub> exists in three allotropic modifications: brookite (rhombohedrical), anatase (tetragonal) and rutile (tetragonal) [14]. Annealing temperatures above 250° C yield anatase crystalline structure, and rutile structure begins to appear at annealing temperatures of roughly 450° C to 500° C [15], leading to a mixture of predominantly anatase and rutile. The ratio of rutile to anatase then increases with annealing temperature [14]. The oxide layer thickness of titanium and its alloys also increases with annealing time [16]. It has also been found that aluminum tends to pile up near the external surfaces of the oxide scale[17]. The formation of this layer is critical to determine the corrosion resistance of the implant surface. Both commercially pure titanium and Ti-6Al-4V alloy show enhanced corrosion resistance with thermally produced oxide layers 100 nm thick in comparison to polished controls with native oxide layers [16].

The difference in structure of anatase and rutile, or a mixture of the two, of TiO<sub>2</sub> thin films also lends to differences in the electrochemical and conductivity properties. It is reported that anatase has higher electron mobility than rutile. The Fermi level of anatase is higher than that of rutile by about 0.1 eV, but the band gap of rutile is smaller than that of anatase [18]. In electrochemical impedance spectroscopy studies (EIS), rutile thin films have displayed lower capacitance and higher polarization resistance in electrolyte solutions than anatase thin films [19]. Musa and Rusop [20] showed that with I-V studies of anatase and predominantly anatase thin films, the conductivity increases and resistance decreases with increasing annealing temperature up to 550° C.

The cellular response to thermally oxidized titanium and its alloys has been extensively studied. Lee et al. [21] compared titanium samples thermally treated at

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300° C, 500° C and 750° C to a smooth polished control. In the surface characterization, it was found that surface roughness and surface energy increased while water contact angle decreased with increasing annealing temperatures. There were no significant differences between groups regarding cell viability, but increased ALP activity, a marker for osteoblast-like activity, increased with increasing annealing temperature. Similarly, the surface properties of thermally oxidized Ti-6Al-4V can affect the cellular response as it relates to osseointegration. Garcia-Alonso et al. [22] showed that with thermal oxidation, the oxide layer was predominantly TiO<sub>2</sub>, with small amounts of TiO and Ti<sub>2</sub>O<sub>3</sub> closer to the metal/oxide interface. Al<sub>2</sub>O<sub>3</sub> was also observed at the oxide/air interface. Additionally, the surface roughness has been found to increase with Ti-6Al-4V annealed at 700° C versus 500° C. Increased cellular adhesion within 24 hours and increased osteoblast-like activity has been reported for thermally oxidized titanium alloy at higher temperatures [22, 23].

# 1.2.2 Titanium Dioxide Nanotubes by Electrochemical Anodization

The use of nano-network substrates is becoming more attractive for application in biological systems. The ability of titanium to form ordered titanium dioxide nanotubes (TNTs) is of significant interest for use in biomedical implants. They provide increased surface area for protein adsorption and cellular adhesion, increased hydrophilicity for biocompatibility, and provide the opportunity for altering the electrochemical properties by doping, for drug-loading, and for a number of other applications. The synthesis of TiO<sub>2</sub> nanotubes can be achieved by sol-gel, template-assisted, hydro/solvothermal and electrochemical methods. The nanotubes used in this study were fabricated by electrochemical anodization means in which nanotubes form by fluoride etching and metal ion dissolution [24]. The length and diameter of the TiO<sub>2</sub> nanotubes can be controlled by the polarization voltage, anodization time, pH and electrolyte solution [25]. TiO<sub>2</sub> nanotubes of Ti-6Al-4V were fabricated by 2 hours of anodization at 60V v. Cu in ethylene glycol/ammonium fluoride solution. Figure 1.3 shows the comparison of current v. time in the formation of TiO<sub>2</sub> nanotubes versus a compact TiO<sub>2</sub> layer. With titanium dioxide nanotubes, as the surface becomes more porous, a sharp increase in conductivity appears. However, as the thickness of the nanotubes increase, the current decreases and the resistance increases [25, 26]. Still, the conductivity of the nanotubes is higher than that of the compact oxide surface, which could have implications regarding the conduction of current to cells at an implant interface as well as to aspects of corrosion rates due to current densities.



**Figure 1.3** Comparison of current v. time for TNTs and compact oxide via anodic oxidation [25].

The fabrication of surfaces with nano-network topography on a variety of biomaterials, including carbon, aluminum, cobalt-chromium and titanium, have proven to increase hydrophilicity, ion and protein adsorption along with cellular adhesion in comparison to their smooth substrates. It has also been found that the increase in cellular adhesion has lead to increases in cellular viability, proliferation, and osteogenic differentiation [27-32]. The increased hydrophilicity and protein adsorption is important as it has been reported that cells are constantly in contact with biomaterial surfaces that have previously adsorbed water and proteins from fluids rather than with the bare surface. The cellular adhesion process begins with an attachment phase dominated by van der Waals forces and is followed by an adhesion phase by anchoring to the implant surface via fibronectin and vitronectin proteins to form focal adhesions at cell membrane integrins. The spreading if fingerlike projections (filopodia) for increased anchorage ensues [33]. The effects of  $TiO_2$ nanotube diameter have also been investigated and it has been reported that the optimal nanotube diameter for bone cell formation and activity is on the order of 70 nm [27, 33]. In addition to *in vitro* studies of cellular responses to  $TiO_2$  nanotubes, *in* vivo studies have also reported positive outcomes [34, 35]. Wang et al. [35] investigated the effect of anatase TiO<sub>2</sub> nanotube diameter of implants on osseointegration in minipigs. The expression of osteogenesis-related genes, such as alkaline phosphatase (ALP), were significantly increased in comparison to a machined control. The highest levels of gene expression occurred with the 70 nm nanotube implants.

#### 1.2.3 Electrochemical Anodization Followed by Thermal Oxidation

In brief, titanium and titanium alloy anodized to produce surface nanotubes can also be thermally oxidized to change the crystalline structure of the titanium dioxide and grow compact oxide beneath the nanotube layer by combining the aforementioned processes. Amorphous TiO<sub>2</sub> nanotubes can be converted to anatase crystalline structure at temperatures higher than roughly 280° C in air, and to a mixture of anatase and rutile at temperatures higher than roughly 450° C in air. The semiconducting properties of the nanotubes are characterized by their photoresponse and capacitance behavior using Mott-Schottky plots. Additionally, anatase is found primarily on the walls of the nanotubes and vacancy type defects were found to diffuse toward the surface with increasing annealing temperature. Thermal oxidation at 600° C leads to a healing of these vacancies [36]. The conductivity of annealed nanotubes is dependent on both the length of the nanotubes as well as the crystalline structure and composition of the oxide layer. As amorphous TiO<sub>2</sub> is converted to anatase by annealing at lower temperatures ( $\sim 250^{\circ}$ C), a decrease in resistivity and increase in conductivity can be observed. As the annealing temperature continues to increase, there is a critical point at which the increased presence of rutile in the oxide layer causes the resistance to increase and conductivity to decrease. Tighineanu et al. [37] reported this critical annealing temperature to be 450° C.

Research indicates that pre-osteoblasts respond positively to titanium dioxide nanotube surfaces after annealing to form either anatase or a mixture of anatase and rutile nanotubes. Increases in the acceleration of the growth rate of osteoblasts on titanium dioxide nanotubes as much as 300-400% after annealing at 500° C compared to a smooth surface have been reported. The number of adhered cells and ALP activity was also significantly higher compared to non heat-treated, amorphous TiO<sub>2</sub>. Additionally, it has been shown that proliferation and Mesenchymal Stem Cell (MSC) differentiation increase with increasing annealing temperature of titania nanotubes [30, 38].

#### 1.3 Surface Characterization Techniques

#### 1.3.1 Wettability

Water contact angle (WCA) analysis measures the hydrophobicity or wettability of the sample. A drop of water is placed on the sample, and the angle of contact is measured using a goniometer. It has been shown that the size of the water droplet can affect water contact angle measurements in relation to Young's equation [39]. However, generally speaking the larger the contact angle is, the more hydrophobic the surface of the sample. For the purposes of this study, it is should be understood that it surface wettability has also been linked to surface roughness, that is, rougher surfaces exhibit more hydrophilic behavior [40]. This is an important characterization method as *in vivo* solution environments are aqueous in nature, and surface charges of cellular adhesion proteins, such as integrins, function better on hydrophilic surfaces.

# 1.3.2 Fourier Transform Infrared Spectroscopy

Fourier Transform Infrared Spectroscopy (FTIR) is a method of obtaining information about the chemical structure of a sample due to the types of bonds that occur. Infrared radiation is used to excite molecules in the sample. This causes polar bonds to oscillate by stretching and bending, resultant of absorption at characteristic frequencies. Different types of bonds between atoms have different absorption frequencies, and thus the chemical structure can be distinguished by analyzing these resultant frequencies [41].

#### 1.3.3 Field Emission Scanning Electron Microscopy

A field emission scanning electron microscope (FESEM) presents a useful tool for surface imaging at the nano-scale. A beam of electrons scans the surface of a sample. As the electrons collide with atoms in the sample, they scatter. Inelastic scattering results in the transfer of energy from an electron to an atom in the sample, and the atom emits a secondary electron. SEM images are produced by recording the production of these secondary electrons [41].

### 1.3.4 Surface Roughness Metrology

Surface roughness is a quantitative measurement of deviations from an idealized flat surface. One way to quantify this is through optical measurements by white light interferometry. This method uses a light source and a detector to measure interference patterns from the various wave paths taken through the material's surface in order to create a three-dimensional image. The parameter often used to characterize the surface roughness is the R<sub>a</sub> value, which represents the mean of the absolute values of the surface profile heights measured from a mean line average over the surface profile.

#### 1.3.5 Brunauer-Emmett-Teller Surface Area Analysis

The Brunauer-Emmett-Teller (BET) method of calculating surface area extends the Langmuir theory of monolayer molecular gas adsorption to multilayer

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gas adsorption (Figure 1.4). The BET method may utilize different adsorbates, for example nitrogen and krypton gas, and uses the equilibrium and saturation pressures of these adsorbates in multiple monolayers to determine the BET constant. Graphical analysis is used to determine a 3-point fit from which the surface area per unit mass is determined [42].





#### 1.3.6 Cell Culture: Osteoblasts and Cellular Adhesion

In order to examine the effects of cellular growth and osseointegration on electrochemical processes at the implant interface, it is necessary to understand the mechanisms involved in cellular adhesion, beginning with ion adsorption and protein adsorption to the implant surface. Pure and alloyed titanium, as well as other metals used as biomaterials that form thin film oxides, adsorb a plethora of inorganic ions to the metal oxide surface. Two surface properties govern the favorability of adsorption to a metal surface: surface hydrophobicity and surface charge. Surface hydrophobicity essentially describes how the surface responds to interaction with water, and is often measured by water contact angle (WCA). Surface charge can cause attraction or repelling of ions and proteins [41]. Additionally, alteration of surface topography is a characteristic that can change the adsorption of ions and proteins. Titanium dioxide (TiO<sub>2</sub>) surfaces carry a negative surface charge due to the oxide anions, and thus readily bind positively charged ions via electrostatic interactions [43, 44].

The role polyvalent cations play in protein adsorption and cellular adhesion has been extensively investigated. Zreiqat et al. [45] concluded that osteoblasts can bind directly to an implant surface, in their case aluminum oxide, via  $\alpha 5\alpha 1$  and  $\beta 1$ integrin proteins of human bone-derived cells (HBDCs). These functional integrins are transmembrane proteins that consist of non-covalently linked  $\alpha$  and  $\beta$  subunits. The extracellular domain of the  $\alpha$  subunits binds divalent cations (Ca<sup>2+</sup>, Mg<sup>2+</sup>, Mn<sup>2+</sup>) and alterations of the concentration of these ions in the extracellular space can modify the integrin-ligand affinities. It was shown that the aluminum oxide surface modified to contain adsorbed magnesium ion led to higher integrin expression, cell spreading and cell adhesion of HBDCs.

Additionally, the adsorption of cations, specifically calcium ion, to a  $TiO_2$  surface increases the adsorption of anionic calcium-dependent proteins when exposed to body fluids (Figure 1.5). Ellingsen [44] showed that both the native oxide of c.p. Titanium exposed to  $CaCl_2$  and hydroxyapatite selectively adsorbed albumin, pre-albumin and immunoglobulin g (IgG) from human serum. Calcium was identified to a depth of 17 nm within the oxide layer. It was concluded that the results were consistent with the proposal of calcium being an important mechanism by which proteins adsorb to  $TiO_2$ . Klinger et al. [46] furthered this proposal by

studying the mechanism of human serum albumin (HSA) adsorption to titanium powder *in vitro*. Titanium powder exposed to CaCl<sub>2</sub>, MgCl<sub>2</sub> or KCl was suspended in solutions of varying concentration of HSA. Albumin adsorption mostly occurred within the first hour and plateaued at a concentration of 0.8 mg/ml. HSA adsorption was found to be significantly higher on the titanium powder treated with either CaCl<sub>2</sub> or MgCl<sub>2</sub>. The highest amount of protein adsorption occurred with CaCl<sub>2</sub> treatment at physiological pH, indicating the importance of divalent ions, and specifically calcium ion, in the electrostatic mechanism of albumin adsorption.

These mechanisms remain an important factor to consider when evaluating the performance of surface modifications to the titanium alloy substrate. It has been discussed that the increased surface area of TNTs allows for increased cation and protein adsorption [47], which ultimately results in greater cellular adhesion. In particular, an increased number of sites for focal adhesions can be found, where divalent ions are necessary for integrin function.



**Figure 1.5** Schematic of divalent cations, phosphate ion, and protein adsorption to the charged TiO<sub>2</sub> surface.

## 1.4 Titanium Dioxide Thin Films as Semiconductors

In order to further understand the movement of charge within the oxide thin film systems produced by the aforementioned surface modifications, it is useful to discuss the semiconducting theories surrounding titanium dioxide. In particular, band gap theory and Fermi levels will be discussed.

# 1.4.1 Semiconductors: Band Gap Theory

Solids are classified based on their energy-band structure as conductors, insulators or semiconductors. Due to the number of atoms that must be considered, and consequently the number of molecular orbitals, energy differences between adjacent molecular orbitals within a given energy band are negligible and the band is treated as a continuum of energy levels [48]. These energy bands often contain multiple energy levels within each band, and require little energy for electrons to move to higher energy levels within the same band [49]. The movement of electrons can only occur at certain bands of energy, and any band that is completely occupied by electrons cannot conduct electric current [49]. The highest energy band completely occupied by electrons is known as the valence band, and the lowest energy band that is not completely filled is the conduction band.

In conductors, the conduction band is usually partially filled or overlaps the valence band, but generally these bands are separated by a region of energy in which no electron can reside. This region is known as the band gap [48]. In insulators, the band gap is too large to promote electrons from the valence band to the conduction band. However, in semiconductors, a narrowing of the band gap allows for such promotion. As electrons move from the valence band to the conduction band, positively charged vacancies, or holes, are left behind. These holes are considered mobile as they can move by transfer of electrons [48].

Doping can alter the electrochemical properties of a semiconductor. Doped semiconductors are referred to as *extrinsic* semiconductors and are characterized as either n-type or p-type. This concept is important as the TiO<sub>2</sub> layer is often considered to be an n-type semiconductor [50]. The dominant charge carriers of n-type semiconductors are electrons, while the dominant charge carriers of p-type semiconductors are holes (Figure 1.6).



**Figure 1.6** Schematic of band gap theory for n-type semiconductors (left) and p-type semiconductors (right) [51].

#### 1.4.2 Fermi Level and the Oxide-Solution Interface

Another important concept in conduction theory is the Fermi level. The Fermi level in essence is a hypothetical energy level of an electron at which the probability of occupation by thermal equilibrium is 50% [48]. This relates to voltage, as voltage is a measure of the chemical potential of electrons in a circuit. For an *intrinsic* semiconductor, the Fermi level lies at the midpoint between the highest energy of the valence band and the lowest energy of the conduction band, however, semiconductor doping alters the Fermi level. The Fermi level for n-type semiconductors lies just lower than the conduction band in energy, and can change depending on the polarization of the semiconductor electrode. At equilibrium, there is no net transfer of charge and the potential is referred to as the flatband potential (E<sub>fb</sub>). Potentials anodic and cathodic to the flatband potential cause depletion and accumulation layers respectively in the space charge region of n-type semiconductors, leading to energy band bending at the electrode-solution interface. [14, 48]. The accumulation layers of cathodically polarized n-type semiconductors provide an abundance of electrons for reduction reactions at the surface, while the

depletion layers of anodically polarized n-type semiconductors limit electron availability. These concepts are integral in understanding the properties of the interface of titanium and TiO<sub>2</sub> on implant surfaces as it relates to corrosion and passivation, and the movement of charge through the semiconducting oxide layer. Because this interface can be described as ohmic (having linear and symmetric current-voltage curves) and heavily doped, it can be implied that current can readily flow between the titanium substrate and its oxide layer [49].

## 1.5 Titanium and Titanium Alloy Corrosion Testing

#### 1.5.1 Aspects of Implant Corrosion

To understand the processes titanium and other metals used in biological systems undergo in a body environment, it is necessary to understand the corrosion mechanisms that take place *in vivo*. Various forms of corrosion and tribocorrosion of metallic biomedical implants that lead to implant failure include pitting, fretting, galvanic and stress corrosion. Pitting corrosion is caused by the depassivation of a small area of the metal surface. This area becomes anodic while a larger area becomes cathodic, leading to galvanic corrosion, which corresponds to spontaneous electrochemical cells. Fretting corrosion refers to the mechanical wear induced by a load that results in metallic debris [6]. In body fluids such as synovial fluid, blood and saliva, biological molecules consume products of anodic or cathodic reactions, altering the equilibrium of the corrosion redox reactions. The acceptable corrosion rate for metallic implants is about 2.5×10<sup>-4</sup> mm/yr [2]. Two physical characteristics that determine implant corrosion are thermodynamic forces and kinetic barriers such as surface oxide layers [2]. Additionally, wear/fretting can lead to the

acceleration of implant corrosion. The metal ions released from corrosive processes can induce inflammation and implant failure.

Corrosion of certain metals is also dependent upon the pH of the solution in which it is immersed. A common tool used to identify the tendency of a metal to corrode under certain conditions is a Pourbaix diagram [50], which yields the regions of corrosion and non-corrosion as a function of the electrochemical cell potential and the pH derived from the Nernst equation (Equation 1.1). The Pourbaix diagram portrays the conditions under which titanium corrodes, is immune to corrosion and experiences passivation, or the formation of a passive oxide layer. The thin oxide film can induce corrosion resistance by slowing the rate of corrosion [41]. These concepts are relevant to implant corrosion as the pH of body fluids can be subjected to change. Bacterial infection, wound sites, and certain beverages can lower the pH of their respective body fluids having adverse effects on titanium orthopedic and dental implants.

$$E = E^{0} - \frac{RT}{nF} \ln \left[ \frac{M_{1}^{n+}}{M_{2}^{n+}} \right].$$
 (1.1)

The corrosion behavior of metals in biological solutions has been extensively studied. As it relates to titanium implants in bone, it has been shown that the formation of calcium phosphate deposits on titanium decrease both the corrosion rate at open circuit potential (OCP) and the anodic reaction current in high anodic potential ranges greater than 2.6V. It was also shown that the addition of bovine serum albumin shifted OCP in the cathodic direction and inhibited the cathodic corrosion reaction [52]. These results illustrate the importance and synergism between the biological aspects of implant surfaces as well as resisting corrosion. This correlation is seen in other aspects regarding corrosion and the cellular response.

#### 1.5.2 The effects of corrosion potentials and currents on osteoblast cells

The field of tribocorrosion incorporates the mechanical effects of wear and friction and the chemical environment effects on the corrosive behavior of metallic implants. While a diverse array of corrosion resistant materials depend on thin surface oxide layers that create a passive film, mechanical wear processes and anodic polarization can lead to the removal of the passive film [53]. Mechanical disruption of the passive film through fretting corrosion is considered one of the principal causes of orthopedic and dental implant failure [53]. Goldberg and Gilbert [8] performed scratch tests on freely corroding Ti-6Al-4V samples. They found that physical disruption of the oxide layer and disturbance of the double layer, followed by repassivation, led to transient electric fields (Figure 1.7). They indicate that the current densities at the scratch site can reach upward of 10-100 A/cm<sup>2</sup>. It was also proposed that the data supports the supposition that the electrical transients propagate away from the scratch site. Additionally, fretting-corrosion and repassivation of titanium and cobalt chromium alloys in biological solutions have reported spontaneous voltages as negative as -1000 mV v. AgCl [9, 54]. Other reports have indicated that fretting corrosion of commercially pure Ti samples has led to OCP shifts as much as -500 mV v. SCE and mechanically abraded voltage shifts as much as -840 mV v. SCE, and that the rate of repassivation and ion dissolution are orders of magnitude higher than undisrupted surfaces when oxide films are abraded [55]. Gilbert et al. [55] monitored the concentration profile for oxygen near the commercially pure titanium surfaces under different polarized conditions. It was found that between the potentials of -100 and -500 mV v. AgCl the oxygen concentrations decreased by 15-25% within 2  $\mu$ m of the surface, and at potentials below -600 mV v. AgCl the concentration dropped rapidly to near zero values.



**Figure 1.7** Schematic of breaks in the passive oxide film that can occur during mechanical abrasion or galvanic corrosion [55].

The effects of spontaneous voltages at the implant surface have implications for the cellular response as it relates to osseointegration and implant failure. Additionally, with regard to galvanic corrosion, it allows for the understanding of how anodic and cathodic reactions due to corrosive processes affect the local cellular environment. Particularly, cathodic voltages and current densities will be discussed due to the prominence in the literature as it relates to both fretting corrosion and galvanic corrosion. Two hours of cathodically polarized (-1000 mV v. SCE) passivated titanium samples seeded with osteoblasts yielded decreased cell spreading and round morphology [55]. Haeri et al. [7] continued to explore the effects of voltage regions on pre-osteoblast cells, determining a voltage viability range and mode of cell death on CoCrMo surfaces. It was found that cathodic and

anodic voltages outside the ranges of -400 mV and +500 mV v. AgCl led to intrinsic apoptotic and necrotic cell death respectively after 24 hours of polarization. The magnitudes of current densities associated with cell death were 0.1  $\mu$ A/cm<sup>2</sup> and  $10\mu$ A/cm<sup>2</sup> for cathodic and anodic polarization respectively. They additionally investigated the cathodic polarization range of pre-osteoblasts seeded on c.p. Ti and on an anodically polarized TiO<sub>2</sub> film substrate. Electrochemical impedance increased over 24 hours of polarization with the oxide layer samples, keeping current densities lower and increasing the voltage viability range in comparison to the native oxide surface of the c.p. Ti control [56]. Voltages between -600 and -1000 v. AgCl have shown a reduction in cell spreading and cell viability on c.p. Ti for 24 hours of polarization [49]. This indicates the importance of oxide layer thickness in protection of voltages that may arise from fretting-corrosion or galvanic corrosion. The implications of the aforementioned studies have a potentially two-fold impact on cells, osseointegration and implant failure, the first being the effect of destructive currents that arise from polarization and the second being the reduction of oxygen concentration at the implant surface available for cellular respiration and vitality.

In discussing cellular viability, there are generally two modes of cell death that are considered in discussion: necrosis and apoptosis. Necrosis is caused by sudden injury to the cell, leading to a disruption of the plasma membrane and a release of intracellular contents into the surrounding extracellular space. Inflammation often results from cell necrosis. Alternatively, apoptosis is a method of programmed cell death via internal cellular signaling pathways and leads to little or no inflammation. There are two separate pathways that lead to apoptosis: *intrinsic* 

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and *extrinsic*. The intrinsic pathway is initiated from within the cell due to DNA damage or other severe cellular stresses. Conversely, the extrinsic pathway results from a proapoptotic ligand to the death receptor on the cellular membrane. These apoptotic pathways can be distinguished from one another by examining the type of caspase proteins released during the process, caspase 8 being the initiator caspase in the extrinsic pathway and caspase 9 being the initiator caspase in the intrinsic pathway [7]. Extreme cathodic potentials applied to cobalt chromium cultured with preosteoblast cells appear to activate the intrinsic pathway [7]. This could be attributed to the presence of reduction reactions at the metal surface potentially producing compounds that are toxic to the local cellular environment, in particular, the generation of reactive oxygen species (ROS) such as super oxide anion, hydroxyl, peroxyl, alkoxyl and hydrogen peroxide. High concentrations of these compounds damage DNA, initiate disruption of cellular processes and can lead to apoptosis and necrosis. It has been shown that ROS can generate from cathodically polarized Ti-6Al-4V with osteoblast cell culture and that oxidative stress and hydrogen peroxide generation can induce apoptosis and alter cellular adhesion [57, 58]. While normal levels of ROS are necessary to the homeostatic balance of cellular signaling processes and function as redox messengers, high concentrations of ROS modify cellular macromolecules, inhibit protein function, and lead to cell death by necrosis and apoptosis [59, 60]. As it relates to the use of titanium and its alloys, higher concentrations of TiO<sub>2</sub> nano particles compared to fine particles induce both extrinsic and intrinsic apoptosis in mouse epidermal JB6 cells. Increased levels of caspase 8, as well as increased levels of mitochondrial cytochrome c release and
membrane permeability, have been observed [61, 62]. The results regarding the cellular response to corrosion voltages and associated redox reactions are indicative of the necessity of investigating cellular viability, proliferation, and specifically spreading and adhesion with new nanoscale surface treatments to titanium substrates. In conjunction with osteogenic cellular responses, it is necessary to perform potentiodynamic and electrochemical impedance spectroscopy corrosion tests in cell culture media to better understand the electrochemical surface properties and how it may affect the local environment.

### 1.5.3 Potentiodynamic Testing

Potentiodynamic polarization is an integral method used to characterize the corrosion and passivation kinetics of a substrate. The method generally involves a three-electrode system in which the substrate is used as a working electrode, in conjunction with a counter electrode and an inert reference electrode in an electrolyte solution. The polarization scan offers critical information regarding the corrosion, passivation and pitting or transpassivation dissolution kinetics regarding different regions of cathodic or anodic polarization. Two important factors to consider are the corrosion potential and corrosion current density, which are estimated using Tafel's method of finding the intersection of cathodic and anodic currents (Figure 1.8) [63, 64]. The corrosion potential, or E<sub>corr</sub>, is representative of the substrate's tendency to corrode, while the corrosion current density, or I<sub>corr</sub>, is representative of the corrosion rate. In determining I<sub>corr</sub>, it is necessary to know the electrode surface exposed to the electrolyte solution. While this estimation is simpler with polished electrode samples, it is more difficult with samples of higher

surface roughness, such as thermally oxidized and electrochemically anodized samples. It is for this particular reason that BET surface analysis has been discussed and was utilized for estimating the true surface area of TNTs.

In addition to corrosion rates determined by potentiodynamic polarization, passivation and pitting kinetics can also be observed (Figure 1.8). Reaction's 1 and 2 display the chemical mechanisms by which titanium metal forms a passive oxide layer. In essence, as passivation occurs at a particular potential ( $E_{pass}$ ), the current density ( $I_{pass}$ ) remains relatively constant. This is because a passive oxide film is forming and preventing the dissolution of metal ion into the solution, thus protecting the surface from corrosion. Under higher anodic potentials, this passive film can break down, or pitting can begin to occur, resulting in higher current densities at the pitting potential ( $E_{pitt}$ ). Thus, different regions of a potentiodynamic polarization scan can illustrate the kinetics of corrosion and passivation, helping to electrochemically characterize the substrate's resistance to corrosion behavior.

Reaction 1: Ti +  $O_2 \rightarrow TiO_2$ 

 $\Delta G = -203.8 \text{ kcal/mol}$ 

 $\Delta G = -82.92 \text{ kcal/mol}$ 

Reaction 2: Ti +  $2H_2O \rightarrow TiO_2 + 4H^+ + 4e^-$ 



**Figure 1.8** Example graphs of potentiodynamic polarization displaying the Tafel method (left) and passivation/pitting (right).

Regarding the surface modifications of thermal oxidation, electrochemical anodization, and the combination of the two, some research has been preformed surrounding the corrosion resistance of titanium and titanium alloy in different electrolytic media. For example amorphous TNTs have been shown to increase corrosion resistance [65]. Potentiodynamic testing revealed that the open circuit potential was higher, and that the current densities were lower for amorphous TiO<sub>2</sub> nanotubes in comparison to a smooth polished control [65]. Current densities were lower for both corrosion and passivation. It has also been shown that temperature, pH and the presence of serum proteins affect the corrosive properties of Ti and Ti alloy [66-68]. Additionally, annealing cpTi and Ti alloy at temperatures yielding a mixture of anatase and rutile phases for at least 8 hours has been shown to increase the resistance to corrosion [69-71]. Both amorphous and annealed  $TiO_2$  nanotube foil substrates have also been shown to improve corrosion resistance in electrolyte and without serum proteins. solutions with Ouantitative analysis of potentiodynamic and EIS studies have shown decreased corrosion current densities and more noble corrosion and open circuit potentials, as well as increased polarization resistance [65, 72-74]. For annealed TNTs, this was attributed to anatase having larger crystalline size than amorphous and greater stability, causing an increase in the resistance of the outer tube layer.

## 1.5.4 Electrochemical Impedance Spectroscopy

Electrochemical Impedance Spectroscopy (EIS) is a useful tool for studying the resistive and capacitive nature of the corrosion of metal as it relates to biomedical implants and the redox reactions that occur at the implant surface. EIS

relies on alternating current (AC) circuit theory. A circuit with an ideal resistor can be described by Ohm's law:  $R = \frac{E(t)}{I(t)}$ , where R is the resistance, E is the potential and I is the current, both being functions of time. However, immersing titanium with a passive oxide film in an electrolyte solution represents a more complex circuit element. The addition of proteins and cells further complicates the circuit. In these cases, the more general parameter impedance is used to characterize the resistance to current flow. Impedance is measured by applying a sinusoidal potential and by measuring the current through the electrode. A different equation to represent impedance (Z) is given as  $Z = \frac{E(t)}{I(t)}$ . Again, E and I are the potential and current respectively. With AC current, they obey the equations  $E = E_0 \cos(\omega t)$  and  $I = I_0 \cos(\omega t - \phi)$ , where  $E_0$  and  $I_0$  are the potential and current amplitudes respectively and  $\Phi$  represents the phase angle. The impedance can incorporate properties of resistance, capacitance and inductance into a circuit element.

In the case of the corrosion of titanium alloys and their oxide layers in biological solutions, inductance can be ignored and the elements of resistance and capacitance are analyzed through EIS. Equations 1.2-1.4 give the derivation of complex impedance in which the real portion of the solution represents the resistance and the imaginary portion of the solution represents the reactance, or in this case, capacitance. The Nyquist plot (Figure 1.9) utilizes this calculation to show the relationship between the resistance and capacitance that represents the impedance to current flow. Additionally, Bode plots (Figure 1.9) of the absolute value of the impedance (or log of the absolute value of impedance) plotted against the log of the frequency, along with the phase angle (or negative phase angle) plotted against the log of the frequency, provides valuable information as to the differences between the input and output signals. That is, the effect a metal electrode may have on the impedance of the AC current can be seen comparing any lag in the current or changes in the absolute value of the impedance.

Impedance: 
$$Z = \frac{E(t)}{I(t)} = \frac{E_0 \cos(\omega t)}{I_0 \cos(\omega t - \phi)} = Z_0 \frac{\cos(\omega t)}{\cos(\omega t - \phi)}.$$
(1.2)

Euler's Formula:  $e^{ix} = \cos(x) + i\sin(x)$ ....(1.3)

Complex Impedance: 
$$Z = Z_0 \frac{e^{i\omega t}}{e^{i(\omega t-\phi)}} = Z_0 e^{i\phi} = Z_0 [\cos(\phi) + isin(\phi)]$$
.....(1.4)



Figure 1.9 Example graphs of Nyquist (left) and Bode (right) plots.

Polarization resistance is an important measurement in describing the resistive nature of the electrode. Whenever the potential of the electrode is moved away from the open circuit potential, the electrode is considered to be polarized, and current can flow through the electrode due to the electrochemical reactions at the surface. The magnitude of the current is determined by the reaction kinetics. As

previously discussed, there are two simultaneous reactions that are occurring in equilibrium at OCP, cathodic and anodic, when a metal freely corrodes. The open circuit potential is also known as the mixed potential, and the current for either reaction is known as the corrosion current (I<sub>corr</sub>).

#### 1.5.4 Circuit Modeling: Electrolyte and Biological Solutions

Circuit modeling is an integral part of fitting EIS data and gaining a better understanding of the physical properties of electrochemical processes. One of the simplest circuits of an electrode in an electrolyte solution is the Randle's Circuit, which incorporates the capacitance and resistance of the double layer in parallel, and this element is in series with the solution resistance. This circuit can also be modeled with a constant phase element (CPE) in place of a capacitor at the double layer (Figure 1.10). The CPE represents a non-ideal capacitor and provides a better model for imperfect dielectrics.



**Figure 1.10** Randle's circuits incorporating a capacitor (left) and constant phase element (right).

When a semiconductor is immersed in an electrolyte solution, in this case referring to titanium or titanium alloy with an oxide layer, because the Fermi level

of the oxide layer and the redox potential of the solution are often different, an equilibrium will be reached. This leads to a redistribution of charge between the semiconductor and the electrolyte solution, forming a double layer at the surface. The excess charge of the semiconductor can extend into the electrode creating a space charge region and a correlating voltage drop and electric field [48]. Thus, when immersed in solution, there are two double layers to consider when modeling, the space charge double layer and the electrode-electrolyte double layer. The electrode-electrolyte double layer has can be considered in three different ways. The first is referred to as the Helmholtz layer, and is represented as a simple capacitor. Further modifications of this concept were made by Gouy and Chapman (the Gouy-Chapman layer), and treat the double layer as diffuse in which the potential decreases exponentially as one moves away from the surface interface into the fluid bulk. These concepts were then combined into the Stern layer, and are characterized by the Helmholtz layer surrounded by an outer diffuse layer (Figure 1.11).

However, with the introduction of the space charge region within a  $TiO_2$  layer, the capacitance of the space charge region and the double layer are in series when immersed in an electrolyte solution. The total capacitance is thus represented as the inverse of the sum of their reciprocals. Because the space charge capacitance is much smaller than the double layer capacitance, when calculating the sum of their reciprocals the impact of the double layer is negligible. Thus, in the case of a semiconducting oxide layer, the capacitance (or CPE) is often represented as that of the space charge region only [48, 75].



**Figure 1.11** Schematic of oxide space charge region and stern double layer (left) with the associated Randle's circuit (right).

For the purposes of this study, it is also important to investigate the modeling of circuits involving TiO<sub>2</sub> nanotubes as well as thermal oxidation of Ti-6Al-4V alloy. As previously discussed, when fabricating nanotubes, a compact oxide layer is first formed during the anodization process, followed by nano-pores and then the formation of nanotubes. Thus, when creating a circuit model, it is important to incorporate the charge transfer with the semiconducting compact oxide layer and the semiconducting nanotubes [76]. Figure 1.12 shows a modified equivalent circuit used to represent TiO<sub>2</sub> nanotubes in which CPE<sub>1</sub> and R<sub>1</sub> are the constant phase element and charge transfer resistance respectively, and CPE<sub>2</sub> and R<sub>2</sub> are the constant phase element and resistance [77]. Figure 1.12 also shows an equivalent circuit for thermally oxidized Ti-6Al-4V in which CPE<sub>film</sub> and R<sub>film</sub> represent the constant phase element and resistance to charge transfer of the double layer, while CPE<sub>oxide</sub> and  $R_{oxide}$  represent the constant phase element and resistance of the oxide layer. These values can change at different annealing temperatures, and it has been shown that the oxide resistance of titanium alloy annealed at 700° C was lower than that annealed at 500° C. This was attributed to an increasing amount of  $Al_2O_3$  and alumina nuclei in the rutile layer [22].



**Figure 1.12** Equivalent circuits used for TNT (left) and thermally oxidized (right) titanium alloy in EIS analysis [22, 77].

As titanium and its alloys are immersed in body fluids, EIS can be measured as a function of time. The contents of the solution and their interaction with the metal surface will affect the measured impedance. As a compact passive layer grows at the metal surface, the impedance increases [78]. Huang [79] performed *in situ* electrochemical impedance spectroscopy analysis at open circuit potential (OCP) of osteoblast-like U-2 OS cells on polished Ti and Ti-6Al-4V alloy during 72 hours of incubation. The modeling of the circuit included a resistor and capacitor in parallel, representing the space charge region of the oxide layer, and a resistor and capacitor in parallel, representing the cellular adhesion layer (this took into account the effect other adsorbed biomolecules). The polarization resistance ( $R_{p}$ ) was defined as the addition of oxide resistance to the cellular resistance ( $R_{ox}+R_{cell}$ ). Over the 72-hour incubation period, it was shown that the impedance and  $R_{p}$  increased. The increase in resistance was attributed to cell adhesion, cell spreading and cell proliferation affecting ion transportation through the cell adhesion layer. During the cellspreading period (2h-24h) the determined  $R_p$  values of Ti and Ti-6Al-4V alloy went from 3.9 and 3.7 M $\Omega$ cm<sup>2</sup> to 4.7 and 4.5 M $\Omega$ cm<sup>2</sup> respectively, and during the proliferation period (24h-72h), the determined  $R_p$  values of Ti and Ti-6Al-4V alloy reached 10.8 and 8.0 M $\Omega$ cm<sup>2</sup> respectively [79]. These results have significant implications of the effect increased cellular adhesion plays in corrosion resistance and potential protection from polarization potentials and subsequent redox reactions that occur during corrosion (Figure 1.13).



**Figure 1.13** Equivalent circuit and physical representation of EIS using osteoblast cells [79].

Similar work was continued culturing cells on titanium with a nano-network surface layer [80]. Three anodic currents of unmentioned magnitude were used with c.p. Ti disks in 5M NaOH solution to create the Ti nano-network. EIS was performed on the samples in culture medium both with and without cells. The results showed a largely capacitive effect of the combined oxide layer and cellular

adhesion layer in the cell-cultured substrates. Additionally, impedance of the control without cell culture remained stagnant and significantly lower than control and anodized samples with cell culture over the course of 5 days. As soon as 12 hours, a significant increase in impedance could be seen in cell-cultured anodized samples, with the sample that received the larger magnitude current showing highest impedance (~5 M $\Omega$ cm<sup>2</sup>). It was proposed that the impedance of non cell-cultured samples was related to the oxide layer and adsorbed proteins from the culture medium (mainly albumin) while for the cell-cultured substrates the impedance was again related to cell adhesion, cell spreading and proliferation. Moreover, the increased surface area of the nano-network substrates led to greater protein adsorption to the anodized substrates as opposed to the untreated substrates. This increased protein adsorption also led to an increase in electrochemical impedance. In turn, the enhanced protein adsorption led to enhanced cellular adhesion, furthering the role of the cellular layer in increasing the electrochemical impedance as well [80]. The biological cellular layer can be an important factor in corrosion prevention of the substrate surface as it can prevent the diffusion of ions through the electrolytic system [81].

## 1.6 Summary and Objectives

Much is known about the nature of titanium and titanium alloy surface interactions, both biologically as well as electrochemically. Additionally, as nanoscale topography is continued to be investigated from a biological response position, there still exists a gap in characterizing the corrosive behavior of asreceived and annealed titania nanotubes. Specifically, given the change in

semiconducting properties of nanotubes before and after annealing, the vast increase in exposed surface area, and the incorporation of physiological conditions of temperature and presence of proteins, the current literature lacks a discussion of these particular parameters regarding the corrosive nature of titania nanotube or nanoporous substrates. This study aimed to accomplish three objectives, the first, to characterize the surface wettability of substrates anodized, thermally oxidized, and both anodized then thermally oxidized over the course of 25 days aging, the second, to evaluate the electrochemical behavior and corrosion/passivation behavior of these substrates, and third, to propose next-step research on the cellular response to these substrates when anodically or cathodically polarized.

#### Chapter 2

# Surface Characterizations of Anodized and Thermally Oxidized Ti6Al4V Abstract

The characterization of modified surfaces for biomedical application is an integral process in evaluating their performance. In this study, Ti-6Al-4V alloy discs were separated into four treatment groups, a polished "smooth" control, thermal oxidation (600° C, 3 hours), electrochemical anodization (60 V, 2 hours, ethylene glycol/ammonium fluoride solution), and a combination of the treatments with anodization followed by thermal oxidation. Surface characterizations included wettability, Fourier transform infrared spectroscopy, field emission scanning electron microscopy and Brunauer-Emmett-Teller surface area analysis of the anodized samples. Anodized+thermally oxidized samples exhibited super hydrophilic behavior and retained lower water contact angles over 25 days of aging than other treatments. Spectroscopy results yielded predominantly rutile on thermally oxidized surfaces, a mixture of amorphous and anatase titania nanotubes on anodized surfaces, and a mixture of rutile and anatase titania nanotubes on the combined treatment surfaces. Average nanotube diameter and length was ~100 nm and  $\sim 5 \mu m$  respectively. Additionally, the surface area measured for anodized samples was roughly 0.33 m<sup>2</sup> per gram.

# 2.1 Introduction

Titanium and titanium alloy have been widely used in applications for biomedical implants, specifically in hip and dental implants, due to factors of high strength, low modulus, low density, and general resistance to corrosion [6]. Despite strong relative corrosion resistance, the release of metal ions from Ti-6Al-4V alloy still remains a concern, as Al and V cations can cause inflammation and local toxicity, resulting in tissue damage, joint loosening and a need for revision surgeries [1]. Additionally, osseointegration is an integral component of the success of an implant in bone tissue. Because Ti is bioinert, it often shows poor cellular adhesion [82]. In an attempt to alleviate these clinical problems, numerous substrate surface modifications are currently being investigated. The surface modifications of interest regarding Ti-6Al-4V alloy include the formation of TiO<sub>2</sub> nanotubes (TNTs) via electrochemical anodization and the formation of thick oxide layers (compared to native) and change in oxide crystalline structure to anatase/rutile via thermal oxidation (TO). The formation of titania nanotubes via electrochemical anodization is attractive for application due to self-organization in an array perpendicular to the substrate, the ability to control the dimensions based on applied voltage and duration, and the ability to couple with other surface treatments such as thermal oxidation (as with this study), coatings, nanotube doping, and drug-loading [25]. These surface modifications have been studied extensively as to their effect on osteogenic response and improved biocompatibility. This response is dependent on surface topography, physiochemistry, mechanics and electronics, and is manifested by changes in adhesion, proliferation, morphology, migration and differentiation of cells in the osteogenic lineage [33]. The presence of nanophase surfaces and amorphous TiO<sub>2</sub> nanotubes (30-120 nm in diameter) on Ti and Ti-alloy substrates have been shown to increase wettability, protein adsorption, cellular adhesion, proliferation and differentiation of osteogenic cells in vitro [83]. Similar results

regarding accelerated cellular adhesion and differentiation have been seen in vivo as well [34]. Increased cellular adhesion has also been observed in vitro with thermally oxidized Ti-6Al-4V, and thermally oxidized TiO<sub>2</sub> nanotubes, with greater annealing temperature leading to accelerated cellular adhesion. That is, annealing temperatures >550° C that yield mixtures of anatase and rutile (predominantly rutile) led to greater osteoblast adhesion than amorphous and anatase compact and nanotube surfaces [84]. This effect was attributed not only to the nanoscale cellular cues promoted by the nanotube array, but also by the regularity in crystallinity (whether anatase or anatase/rutile) that led to increased regularity in cytoskeleton structure and filopodia spreading of osteoblasts [38]. In further testing the aforementioned surface modifications for their performance in corrosion resistance for application as a biomedical implant surface, it is necessary to characterize the surface using an array of techniques. In particular, this work provides novel surface area analysis of titania nanotubes produced by electrochemical anodization using Brunauer-Emmett-Teller multilayer gas adsorption techniques.

# 2.2 Experimental Procedure

# 2.2.1 Materials and surface modifications

Twenty Ti-6Al-4V alloy discs of 15 mm diameter and 3 mm thickness (Mac-Master Carr, Elmhurst, IL) were mechanically wet-ground using a series of abrasive pads (#320, #400, #600 and #800) (Carbimet 2, Buehler, Lake Bluff, IL). Samples were then polished using diamond paste (MetaDi 9-micron, Buehler, Lake Bluff, IL) with lubricant (MetaDi Fluid, Buehler, Lake Bluff, IL) on polishing cloth (TexMet polishing cloth, Buehler, Lake Bluff, IL), and brought to mirror finish using colloidal silica polishing suspension (MasterMet, Buehler, Lake Bluff, IL) on polishing cloth (Chemomet I, Buehler, Lake Bluff, IL). Samples were then divided into 4 groups: smooth (control), TO (formation of rutile/anatase by thermal oxidation at 600° C for 3h), Ad (formation of amorphous TNTs by electrochemical anodization at 60V for 2h) and Ad+TO (formation of rutile/anatase TNTs by 60V, 2h anodization followed by 600° C, 3h thermal oxidation).

The thermal oxidation process of the annealed sample group was performed in a Single-zone Quartz Furnace (Lindberg, S# 54032) in air at ambient pressure. The samples were ultrasonically cleaned in ethanol and dried with nitrogen gas prior to annealing. Once the target temperature of 600° C was obtained, samples were loaded 5 cm per 5 minutes into the region closest to the thermocouple to ensure gradual temperature change within the samples to avoid micro-cracks from thermal shock, and to ensure the accuracy of final sample temperature. The samples remained at the final position in the furnace for 3 hours, and then were removed 5 cm per 5 minutes to ensure gradual cooling. From the thermal oxidation process, it was expected to obtain a mixture of anatase and rutile as the predominant phases within the oxide layer.

For the electrochemical anodization process, samples were ultrasonically cleaned in acetone for 30 minutes prior to anodization. Ti-6Al-4V were connected to a voltage source (Keithley 2400 SourceMeter) and functioned as the working electrode while a copper rod functioned as the counter electrode. The two-electrode system was submerged in electrolyte containing 4.0 vol. % DI-water (3.85 ml), 0.2 wt. % NH<sub>4</sub>F (0.21 g), and ethylene glycol (EG, 96.15 ml). Samples were anodized at

60 V at room temperature for 2 hours. After anodization, samples were washed with DI-water, air dried and wrapped in sterile tissue (Kimwipe, Kimtech Science) and stored in a glass petri dish (KIMAX® Petri Dish).

For the Ad+TO sample group, both of the aforementioned processes were used. Samples were first subjected to the electrochemical anodization protocol of 60V for 2 hours in 4.0 vol. % DI-water (3.85 ml), 0.2 wt. % NH<sub>4</sub>F (0.21 g), and ethylene glycol (EG, 96.15 ml) electrolyte, followed by the thermal oxidation protocol of 600° C for 3 hours.

#### 2.2.2 Surface characterizations

(a) Water contact angle (WCA) measurement: A goniometer (Rame-Hart, NRL, CA) was used to measure the WCA of 5 μl water droplets placed onto the smooth, TO, Ad, and Ad+TO samples via a micro-syringe (Hamilton, 802RN). ImageJ software was used to determine to WCA measurements. Before WCA measurement, samples were removed from storage and sprayed with N<sub>2</sub> gas for 30 seconds to remove sterile tissue residue. After WCA measurements, samples were returned to sterile tissue wrap for storage. Day 0 was determined to be immediately after treatment for the TO, anodized, and anodized + TO groups. WCA was measured at the following time points in days: 0, 1, 2, 5, 7, 14, and 25. For the aging period all samples were removed from sterile tissue wrap and sprayed with N<sub>2</sub> gas for 30 seconds to remove residue prior to WCA measurements. After WCA measurements, samples were returned to sterile tissue wrap for storage.

(b) Fourier transform infrared spectroscopy (FTIR): Diffuse reflectance FTIR (Nicolet S#ADU9700221, Thermo Scientific, Waltham, MA, USA) was used to

perform surface composition characterization of anodized and thermally oxidized surfaces using the polished control as a background. Prior to the FTIR scans, samples were purged for 1 hour to minimize noise. The FTIR spectrum ranged from 400 to 4000 cm<sup>-1</sup> and was obtained with 2 cm<sup>-1</sup> resolution and 512 scans. Deconvolution was carried out using XPS Peak software.

(c) Field emission scanning electron microscopy (FESEM): A JEOL JSM-6320F FESEM was used to characterize sample surfaces after Ad, TO, and Ad+TO treatments. In order to obtain images of the different dimensions of TNTs, the anodized sample surfaces were scraped to remove TNTs onto double-sided conductive carbon tape, which was mounted onto an aluminum stub. Images displaying the length of the TNTs were produced with 10k magnification and a scale of 1  $\mu$ m. Images displaying the top and bottom of TNTs were produced using 50k magnification and a scale of 100nm. ImageJ software was used to measure TNT dimensions.

(d) Brunauer-Emmett-Teller (BET): BET surface area analysis was performed on a smooth and anodized sample using a Quantochrome Autosorb (Quantochrome Instruments, Boynton Beach, FL, USA). Krypton was the adsorbate and the sample mass for the Ad group was 0.67 g.

#### 2.2.3 Statistical analysis

Wettability was evaluated based on average WCA for each time period of aging. Parameters were tested separately using one-way ANOVA to examine the effects of the different surface treatments. Tukey test analysis was used for pair-

wise comparison between treatment groups. Statistical tests were performed using SPSS software (IBM, Armonk, NY, USA) and a critical level of significance of p=0.05.

#### 2.3 Results

## 2.3.1 Surface Wettability

Figures 2.1 and 2.2 show qualitative and graphical analysis of WCA measurements for the various sample groups over 25 days of aging. WCA measurements for the smooth group for days 0, 1, 2, 5, 7, 14 and 25 were 35°±4°. 61°±3°, 69°±9°, 69°±3°, 68°±5°, 71°±4° and 84°±4° respectively. WCA measurements for the TO group for days 0, 1, 2, 5, 7, 14 and 25 were  $25^{\circ}\pm4^{\circ}$ ,  $26^{\circ}\pm4^{\circ}$ , 34°±2°, 44°±7°, 58°±6°, 70°±9° and 79°±5° respectively. WCA measurements for the Ad group for days 0, 1, 2, 5, 7, 14 and 25 were 26°±2°, 26°±5°, 31°±3°, 37°±4°, 48°±1°, 64°±5° and 73°±3° respectively. Finally, WCA measurements for the Ad+TO group for days 0, 1, 2, 5, 7, 14 and 25 were 7°±1°, 18°±2°, 20°±3°, 24°±1°, 28°±4°, 35°±4° and 43°±1° respectively. WCA analysis of the aged sample groups showed significantly lower contact angles for the Ad+TO group compared to smooth throughout the 25-day aging period (p<0.001). Compared to the TO group. Ad+TO showed significantly lower WCA on days 0 (p<0.001), 2 (p=0.036), and 5-25 (p<0.001), while compared to the Ad group, Ad+TO showed lower WCA on days 0 (p<0.001), 5 (p=0.005), and 7-25 (p<0.001). Additionally, the Ad and TO groups showed significantly lower contact angles compared to smooth for days 0-14 (p=0.03), and were not statistically significant from one another throughout the aging process (p>0.05).



**Figure 2.1** Qualitative evaluation of water droplets on aged Ti alloy surfaces.



**Figure 2.2** Water contact angle data for smooth, TO, Ad, and Ad+TO samples collected at days 0, 1, 2, 5, 7, 14, and 25 days of aging. (\* p<0.05, \*\* p<0.01, \*\*\* p<0.001).

## 2.3.2 Fourier Transform Infrared Spectroscopy

The annealing temperature of 600° C for 3 hours was expected to yield polycrystalline anatase and rutile phases in the barrier layer (oxide layer) [36]. FTIR data (Figure 2.3) yielded a significant peak for the TO (Figure 2.4) and Ad+TO (Figure 2.6) groups of  $TiO_2$  at 832 cm<sup>-1</sup>. Deconvolution was used to produce the summations of peaks. The TiO<sub>2</sub> peak at 832 cm<sup>-1</sup> was presented as predominantly rutile (830 cm<sup>-1</sup>) for the TO group, and a summation of peaks at 830 cm<sup>-1</sup> and 870 cm<sup>-1</sup>, indicating the presence of rutile and anatase respectively, in the Ad+TO group [16]. Additional peaks found in the TO group for oxides of the alloying metals were VO<sub>2</sub> (715 cm<sup>-1</sup>) [85], Al-O (835 cm<sup>-1</sup>) [86], and Ti-O (940 cm<sup>-1</sup>) [16]. The presence of anatase appears consistent with the anodization process at 60V [16]. Both amorphous Ti oxide and anatase were present in the deconvolution of peaks from the Ad group (Figure 2.5) at both 939 cm<sup>-1</sup> and 769 cm<sup>-1</sup>, and 870 cm<sup>-1</sup> respectively [16]. Additional peaks representing Ti-OH were found at 1136 cm<sup>-1</sup> and 1007 cm<sup>-1</sup> and Al-OH at 979 cm<sup>-1</sup> and 1072 cm<sup>-1</sup> in the Ad group. Similarly, in the Ad+TO group, Ti-OH presented at 1048 cm<sup>-1</sup> [87], Al-OH at 980 cm<sup>-1</sup> [88] and alumina at 935 cm<sup>-1</sup> [16].



Figure 2.3 FTIR curves for all treated samples against the smooth background.



Figure 2.4 FTIR deconvolution for the TO samples.



Figure 2.5 FTIR deconvolution for the Ad samples.



Figure 2.6 FTIR deconvolution for the Ad+TO samples.

# 2.3.3 Field Emission Scanning Electron Microscopy

FESEM dimensional analysis: FESEM imaging showed evidence of similarly formed TNTs in a honeycomb pattern indistinguishable for both Ad and Ad+TO

sample groups (Figure 2.7-13). It should be noted that the honeycomb pattern is indicative of a nanoporous layer on top of the nanotube layer. This occurs due to reaccumulation of titanium ion after fluoride etching on the surface, and then reetching to create nanopores. Thus, the top images of the TNTs are in fact the openings of the nanopores, and give rise to the corresponding inner diameter of the scratched TNTs. Images of the top of scratched TNTs (Figure 2.7, Figure 2.10) were used to determine the average inner diameter of the Ad and Ad+TO TNTs and nanopores as 97.3  $\pm$  15 nm, images of the bottom of scratched TNTs (Figure 2.8, Figure 2.11) were used to determine the average outer diameter of the Ad and Ad+TO TNTs as 167.8  $\pm$  15 nm, and side images of scratched TNTs (Figure 2.9, Figure 2.12) were used to determine the average length of the Ad and Ad+TO TNTs as 5.3  $\pm$  0.75 µm. It was also shown that the TO group did not consist of a nanotube surface (Figure 2.13).



**Figure 2.7** Top view of scratched TNTs from samples anodized at 60V for 2 hours. In this image, one can see the top surface and the nanoporous layer openings.



**Figure 2.8** Bottom view of scratched TNTs from samples anodized at 60V for 2 hours. In this image, one can see the bottom of the nanotube layer, where individual nanotubes are closed at the interface with the compact oxide layer.



**Figure 2.9** Side view of scratched TNTs from samples anodized at 60V for 2 hours. In this image, one can see the length of the nanotube layer. Broken nanotubes are observed due to the scratching technique for imaging.



**Figure 2.10** Top view of scratched TNTs from samples anodized at 60V for 2 hours and annealed at 600° C for 3 hours. In this image, one can see the top surface and the nanoporous layer openings.



**Figure 2.11** Bottom view of scratched TNTs from samples anodized at 60V for 2 hours and annealed at 600° C for 3 hours. In this image, one can see the bottom of the nanotube layer, where individual nanotubes are closed at the interface with the compact oxide layer.



**Figure 2.12** Side view of scratched TNTs from samples anodized at 60V for 2 hours and annealed at 600° C for 3 hours. In this image, one can see the length of the nanotube layer. Broken nanotubes are observed due to the scratching technique for imaging.



**Figure 2.13** Top view of samples annealed only at 600° C for 3 hours. It can be observed that nanotubes or nanopores are not present.

# 2.3.4 Brunauer-Emmett-Teller Surface Area Analysis

Surface area estimation by BET: BET surface area analysis also provided a quantitative approach to estimating the exposed surface area of anodized samples. While the smooth disc did not have enough total surface area to register, the anodized disc yielded a total surface area of  $0.33 \text{ m}^2/\text{g}$ . Using the sample mass of 0.67 g and the assumption of the surface area of the base and side of the disc being comparably negligible to that of the nanotube surface, the estimated exposed surface area of the anodized sample was roughly 300 cm<sup>2</sup> (Table 2.1).

**Table 2.1** BET surface area for anodized samples.

Treatment	BET (m²/g)	Correlation	BET Exposed Surface Area (cm²)
Anodized	0.33	0.998	~300

#### 2.4 Discussion

Enhanced surface wettability of all treated groups as received is explained by both increased surface roughness and anatase/rutile crystalline phases for thermally oxidized groups, and by increased surface roughness and presence of hydroxyl groups (Ti(OH)<sub>4</sub>) from the electrochemical anodization process [Shin, 2011 #51], verified by FTIR analysis (Figure 2.3). For the Ad group, aging led to increased WCA due to replacement of  $Ti(OH)_4$  by  $TiO_2$  [83], and it appears that annealing the TNTs increased their stability and slowed the process of hydroxyl group replacement. These results indicate the Ad+TO samples superior wettability and conduciveness to a cell culture environment. Dimensions were consistent with TNT literature regarding similar voltage, anodization solvent and duration [25]. The determination of the surface area exposed to the electrolyte proved to be challenging, yet it is an integral part of analyzing EIS and potentiodynamic data in the following section, and often is not discussed in nanotube corrosion literature. Thus, it was useful to provide quantitative measurements in order to later estimate resistance, capacitance and current densities across the substrate surface-solution interface. The BET method is a sensitive and accurate method for measuring surface area, and has been used to estimate titania nanotube surface area (in some cases as much as 30  $m^2/g$ ) [25], however with the treated alloyed substrates, the surfaces are not uniform. That is, the treatments (specifically anodization) only occur on one face of the disc. Though most of the surface area can be attributed to the nanotubes, it is important to understand that the surface area analysis yields an estimation, and thus calculations that incorporate the surface area are also estimations. Nevertheless, qualitative comparisons of inadequate total surface area for BET analysis with smooth samples as well as average nanotube dimensions suggest vastly increased surface area in anodized samples (Ad and Ad+TO) compared to smooth and TO samples.

# 2.5 Conclusions

The following can be derived from this study:

- Electrochemical anodization was effective in producing anatase/amorphous TiO<sub>2</sub> nanotubes on Ti-6Al-4V substrates, while thermal oxidation (600° C) effectively changed the crystalline structure to an anatase/rutile mixture.
- The anodization voltage and duration yielded TNTs with average diameter close to 100nm and average length of 5 microns. The annealing process did not appear to disrupt the morphology of the TNT layer.
- Annealed nanotubes enhanced surface area, surface roughness and wettability, due to the preservation of hydroxyl groups on the substrate surface over 25 days of aging.

## Chapter 3

# The Effect of Electrochemical Anodization and Thermal Oxidation on Corrosion Resistance of Ti6Al4V

#### Abstract

The negative impact of *in* vivo corrosion of metallic biomedical implants remains a complex problem in the medical field. We aimed to determine the effects of electrochemical anodization (60 V, 2h) and thermal oxidation (600° C) on the corrosive behavior of Ti-6Al-4V, with serum proteins, at physiological temperature. From the previous study, it was determined that anodization produced a mixture of anatase and amorphous TiO<sub>2</sub> nanotubes, while the annealing process yielded an anatase/rutile mixture TiO<sub>2</sub> nanotubes. The Brunauer-Emmett-Teller surface area analysis yielded results of anodized samples approximately 3 orders of magnitude higher than that of polished control samples. In this study, corrosion resistance was evaluated on the parameters of open circuit potential, corrosion potential, corrosion current density, passivation current density, polarization resistance and equivalent circuit modeling. Samples both anodized and thermally oxidized exhibited shifts of open circuit potential and corrosion potential in the noble direction, indicating a more stable nanotube layer, as well as lower corrosion current densities and passivation current densities than the smooth control. They also showed increased polarization resistance and diffusion limited charge transfer within the bulk oxide layer. As per the findings, the treatment groups analyzed can be ordered from greatest corrosion resistance to least as Anodized+Thermally Oxidized > Anodized > Smooth > Thermally Oxidized.

# 3.1 Introduction

It has been shown that temperature, pH and the presence of serum proteins affect the corrosive properties of Ti and Ti alloy [66, 67, 89]. Additionally, annealing cpTi and Ti alloy at temperatures yielding a mixture of anatase and rutile phases for at least 8 hours has been shown to increase the resistance to corrosion [69, 70]. Both amorphous and annealed TiO<sub>2</sub> nanotube foil substrates have also been shown to improve corrosion resistance in electrolyte solutions with and without serum proteins. Quantitative analysis of potentiodynamic and EIS studies have shown decreased corrosion current densities and more noble corrosion and open circuit potentials, as well as increased polarization resistance [65, 72-74]. However, the effect and quantification of increased surface area due to titania nanotubes is not expressed in current literature with regard to incorporation into current density, resistance and capacitance measurements. These parameters are important to describe per unit area to gain a true understanding of what is happening at the substrate surface, and such analysis and discussion will be considered in this study.

As current research explores surface treatments to enhance implant osseointegration, it is necessary to understand the effects on the corrosion properties at the implant surface in a biofluid and cell culture environment. The present work aimed to test the wettability, cellular adhesion response, and corrosive behavior of Ti-6Al-4V alloy discs with novel surface treatments and electrolyte regarding thermal oxidation temperature and duration, electrochemical anodization voltage and duration, and combining the two processes of electrochemical anodization followed by thermal oxidation, in cell culture media with serum proteins. Additionally, a novel method of surface area analysis was used from the previous study to incorporate true surface area increases due to the titania nanotube arrays in order to describe the effects of substrate polarization on a per unit area basis. In furthering the understanding of the corrosive properties of the aforementioned surface treatments of Ti-6Al-4V alloy, we expected the thermally oxidized titania nanotubes of higher rutile content to display the greatest resistance to corrosion due to the increased surface area of the nanotube array, added stability of the nanotubes through crystalline structure, higher resistance of rutile compared to anatase, and growth of a thick passive oxide layer beneath the nanotube array [25].

# 3.2 Experimental Procedure

## 3.2.1 Materials and surface modifications

Twenty Ti-6Al-4V alloy discs of 15 mm diameter and 3 mm thickness (Mac-Master Carr, Elmhurst, IL) were mechanically wet-ground using a series of abrasive pads (#320, #400, #600 and #800) (Carbimet 2, Buehler, Lake Bluff, IL). Samples were then polished using diamond paste (MetaDi 9-micron, Buehler, Lake Bluff, IL) with lubricant (MetaDi Fluid, Buehler, Lake Bluff, IL) on polishing cloth (TexMet polishing cloth, Buehler, Lake Bluff, IL), and brought to mirror finish using colloidal silica polishing suspension (MasterMet, Buehler, Lake Bluff, IL) on polishing cloth (Chemomet I, Buehler, Lake Bluff, IL). Samples were then divided into 4 groups: smooth (control), TO (formation of rutile/anatase by thermal oxidation at 600° C for 3h), Ad (formation of amorphous TNTs by electrochemical anodization at 60V for
2h) and Ad+TO (formation of rutile/anatase TNTs by 60V, 2h anodization followed by 600° C, 3h thermal oxidation).

The thermal oxidation process of the annealed sample group was performed in a Single-zone Quartz Furnace (Lindberg, S# 54032) in air at ambient pressure. The samples were ultrasonically cleaned in ethanol and dried with nitrogen gas prior to annealing. Once the target temperature of 600° C was obtained, samples were loaded 5 cm per 5 minutes into the region closest to the thermocouple to ensure gradual temperature change within the samples to avoid micro-cracks from thermal shock, and to ensure the accuracy of final sample temperature. The samples remained at the final position in the furnace for 3 hours, and then were removed 5 cm per 5 minutes to ensure gradual cooling. From the thermal oxidation process, it was expected to obtain a mixture of anatase and rutile as the predominant phases within the oxide layer.

For the electrochemical anodization process, samples were ultrasonically cleaned in acetone for 30 minutes prior to anodization. Ti-6Al-4V were connected to a voltage source (Keithley 2400 SourceMeter) and functioned as the working electrode while a copper rod functioned as the counter electrode. The two-electrode system was submerged in electrolyte containing 4.0 vol. % DI-water (3.85 ml), 0.2 wt. % NH<sub>4</sub>F (0.21 g), and ethylene glycol (EG, 96.15 ml). Samples were anodized at 60 V at room temperature for 2 hours. After anodization, samples were washed with DI-water, air dried and wrapped in sterile tissue (Kimwipe, Kimtech Science) and stored in a glass petri dish (KIMAX® Petri Dish).

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For the Ad+TO sample group, both of the aforementioned processes were used. Samples were first subjected to the electrochemical anodization protocol of 60V for 2 hours in 4.0 vol. % DI-water (3.85 ml), 0.2 wt. %  $NH_4F$  (0.21 g), and ethylene glycol (EG, 96.15 ml) electrolyte, followed by the thermal oxidation protocol of 600° C for 3 hours.

### 3.2.2 Corrosion Tests

All electrochemical tests were performed using a potentiostat (SP-240, BioLogic, Claix, France) and a custom made electrochemical cell. Corrosion measurements were recorded using EC-Lab v.10.23 software (BioLogic, Claix, France). A 3-electrode configuration was used with the Ti alloy sample as the working electrode, a standard calomel reference electrode (SCE), and a graphite rod counter electrode. Dulbecco's Modified Eagle Medium (DMEM) + 10% fetal bovine serum (FBS) (Gibco, Life Technologies, Grand Island, NY, USA) served as the electrolyte solution to mimic cell culture media (volume = 10 mL) (Figure 3.1). Prior to testing, all samples were ultrasonically cleaned in acetone for 15 minutes followed by ultrasonic cleaning in deionized H<sub>2</sub>O for 10 minutes. Samples were mounted to the corrosion cell, and the electrochemical setup was kept at 37° C using a cell culture incubator (WJ501-T, Baxter, Deerfield, IL, USA) throughout electrochemical testing.



Figure 3.1 Schematic diagram of the experimental apparatus for corrosion testing.

Electrochemical tests performed included open circuit potential (OCP), electrochemical impedance spectroscopy (EIS), and cyclic polarization. Three trials (n=3) were performed for each group. After a potentiostatic electrochemical cleaning stage at -0.9 V vs. SCE, OCP was performed for 1 hour to allow the system to stabilize. Following OCP, EIS measurements were obtained over a frequency range of 100K-0.005 Hz ( $\pm$  10 mV vs. SCE at corrosion potential). EIS data was used to determine equivalent circuit models and thereby estimations of effective circuit resistance (R<sub>eff</sub>) and effective circuit capacitance (CPE<sub>eff</sub>) using constant phase element (CPE) equivalents. EC-Lab v. 10.23 software was used to perform the z-fit analysis of equivalent circuit models over a range of 1000-0.01 Hz. Nyquist and Bode plots were used to estimate polarization resistance (R<sub>p</sub>). Finally, samples were cyclically polarized from -1 V vs. SCE to 1.8 V vs. SCE and back to -1 V vs. SCE (2 mV/s scan rate). Corrosion parameters evaluated from the cyclic polarization scan included corrosion potential ( $E_{corr}$ ), corrosion current density ( $I_{corr}$ ) and passivation current density ( $I_{pass}$ ).  $E_{corr}$  and  $I_{corr}$  were estimated using Tafel's method (± 25 mV from  $E_{corr}$ ).  $I_{pass}$  was determined from current density measurements within the passivation region of the cyclic scan.

### 3.2.3 Statistical analysis

Corrosion behavior was evaluated based on parameters of OCP,  $E_{corr}$ ,  $I_{corr}$ ,  $I_{pass}$ ,  $R_{eff}$ ,  $CPE_{eff}$  and  $R_p$ . Corrosion parameters were tested separately using one-way ANOVA to examine the effects of the different surface treatments. Tukey test analysis was used for pair-wise comparison between treatment groups. Statistical tests were performed using SPSS software (IBM, Armonk, NY, USA) and a critical level of significance of p=0.05.

### 3.3 Results

## 3.3.1 Open Circuit Potential

Evolution of free potential: Initially, OCP tests were conducted prior to EIS to determine the free potential of the surfaces. There are statistical differences between all sample groups (p<0.01) and can be seen in Figure 3.2 and Figure 3.3. The OCP for the smooth, TO, Ad and Ad+TO groups were -525 ± 44 mV vs. SCE, -330 ± 25 mV vs. SCE, -204 ± 48 mV vs. SCE and -46 ± 27 mV vs. SVE respectively (Table 3.1).



**Figure 3.2** OCP curves for smooth, TO, Ad, and Ad+TO samples.



**Figure 3.3** Graphical representation of OCP values for smooth, TO, Ad, and Ad+TO samples.

### 3.3.2 Potentiodynamic Polarization

Changes in corrosion potential, corrosion current, and passivation behavior: The Tafel's slope method was to calculate E<sub>corr</sub> and I<sub>corr</sub> for the various sample groups. Figure 3.4 corresponds to the incorporated BET surface area estimation. Statistical analysis yielded significant differences between Ad+TO and all other treatment group means (p<0.001). The TO group also showed a significant shift in the noble (positive) direction compared to the Ad group (p=0.009) and smooth (p<0.001). E<sub>corr</sub> among the sample groups was, from greatest to smallest (or smallest to greatest in magnitude), -317±57 mV vs. SCE for Ad+TO, -678±17 mV vs. SCE for TO, -776±12 mV vs. SCE for Ad, and -875±17 mV vs. SCE for smooth (Table 3.1, Figure 3.5). Similar to the incorporation of estimated surface area for samples with TNTs for EIS analysis, BET estimated surface area was included in the calculation of the corrosion current density, which serves an indicator of the corrosion rate. Icorr among the sample groups was, from smallest to greatest, was  $6.0 \times 10^{-5} \,\mu\text{A/cm}^2$  for Ad+TO,  $2.5 \times 10^{-3} \mu A/cm^2$  for Ad,  $3.5 \times 10^{-1} \mu A/cm^2$  for TO, and  $2.2 \times 10^{-1} \mu A/cm^2$  for smooth (Table 3.1, Figure 3.6). I<sub>corr</sub> for Ad+TO and Ad treatments were significantly lower than TO and smooth (p<0.001). The forward scan of the cyclic polarization test was used to estimate the passivation current density (I<sub>pass</sub>) at 1.5 V vs. SCE (Figure 3.4) and to analyze the passivation behavior. Estimated TNT surface area was incorporated in the same method as for I<sub>corr</sub> calculations. From smallest to greatest,  $I_{pass}$  for the different sample groups was determined to be  $2.8 \times 10^{-2} \,\mu\text{A/cm}^2$ for Ad,  $8.6 \times 10^{-2} \mu$ A/cm<sup>2</sup> for Ad+TO,  $2.1 \mu$ A/cm<sup>2</sup> for TO, and  $12.6 \mu$ A/cm<sup>2</sup> for smooth (Table 3.1, Figure 3.7). Ipass for Ad+TO and Ad treatments were significantly lower

than TO and smooth (p<0.001).

**Table 3.1** OCP and cyclic polarization testing. OCP values, values of  $E_{corr}$  and  $I_{corr}$  determined from Tafel's method, and  $I_{pass}$  values with standard deviations for the different treatment groups.

Treatment	E <sub>OCP</sub>	E <sub>corr</sub>	I <sub>corr</sub>	pass
Smooth	525 (44)	875 (17)	2.2×10 <sup>-1</sup> (0.36)	12.6×10 <sup>0</sup> (0)
TO	330 (25)	678 (17)	3.5×10⁻¹ (0.23)	2.1×10 <sup>0</sup> (0.32)
Anodized	204 (48)	776 (12)	2.5×10 <sup>-3</sup> (0.7)	2.8×10 <sup>-2</sup> (0.1)
Anodized + TO	46 (27)	317 (57)	6.0×10⁻⁵ (5.6)	8.6×10⁻² (1.5)



**Figure 3.4** Cyclic polarization curves using BET surface area analysis for anodized samples.



Figure 3.5 Graphical analysis of  $E_{corr}$  values for smooth, TO, Ad, and Ad+TO samples.







**Figure 3.7** Graphical analysis of I<sub>pass</sub> values for smooth, TO, Ad, and Ad+TO samples.

## 3.3.3 Electrochemical Impedance Spectroscopy

EIS data and equivalent circuits: Equivalent circuit models for smooth (Figure 3.8a), TO and Ad (Figure 3.8b), and Ad+TO (Figure 3.8c) were used to analyze EIS data, and thereby estimate the effective circuit resistance (R<sub>eff</sub>) and the effective circuit capacitance in the form of a constant phase element (CPE<sub>eff</sub>) (Table 3.3). R<sub>eff</sub> and CPE<sub>eff</sub> were determined by Equation 3.1 and Equation 3.2 respectively. Either R<sub>film</sub> or R<sub>nt</sub>, and CPE<sub>film</sub> or CPE<sub>nt</sub> were used depending on the circuit model, and are further explained.

$$R_{eff} = R_b + R_{film/nt} \dots (3.1)$$

$$CPE_{eff} = \left(\frac{1}{CPE_b} + \frac{1}{CPE_{film/nt}}\right)^{-1}....(3.2)$$

The circuit model for the smooth group consisted of a modified Randle's circuit with electrolyte solution resistance ( $R_{sol}$ ), film resistance ( $R_{film}$ ) and

capacitance ( $CPE_{film}$ ), the TO group consisted of electrolyte solution resistance ( $R_{sol}$ ), barrier resistance  $(R_b)$  and capacitance  $(CPE_b)$  in series with film resistance  $(R_{film})$ and capacitance ( $CPE_{film}$ ), the Ad group consisted of solution resistance ( $R_{sol}$ ), barrier resistance ( $R_b$ ) and capacitance ( $CPE_b$ ) in series with nanotube layer resistance (R<sub>nt</sub>) and capacitance (CPE<sub>nt</sub>) and the Ad+TO group consisted of electrolyte solution resistance ( $R_{sol}$ ), barrier resistance ( $R_b$ ) and capacitance ( $CPE_b$ ) in series with a Warburg diffusion element  $(W_b)$  and nanotube layer resistance  $(R_{nt})$ and capacitance (CPE<sub>nt</sub>). CPE elements were used rather than ideal capacitors due to evidence of time dependence and non-ideal capacitor behavior in the Nyquist and Bode plots (Figures 3.12-15, Figure 3.16-19). Additionally, due to the presence of serum proteins, properties of interface adsorption elements may be included in the TO, Ad, and Ad+TO group layers due to their increased surface roughness and previous use in TNT corrosion studies with serum proteins [72]. It has been shown that surface roughness, even compared to surface wettability, is a strong determinant of protein adsorption [90]. Table 3.2 displays all circuit element values as well as X<sup>2</sup> values determining the goodness of fit. While the total R<sub>eff</sub> was greatest for the smooth group at 7.1M $\Omega$ , it is important to consider the resistance and capacitance values with the associated surface area. For the Ad and Ad+TO groups, the value obtained from BET surface area analysis was used. The order of greatest to smallest  $R_{eff}$  was Ad (1.2×10<sup>8</sup>  $\Omega$ .cm<sup>2</sup>), smooth (2.7×10<sup>6</sup>  $\Omega$ .cm<sup>2</sup>), Ad+TO (2.3×10<sup>6</sup>  $\Omega$ .cm<sup>2</sup>), and TO (7.6×10<sup>5</sup>  $\Omega$ .cm<sup>2</sup>), with a significant difference between Ad and other treatment groups (p=0.017). Regarding the CPE<sub>eff</sub>, the order from greatest to smallest was smooth (4.3×10<sup>-5</sup> F/cm<sup>2</sup>), TO (7.7×10<sup>-5</sup> F/cm<sup>2</sup>), Ad+TO (6.3×10<sup>-8</sup>

 $F/cm^2$ ), and Ad (5.4×10<sup>-8</sup>  $F/cm^2$ ), with significant differences between both Ad+TO and Ad treatments compared to smooth and TO (p<0.001). Ad+TO and Ad effective capacitance were not significantly different (p>0.05). Additionally, the Ad+TO equivalent circuit was fitted with a Warburg diffusion element ( $W_b=1.16\times10^3$ ), indicating diffusion limited charge transfer. Equivalent circuit modeling proved to be difficult across the full EIS frequency range, thus a shorter range of 1,000-0.01 Hz was used for modeling, and then extended to show the full range covered by the data. Nyquist and Bode plots display experimental and equivalent circuit fitting. R<sub>eff</sub> was calculated as an estimation of the equivalent resistance of each circuit. This value is often related to the polarization resistance, however, this made for a difficult comparison due to the varying components of each circuit, for example, the inclusion of a Warburg diffusion element in the Ad+TO group as indicated by the diffusion tail in the Nyquist plot (Figure 3.15). It is known that  $R_p$  can also be estimated by using the y-intercept of the Bode resistance and subtracting R<sub>sol</sub> [91], thus Bode plots were also used to determine the polarization resistance  $(R_p)$  of the sample electrodes (Table 3.3, Figure 3.16-19). The order of sample groups from largest to smallest  $R_p$  value was Ad > Ad+TO > smooth > TO, with significant differences between the Ad treatment and all other treatment groups (p<0.001). The R<sub>p</sub> values determined from Bode plot analysis appear to be more consistent with further discussed indicators of superior corrosion resistance of anodized samples, and may provide a better understanding of the polarization resistance compared to R<sub>eff</sub> (Figure 3.10). C<sub>eff</sub> results also appear consistent with Bode plot analysis (Figure 3.11). Smooth samples (Figure 3.12) yielded high capacitive behavior within low frequencies (<10 Hz) with a phase angle > 80° and low capacitive behavior within high frequencies (>1000 Hz). A similar curve shape can be seen with the Ad samples (Figure 3.14), indicating the similarities among amorphous oxide layers, however the phase angle magnitude at lower frequencies is smaller than that of the smooth group, meaning lower capacitive behavior. The TO samples (Figure 3.13) also showed higher capacitive behavior within low frequencies, but in lower magnitude (phase angle near 60°) than both smooth and Ad samples. Conversely, the Ad+TO group (Figure 3.15) yielded higher capacitive behavior in higher frequencies, with the lowest magnitude phase angle of all groups. There appeared to be capacitive behavior within all frequency ranges of the Ad+TO group, with a range of phase angles from 17° to 56°. The graphical analysis is consistent with CPE<sub>eff</sub> being lowest within the Ad+TO group, and supports its high  $R_p$  due to the thick barrier oxide layer.



**Figure 3.8** Equivalent circuits for smooth (a), TO (b), Ad (b), and Ad+TO (c) used to measure frequency responses of EIS. Individual circuit components were used to estimate  $R_{eff}$  and  $CPE_{eff}$ , and circuit model fitting was used to estimate  $R_p$ .

**Table 1.2** EIS modeling parameters obtained using the equivalent circuits shown in Figure 24 for EIS diagrams presented in Figure 25 and Figure 26 with standard deviations.

Treatment	R <sub>sol</sub>	CPEb	ab	Rb	W <sub>b</sub>	CPE <sub>film/nt</sub>	<b>a</b> film/nt	R <sub>film/nt</sub>	X <sup>2</sup>
	(Ohm)	(F.s <sup>a-1</sup> )		(Ohm)	(Ohm.s <sup>-1/2</sup> )	(F.s <sup>a-1</sup> )		(Ohm)	
Smooth						1.7×10 <sup>-5</sup>		7.1×10 <sup>6</sup>	6.7×10 <sup>-3</sup>
	68 (11)					(0.17)	0.98	(2.2)	
TO		6.4×10 <sup>-5</sup>		2.0×10 <sup>6</sup>		5.6×10 <sup>-5</sup>		3.1×10 <sup>3</sup>	3.7×10 <sup>-3</sup>
	54 (11)	(1.3)	0.67	(1.9)		(1.5)	0.63	(0.31)	
Ad		4.0×10 <sup>-5</sup>		3.8×10 <sup>5</sup>		2.9×10 <sup>-5</sup>		2.7×10 <sup>4</sup>	1.0×10 <sup>-2</sup>
	77 (3)	(1.8)	0.88	(2.0)		(0.52)	0.78	(4.5)	
Ad+TO		1.9×10 <sup>-5</sup>		4.9×10 <sup>3</sup>	1.16×10 <sup>3</sup>	1.5×10 <sup>-5</sup>		2.8×10 <sup>3</sup>	1.7×10 <sup>-3</sup>
	24 (12)	(1.2)	0.66	(2.7)	(0.16)	(0.7)	0.72	(2.6)	

**Table 3.3** Effective resistance and capacitance calculations and  $R_p$ . Values calculated for  $R_{eff}$  and  $CPE_{eff}$  from equivalent circuit modeling as well as  $R_p$  values determined from Figure 26 with standard deviations.

Treatment	R <sub>p</sub> (Ohm.cm <sup>2</sup> )	R <sub>eff</sub> (Ohm.cm <sup>2</sup> )	CPE <sub>eff</sub> (F.s <sup>a-1</sup> /cm <sup>2</sup> )
Smooth	5.5×10 <sup>5</sup> (0.69)	2.7×10 <sup>6</sup> (0.86)	4.3×10 <sup>-5</sup> (0.44)
TO	6.4×10 <sup>4</sup> (1.2)	7.6×10 <sup>5</sup> (7.4)	7.7×10⁻⁵ (1.5)
Ad	1.0×10 <sup>8</sup> (0.18)	1.2×10 <sup>8</sup> (0.74)	5.4×10 <sup>-8</sup> (1.3)
Ad+TO	5.2×10 <sup>6</sup> (1.0)	2.3×10 <sup>6</sup> (0.56)	6.3×10⁻ <sup>8</sup> (3.9)



**Figure 3.9** Estimated polarization resistance for smooth, TO, Ad, and Ad+TO samples.







**Figure 3.11** Estimated effective capacitance for smooth, TO, Ad, and Ad+TO samples using circuit model results.



Figure 3.12 Nyquist plot for smooth samples.



Figure 3.13 Nyquist plot for TO samples.



Figure 3.14 Nyquist plot for Ad samples using BET surface area analysis estimation.



**Figure 3.15** Nyquist plot for Ad+TO samples using BET surface area analysis estimation.



Figure 3.16 Bode plots for smooth samples.



**Figure 3.17** Bode plots for TO samples.



**Figure 3.18** Bode plots for Ad samples using BET surface area analysis estimation.



**Figure 3.19** Bode plots for Ad+TO samples using BET surface area analysis estimation.

### 3.2 Discussion

### 3.4.1 Variation in Electrochemical Potential and Kinetics

The treatment OCP values provide an indication of the electrode's tendency corrode in reaching steady state redox reactions, thus it can be concluded that the Ad+TO group displayed the lowest corrosion tendency due to presenting the smallest difference in potential from the reference electrode. Regarding potentiodynamic testing, analysis of the anodized treatment groups, that is, Ad and Ad+TO, showed excellent corrosion resistance, with the Ad+TO showing the greatest resistance to corrosion. For the Ad+TO group, there appeared to be two Tafel regions within the scan. The region in which the current density fell closest to 0  $\mu$ A/cm<sup>2</sup> was used to calculate E<sub>corr</sub> and I<sub>corr</sub>. The double region may have occurred

due to the presence of polycrystalline TNTs or due to excess hydrogen evolution in the cathodic region. Similar to the OCP testing, the extrapolated E<sub>corr</sub> using the Tafel method serves as an indication of the working electrode's tendency to corrode by displaying the potential at which the anodic current begins to exceed the cathodic. A clear shift of OCP and E<sub>corr</sub> in the noble direction for TNT layer samples was observed, with the greatest being for the Ad+TO group, indicating a decreased tendency to corrode in the cell media electrolyte. This analysis is consistent with previous studies regarding thermally oxidized TNTs, in which the sample material and method of anodization differs from that of this study [72, 92]. Compared to OCP analysis, the  $E_{corr}$  analysis showed that TO had a nobler  $E_{corr}$  than Ad, and all were nobler than the smooth control. The results are indicative that the presence of a thick amorphous and anatase TNT layer provides more stability than the thin spontaneous amorphous oxide layer. It further suggests that the thermal oxidation forming predominantly rutile crystalline structure in the non-anodized group provides a more stable crystalline phase at E<sub>corr</sub>. The heat treatment appears to be additive to the TNT layer compared to the spontaneous layer of the smooth control, and can explain the differences in E<sub>corr</sub> for the various treatment groups. Though both the Ad and Ad+TO had greater surface area exposure to the electrolyte, incorporating BET surface area, the corrosion rate (I<sub>corr</sub>) appears to be roughly two orders of magnitude smaller in the Ad+TO group than the Ad group, and four orders of magnitude smaller than the smooth group. Exact quantification between the groups is difficult to discern due to the surface area estimation method, however, significantly decreased I<sub>corr</sub> due to surface area increases were shown. Low current densities are not only an indicator of slower local corrosion, but are also important in retaining cell viability at a substrate surface as high current densities have been shown to induce apoptosis and necrosis in cell culture[7]. The smooth samples showed little change in corrosion current density, appearing to display passivation until roughly -0.3 V vs. SCE (575 mV greater than  $E_{corr}$ ). The passive layer was then removed and corrosion occurred until stable passivation close to 0 V vs. SCE. The smooth sample also yielded the greatest  $I_{pass}$ . The TO samples showed unstable passivation beginning at roughly -0.5 V vs. SCE (178 mV greater than  $E_{corr}$ ) and most likely transpassivation dissolution at 1 V vs. SCE (1.3 V greater than  $E_{corr}$ ). This trend of depassivation can also be seen, and is more gradual, with the Ad+TO samples.

# *3.4.2 The Effect of Surface Crystalline Structure on Corrosion (Passivation)*

The corrosion/passivation trends may indicate the susceptibility of the different crystalline phases of the alloy oxides to mediate corrosion, passivation or depassivation during anodic polarization, in particular, the mix of anatase, rutile and alumina in the Ad+TO group compared to predominantly rutile in the TO group. While the Ad+TO group did exhibit stable passivation at roughly 0.3 V vs. SCE (617 mV greater than  $E_{corr}$ ), and had  $I_{pass}$  smaller than both smooth and TO samples, it did exhibit corrosive behavior during anodic regions slightly above  $E_{corr}$ . The Ad samples exhibited unstable passivation in the scan immediately after  $E_{corr}$ , and began stable passivation at roughly 0.8 V vs. SCE (1.58 V greater than  $E_{corr}$ ). The instability reflects the composition of the nanotube layer as predominantly a mixture of anatase and amorphous TiO<sub>2</sub>, Depassivation of more unstable amorphous regions create smaller areas of increased corrosion or transpassivation dissolution

until stable passivation is reached. The Ad samples also showed the lowest I<sub>pass</sub>. Compared to the Ad+TO group, it was observed that passivation occurred in the Ad group immediately in anodic regions slightly higher than E<sub>corr</sub>. Additionally, FESEM imaging showed deep corrosion crevices in the surface of the Ad+TO group (Figure 3.20) compared to the Ad (Figure 3.21) due to the anodic corrosion. The higher susceptibility to display corrosion current densities in the Ad+TO group under slight anodic polarization is most likely due to the increased presence of rutile crystalline structure. Increased grain boundaries may lead to increased corrosion, as well as increased resistance to charge transfer and diffusion-limited charge transfer observed in EIS. The interplay of diffusion limited charge transfer correlates to the corrosion and passivation mechanisms regarding the diffusion of metal ion and anionic oxygen species, and will be further discussed.



Figure 3.20 FESEM images of Ad after cyclic polarization.



Figure 3.21 FESEM images of Ad+TO after cyclic polarization.

## 3.4.3 Corrosion Process and Mechanisms

In order to understand the corrosion and passivation behaviors during potentiodynamic polarization, it is necessary to understand the mechanisms and properties of the bulk and porous oxides of the different treatment groups. Figure 3.22 shows a simple proposed mechanism for corrosion and the release of metal ions in to the electrolyte solution coupled with passivation due to anodic polarization. Figure 3.22a and Figure 3.22b are crude representations of the smooth and TO groups, though they differ in thickness and crystalline composition of the oxide layer, while Figure 3.22c and Figure 3.22d are crude representations of the Ad and Ad+TO groups, though they differ in thickness of the bulk oxide layer as well as

crystalline composition of the bulk oxide and nanotube layers. The difference between substrates that may impact the mechanisms are the thickness of the bulk oxide, presence of a porous nanotube layer, composition of both bulk and porous oxide layers, and wettability (due to the presence of serum proteins in the cell culture media). It has been shown that under anodic polarization, growth of the passive layer in Ti-6Al-4V occurs at the metal-oxide interface due to diffusion of anionic oxygen species through the passive layer, rather than at the oxide-solution interface due to metal ion diffusion as it does with Ti [93]. Thus, for passivation to occur, diffusion of oxygen species to the bulk metal is necessary with increasing anodic polarization, while diffusion of metal ion species through the oxide layer(s) to the solution interface is limited. In essence, anionic species are created at the solution front where their concentration remains constant, often from the splitting of water in aqueous solutions[94]. The increased wettability, surface roughness and nanotube topography of Ad+TO may lead to increased protein adsorption. This may be a contributing factor as to why the Ad+TO group experienced stable passivation "later" in the potentiodynamic scan. The exact effect of protein prevalence is unknown, and is beyond the scope of this study, but the presence of a protein layer at the oxide-solution interface may inhibit the creation and diffusion of anionic oxygen species for passivation, while also inhibiting the dissolution of metal ion from the surface. The differences in crystalline structure of both compact and porous oxide lavers also influence the corrosion/passivation kinetics. Polycrystalline materials offer different diffusion pathways for ion species, and understanding the movement of defects in the crystals can provide information as to the corrosion kinetics of polycrystalline Ti alloy oxides. In addition, nanotube layers offer diffusion pathways through nanotube pores as well as nanotube walls (Figure 3.20b). It has been reported that oxygen defects, both interstitial and vacancies, are faster diffusers in anatase compared to rutile [95], and that rutile offers greater resistance to charge transfer [25]. This may indicate the ability of anionic oxygen species to diffuse faster through anatase TiO<sub>2</sub> than rutile, and would explain why the Ad group experienced almost immediate passivation, though unstable, compared to the Ad+TO and TO treatments. There are clear benefits of having a mixture of anatase and rutile crystalline structures in TNTs and bulk oxide layers for wettability, the stability of the TNTs, lower tendency to corrode, and lower current densities. Further research can optimize the annealing temperature and duration, altering the anatase/rutile composition and thickness and thus improving the corrosion/passivation kinetics of the Ad+TO treatment.



**Figure 3.21** Proposed mechanism of corrosion/passivation for (a) smooth, (b) TO, (c) Ad, and (d) Ad+TO substrates showing diffusion of metal ions ( $M^{n+}$ ) and anions, specifically  $O^{2-}$  and  $OH^{-}$ , through the passive oxide layer. For each figure, the circle on the left indicates corrosion, while that on the right indicates passivation.

# 3.6 Conclusions

The following can be derived from this study:

- Electrochemical anodization was effective in producing anatase/amorphous TiO<sub>2</sub> nanotubes on Ti-6Al-4V substrates, while thermal oxidation (600° C) effectively changed the crystalline structure to an anatase/rutile mixture.
- Annealed nanotubes not only enhanced surface roughness and wettability, but also enhanced corrosion resistance with respect to OCP,  $E_{corr}$ ,  $I_{pass}$

and capacitance and polarization resistance behavior. This was most likely due to increased thickness of the bulk oxide layer, and the stability and increased surface area of the polycrystalline nanotubes.

- The Ad+TO group had E<sub>corr</sub> and E<sub>OCP</sub> shifts in the noble direction, as well as decreased I<sub>corr</sub> and I<sub>pass</sub>. Stable passivation was observed as well.
- Both anodized groups (Ad and Ad+TO) exhibited high R<sub>p</sub>, and EIS analysis indicated diffusion dependent limited charge transfer for Ad+TO.
- In slightly anodic regions compared to E<sub>corr</sub>, anatase/amorphous nanotubes exhibited immediate passivation while annealed nanotubes exhibited corrosion and crevices in the surface.
- As per the findings, the treatment groups analyzed can be ordered from greatest corrosion resistance to least as Ad+TO > Ad > Smooth > TO.

#### Chapter 4

## Future Work: The Effect of Applying Static Potentials to Treated Ti6Al4V Surfaces on Cellular Adhesion and Viability of Pre-osteoblasts

### 4.1 Introduction

Analyzing the cellular response to modified titanium alloy surfaces is necessary in order to gain an understanding of how a modified implant surface would behave in an osteogenic environment. Parameters of importance are the ability of the implant surface to facilitate cellular adhesion, proliferation and differentiation. Smooth, hydrophobic titanium surfaces have shown poor cellular growth [26]. Osseointegration remains an important component of the success of orthopedic and dental implants in bone, and thus new nanoscale surface topographies are being investigate to increase implant integration into bone tissue. Of consideration are titania nanotubes (TNTs), fabricated by electrochemical anodization. Besides providing nanoscale topography, the modification can also be used in conjunction with other surface treatments, such as thermal oxidation. In fact, evidence supports that the osteogenic response with regard to adhesion, proliferation and differentiation is enhanced in the presence of titania nanotubes, and more specifically at higher annealing temperatures (>450° C) [12, 38, 84]. Additionally, it is known that spontaneous cathodic potentials can be induced due to fretting, and anodic potentials during corrosion [56]. The effect of these potentials coupled with the electrochemical reactions that accompany them on cellular responses on titania nanotube and annealed titania nanotube surfaces is unknown. This study will aim to determine if the same anodization and annealing conditions carried out in Chapters 2 and 3 regarding surface characterizations and corrosion

resistance will yield an increased osteoblast adhesion response and cellular viability under cathodic and anodic potentiostatic conditions. That is, the differences in cellular adhesion and viability regarding smooth surfaces, amorphous TNT surfaces, and annealed TNT surfaces containing predominantly rutile  $TiO_2$  will be determined.

### 4.2 Experimental Procedure

### 4.2.1 Materials and Surface Modifications

Thirty-six Ti-6Al-4V alloy discs of 15 mm diameter and 3 mm thickness (Mac-Master Carr, Elmhurst, IL) are mechanically wet-ground using a series of abrasive pads (#320, #400, #600 and #800) (Carbimet 2, Buehler, Lake Bluff, IL). Samples were then polished using diamond paste (MetaDi 9-micron, Buehler, Lake Bluff, IL) with lubricant (MetaDi Fluid, Buehler, Lake Bluff, IL) on polishing cloth (TexMet polishing cloth, Buehler, Lake Bluff, IL), and brought to mirror finish using colloidal silica polishing suspension (MasterMet, Buehler, Lake Bluff, IL) on polishing cloth (Chemomet I, Buehler, Lake Bluff, IL). Samples will be divided into 3 groups: smooth (control), Ad (formation of amorphous TNTs by electrochemical anodization at 60V for 2h) and Ad+TO (formation of rutile/anatase TNTs by 60V, 2h anodization followed by 600° C, 3h thermal oxidation).

For the electrochemical anodization process, samples will be ultrasonically cleaned in acetone for 30 minutes prior to anodization. Ti-6Al-4V will be connected to a voltage source (Keithley 2400 SourceMeter) and function as the working electrode while a copper rod functions as the counter electrode. The two-electrode system will be submerged in electrolyte containing 4.0 vol. % DI-water (3.85 ml), 0.2 wt. % NH<sub>4</sub>F (0.21 g), and ethylene glycol (EG, 96.15 ml). Samples will be

anodized at 60 V at room temperature for 2 hours. After anodization, samples will be washed with DI-water, air dried, and wrapped in sterile tissue (Kimwipe, Kimtech Science) and stored in a glass petri dish (KIMAX® Petri Dish).

For the Ad+TO sample group, the aforementioned electrochemical anodization process will be used. Followed by thermal oxidation. The thermal oxidation process will be performed in a Single-zone Quartz Furnace (Lindberg, S# 54032) in air at ambient pressure. The samples will be ultrasonically cleaned in ethanol and dried with nitrogen gas prior to annealing. Once the target temperature of 600° C will be obtained, samples will be loaded 5 cm per 5 minutes into the region closest to the thermocouple to ensure gradual temperature change within the samples to avoid micro-cracks from thermal shock, and to ensure the accuracy of final sample temperature. The samples will remain at the final position in the furnace for 3 hours, and then will be removed 5 cm per 5 minutes to ensure gradual cooling. From the thermal oxidation process, it was expected to obtain a mixture of anatase and rutile as the predominant phases within the oxide layer. Samples will be first subjected to the electrochemical anodization protocol of 60V for 2 hours in 4.0 vol. % DI-water (3.85 ml), 0.2 wt. % NH<sub>4</sub>F (0.21 g), and ethylene glycol (EG, 96.15 ml) electrolyte, followed by the thermal oxidation protocol of 600° C for 3 hours.

4.2.2 Osteoblast Cell Culture

MC3T3-E1 mouse pre-osteoblasts will be cultured in Dulbecco's Modified Eagle Medium (DMEM) with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin (Gibco, Life Technologies, Grand Island, NY, USA). Cells will

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be cultured at 37° C in a cell culture incubator with 5% CO<sub>2</sub> (WJ501-T, Baxter Scientific Products, Deerfield, IL, USA).

### 4.2.3 Electrochemical Tests

To determine the cellular adhesion and viability response, treated Ti-6Al-4V discs will be placed in a culture well plate and seeded with  $2 \times 10^5$  cells/mL for each disc. All potentiostatic tests will performed using a potentiostat (SP-240, BioLogic, Claix, France) and a custom made electrochemical cell. Measurements will be recorded using EC-Lab v.10.23 software (BioLogic, Claix, France). A 3-electrode configuration will used with the Ti alloy sample as the working electrode, a standard calomel reference electrode (SCE), and a graphite rod counter electrode. Dulbecco's Modified Eagle Medium (DMEM) + 10% fetal bovine serum (FBS) (Gibco, Life Technologies, Grand Island, NY, USA) will serve as the electrolyte solution (volume = 10 mL). Prior to testing, all samples will be ultrasonically cleaned in acetone for 15 minutes followed by ultrasonic cleaning in deionized H<sub>2</sub>O for 10 minutes. Samples will be mounted to the corrosion cell, and the electrochemical setup will be kept at 37° C using a cell culture incubator (WJ501-T, Baxter, Deerfield, IL, USA) throughout potentiostatic polarization.

Both immediately prior to, and immediately after, potentiostatic polarization, electrochemical impedance spectroscopy (EIS) measurements will be obtained over a frequency range of 100K-0.005 Hz ( $\pm$  10 mV vs. SCE at corrosion potential) to determine changes in impedance over a 24-hour period. EIS data will be used to determine equivalent circuit models and thereby estimations of effective circuit resistance (R<sub>eff</sub>) and effective circuit capacitance (CPE<sub>eff</sub>) using constant phase

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element (CPE) equivalents. EC-Lab v. 10.23 software will be used to perform the zfit analysis of equivalent circuit models over a range of 1000-0.01 Hz. Nyquist and Bode plots will be used to estimate polarization resistance ( $R_p$ ). After initial EIS measurements, the substrates will be potentiostatically polarized for 24 hours. In the anodic region, 250 mV vs SCE will be used to examine the cellular response in the potential of corrosive behavior of smooth and annealed nanotubes. In the cathodic region, -600 mV vs SCE compared to  $E_{corr}$  will be used, as this potential has been shown to induce apoptosis and necrosis in osteoblast cells [56].

## 4.2.4 Cell Adhesion and Viability

A standard adhesion immunostaining assay using fluorescent-labeled calcein will be used to quantify the cell adhesions after 24 hours of potentiostatic polarization for each of the 3 treatment groups. Additionally, a standard live/dead immunostaining assay will be used to quantify the effect potentiostatic polarization has on the viability of pre-osteoblasts on the treated Ti alloy substrates.

### Chapter 5

### **Clinical Scope**

### 5.1 Relevance and Application

After analyzing the research performed, and proposing new research directions regarding the use of annealed nanoporous and nanotubular Ti alloy substrates as implant surfaces, it is necessary to provide clinical context. The surface treatments of electrochemical anodization and thermal oxidation lend themselves to application in the biomedical device industry as they are relatively inexpensive and can be scaled. An important question to ask regarding these implant surfaces is what will the next generation of metallic biomedical implants for orthopedic and dental purposes look like? As mentioned, the nanotube substrates provide the potential to act as a smart, bioselective surface with its loading capabilities. Antibiotics, osteogenic growth factors, or a combination of both can be loaded into the nanotube surface for timed release, whether it is constant or pH sensitive in the case of antibiotics.

As it was shown in the bulk of the study, the annealing conditions that produced predominantly rutile  $TiO_2$  nanotubes exhibited enhanced corrosion resistance regarding most corrosion parameters, most importantly, the tendency of the surface to corrode. Though the mechanical properties of these surfaces must continue to be examined, we can begin to gain insight into the future scope of these implant surfaces: a multifunctional, smart, bioselective surface with enhanced corrosion resistance. This work aimed to take first steps into examining the danger/protection of metal ion release with specific anodization and thermal oxidation conditions, as that remains a clinical concern and necessity before examining other applicable possibilities of use for these treated surfaces. The goal for the future of these next generation implants must be to optimize the surface treatments so that they provide the best functional surface for both osseointegration as well as protection from corrosion, and to work directly with clinicians to identify any further complications so that as engineers we may continue to tailor the surface of these implants to increase their longevity and functionality. In sum, annealed nanotube surfaces have the potential to reduce the clinical problems of infection, osseointegration, and metal ion release due to corrosion, and thus reduce the risks of implant failure and the need for revision surgeries.

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# VITA

# JOHN C. GROTBERG

## EDUCATION

**University of Illinois at Chicago**, Chicago, IL M.S. in Bioengineering (expected May 2014) GPA: 4.0 Pre-med coursework (Fall 2011-Present) GPA: 4.0

#### Grinnell College, Grinnell, IA

B.A. in General Sciences, concentrations in Psychology and Neuroscience (May 2009)

## LABORATORY AND CLINICAL EXPERIENCE

# Department of Bioengineering, University of Illinois at Chicago, Chicago, IL

*Master's Degree Thesis Research* (Summer 2013) Determining the corrosive behavior and cellular responses to Ti-6Al-4V substrates modified by electrochemical anodization and thermal oxidation as it relates to biomedical implants for orthopedic and dental application.

# Infant Special Care Unit, NorthShore University Health System, Evanston, IL

Clinical Shadowing (Fall 2012-Spring 2013)

Attended morning rounds with neonatologists and observed numerous Caesarean sections as well as other procedures.

## Department of Neonatology, NorthShore University Health System, Evanston, IL

Research Assistant (Summer 2012-Summer 2013)

Conducted experiments related to necrotizing enterocolitis (NEC) in premature infants. Feed and collect tissue samples from neonatal rats overnight.

## Department of Biomedical Engineering, University of Michigan, Ann Arbor, MI

Research Assistant (Fall 2011)

Conducted experiments involved with fluid dynamic properties and forces of unhealthy mucus plug rupture at microscale as related to cystic fibrosis and other pulmonary related diseases and assisted in editing of the publication.

# Department of Biomedical Engineering, University of Michigan, Ann Arbor, MI

## Research Assistant (Summer 2008)

Conducted experiments involved with surfactant replacement therapy research at microscale. Synthesized microchannels working to create methods to utilize bifurcating microchannels for research experiments.

## Child Health Associates Ann Arbor, Ann Arbor, MI

#### Medical Assistant (Summer 2007)

Performed clinical operations such as finger pokes, heights, weights, temperatures, charting, vision and hearing tests, blood pressures, throat swabs, as well as some laboratory operations on pediatric patients.

#### Department of Biomedical Engineering, University of Michigan, Ann Arbor, MI

Research Assistant (Summer 2006)

Conducted experiments involved with surfactant replacement therapy research at microscale. Synthesized microchannels working to create methods to utilize bifurcating microchannels for research experiments.

# Department of Radiology, University of Michigan, Ann Arbor, MI

Research Assistant (Summer 2005)

Executed experiments with in vitro cancer treatment research. Calibrated ultrasound transducers, measured the best frequency transducer for evaporating perfluorocarbon microdroplets.

## Department of Biomedical Engineering, University of Michigan, Ann Arbor, MI

Research Assistant (Summer 2004)

Performed surfactant replacement therapy experiments to establish how surfactant travels through the lungs to determine how to optimize this therapy. Used oils of different viscosities, moving the oil as a plug through a bifurcating tube, measuring the daughter tube to parent tube ratios of the split oil plug.

## PUBLICATIONS AND PRESENTATIONS

J. Grotberg, A. Hamlekhan, S. Patel, D. Royhman, C. Sukotjo, T. Shokuhfar, C. Takoudis, M. Mathew. The effects of electrochemical anodization and thermal oxidation on the corrosive behavior of Ti-6Al-4V. *NACE Corrosion Society*, Ed. M. Ehrensberger. March 10, 2014, San Antonio, USA.

Zheng, Y., H. Fujioka, J.C. Grotberg, and J.B. Grotberg. Effects of inertia and gravity on liquid plug splitting at a bifurcation. *Journal of Biomechanical Engineering*. 128:707-716, 2006.

## PROFESSIONAL ASSOCIATIONS

University of Illinois at Chicago, Chicago, IL Institute of Biometarials, Tribocorrosion and Nanomedicine (Member, August 2013-Present) Advanced Materials Research Laboratory (Member, August 2013-Present) Golden Key Honour Society Member (2012-Present)

# AWARDS AND HONORS/FELLOWSHIPS

**University of Illinois at Chicago**, Chicago, IL *Tuition and Fee Waiver* (Spring 2014) *General Chemistry Award* (2012)

## Grinnell College, Grinnell, IA

Grinnell Trustee Honor Scholarship (2005-2009) Martin Luther King Jr. Scholarship (2005-2009)

# BASKETBALL EXPERIENCE

# Professional Basketball, Europe

*Bayer Giants Leverkusen* (Germany, July 2009-November 2009) *London Capital* (London, November 2009-January2010) *Red Miners Kayl* (Luxembourg, February 2010-June 2011) Led the Red Miners to a Luxembourgish Championship (May 2010), 33 ppg 2011 leading scorer 26 ppg Also assisted with the coaching of 4 youth teams ranging from ages 7 to 17 using both French and German language.

# Grinnell College Pioneers, Grinnell, IA

Varsity Team Member (Sept 2005-May 2009)

Grinnell College Coach's Award (2009)

NCAA record holder for most career 3-point makes (526) 2009

Grinnell College Career Records: Total points (2,848), Scoring Avg. (29.7), FG made (904), Free Throw Percentage (83.6)

#1 in NCAA scoring average (28.1 ppg, 31.0 ppg) #1 in NCAA 3 pt/g (5.8, 6.2) 2007-2009

All-American Honors, All Midwest Region, All Midwest Conference, '08-'09 Captain

All-American Honors, All Midwest Region, All Midwest Conference, '07-'08 Captain (2006-2007).

National All Freshman, All Midwest Region, All Midwest Conference, MVP Grinnell Varsity Basketball (2005-2006)